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Effects of Environment and Variety on Alkylresorcinols in Wheat in the HEALTHGRAIN Diversity Screen†

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Alkylresorcinols (AR), phenolic lipids found in high amounts in whole grain wheat and rye, can be used as biomarkers for these cereals. The content (on a dry matter basis) and homologue composition of AR were determined in 26 wheat varieties grown in Hungary in 2005−2007, as well as in the United Kingdom, France, and Poland in 2007. There was a significant effect of year, location, and variety on both total AR and individual AR homologue content ($p < 0.001$). A warm and dry climate generally resulted in higher AR contents, whereas high precipitation especially during plant development and grain-filling resulted in lower contents. There was a significant negative correlation between AR content and thousand-kernel weight ($p < 0.001$), which may be explained by the warm and dry climate giving smaller kernels with higher AR content. The difference between varieties was generally consistent, with highest and lowest AR contents for the same varieties during different years and at different locations ($p < 0.001$). Total AR content was correlated with the relative proportions of the different homologues, with a relatively lower concentration of homologues C17:0 and C19:0 and a lower C17:0/C12:0 ratio at higher overall contents. The results show that AR content is a highly heritable phytochemical component but that it is also affected by the environment.

KEYWORDS: Alkylresorcinols; biomarker; wheat; whole grain; variety; environment

INTRODUCTION

Alkylresorcinols (AR) are quantitatively important phenolic compounds in wheat and rye (*1*) and are also found in small amounts in barley (*2*). They are amphiphilic 1,3-dihydroxybenzene derivatives with an odd-numbered alk(en)yl chain at position 5 of the benzene ring in the range of 17−25 carbon atoms, giving a mixture of AR homologues C17:0−C25:0 in specific proportions for different cereals. C15:0 and C27:0 may also be found in small amounts (*3*, *4*). AR are located in the intermediate layers between pericarp and testa in the grain (*5*) and, therefore, are found in high amounts only in whole grain and bran products of wheat and rye, but not in refined flour or products (*3*, *6*). They are suggested as markers for whole grain wheat and rye in food (*7*−*9*) and also as biomarkers for human intake of whole grain wheat and rye (*1*, *10*).

The content of AR in wheat has been shown to vary between 300 and 1000 μg/g (*7*, *11*, *12*) and in rye between 360 and 3200 μg/g of dm (*11*, *13*−*16*). Even though the total AR content varies both within and between cereal species, the relative homologue composition is rather constant within species. The relative homologue composition of AR in wheat samples has been shown to be about 3−6% C17:0, 29−42% C19:0, 46−55% C21:0, 4−12% C23:0, and 1−4% C25:0 (*7*, *11*, *12*). The ratio of C17:0 to C21:0 is generally about 0.1 for common wheat (*7*), 0.01 for durum wheat (*4*, *17*), and 1.0 for rye (*11*). Thus, the ratio of C17:0 to C21:0 may be a tool to distinguish between individual types of cereals (*7*).

The content of AR has also been shown to be affected by different environmental factors. Previously, one study with 125 common wheat samples showed that there was a large variation for the same wheat cultivar grown at different locations in three countries (*11*). However, no details on year and location were given. Chen et al. (*7*) found that the content of AR was higher in spring wheat and lower in winter wheat grown in Östergötland (southeastern Sweden) than in Skåne (southern Sweden). Cultivars with a high AR content at one location also tended to have a high content at the other location, which has also been shown for rye (*15*). These findings suggest that both environment and cultivar have important effects on the AR content in cereal grains, but more extensive and controlled field trials are required to better estimate these effects.

The aim of this study was to investigate the effects of environment and variety on AR in a controlled field experiment, for which 26 different wheat varieties were grown for three years (2005−2007) in one location, and at four different locations for one year (2007). The aim was also to investigate if there was a correlation between total AR content and relative proportions of AR homologues, which has not been previously studied in detail. The wheat samples were selected from the HEALTHGRAIN diversity screen (150 wheat lines), which was established to explore the extent of variation in phytochemicals and other bioactive components in the gene pool available for plant breeders (*12*, *18*−*22*).

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Table 1. Alkylresorcinol (AR) Content and Relative Homologue Composition in Winter (n = 24) and Spring (n = 2) Wheat Grown in 2005−2007 in Hungary[a]

	total AR (μg/g of dm)		homologue content (mean ± SD, μg/g of dm)				
year	mean ± SD	range	C17:0	C19:0	C21:0	C23:0	C25:0
2005	458 ± 88a	257−610	21 ± 4a	166 ± 28a	215 ± 49a	43 ± 12a	13 ± 5a
2006	635 ± 137b	335−835	26 ± 5b	204 ± 35b	303 ± 75b	73 ± 20b	28 ± 9b
2007	684 ± 151b	361−981	30 ± 5c	230 ± 41c	326 ± 84b	72 ± 21b	26 ± 8b

[a] Values with different letters within a column are significantly different from each other ($p < 0.001$).

Table 2. Alkylresorcinol (AR) Content and Relative Homologue Composition in Winter (n = 24) and Spring Wheat (n = 2) Grown in 2007 at Four Different Sites[a]

		total AR (μg/g of dm)		homologue content (mean ± SD, μg/g of dm)				
site	n	mean ± SD	range	C17:0	C19:0	C21:0	C23:0	C25:0
Hungary	26	684 ± 151a	361−981	30 ± 5a	230 ± 41a	326 ± 84a	72 ± 21a	26 ± 8a
France	26	570 ± 107b	362−802	24 ± 5b	190 ± 31b	277 ± 65b	58 ± 14b	21 ± 6b
United Kingdom	26	603 ± 105b	544−727	28 ± 6a	215 ± 35ac	281 ± 61b	57 ± 13b	21 ± 5b
Poland	24[b]	608 ± 115b	401−821	27 ± 5a	210 ± 37c	285 ± 59b	62 ± 15b	23 ± 6b

[a] Values with different letters within a column are significantly different from each other ($p < 0.001$). [b] No spring wheats were grown in Poland.

Table 3. Average Content of Total Alkylresorcinols (AR) and AR Homologues in Different Varieties of Wheat Cultivated in Hungary in 2005−2007 (n = 3 Years)

		AR homologue content (mean ± SD, μg/g of dm)					
cultivar	total AR (mean ± SD, μg/g of dm)	C17:0	C19:0	C21:0	C23:0	C25:0	ratio C17:0/C21:0
Tremie	809 ± 187	30 ± 5	255 ± 47	397 ± 98	92 ± 26	35 ± 13	0.08
Claire	738 ± 179	29 ± 7	240 ± 54	359 ± 85	81 ± 24	29 ± 12	0.08
Riband	698 ± 226	24 ± 7	204 ± 58	368 ± 117	77 ± 32	26 ± 13	0.07
Estica	693 ± 112	33 ± 5	245 ± 33	322 ± 49	70 ± 17	24 ± 9	0.10
Avalon	688 ± 161	28 ± 5	224 ± 36	329 ± 82	74 ± 27	28 ± 13	0.09
Malacca	684 ± 162	32 ± 6	252 ± 47	303 ± 74	71 ± 25	26 ± 12	0.11
Disponent	681 ± 89	22 ± 3	208 ± 25	338 ± 46	83 ± 13	30 ± 7	0.07
Atlas-66	670 ± 89	30 ± 3	231 ± 17	312 ± 45	71 ± 17	27 ± 9	0.10
Valoris	670 ± 168	25 ± 6	220 ± 41	327 ± 85	73 ± 26	25 ± 11	0.08
Cadenza[a]	665 ± 129	24 ± 4	194 ± 29	340 ± 69	77 ± 19	29 ± 10	0.07
Rialto	655 ± 187	26 ± 7	222 ± 51	317 ± 96	69 ± 26	21 ± 10	0.08
Herzog	637 ± 130	24 ± 4	199 ± 34	313 ± 66	73 ± 20	28 ± 10	0.08
Tommi	618 ± 106	26 ± 4	205 ± 31	302 ± 55	65 ± 14	21 ± 6	0.09
Chinese-Spring[a]	615 ± 122	29 ± 6	199 ± 38	273 ± 53	76 ± 17	37 ± 8	0.11
Lynx	592 ± 140	22 ± 5	183 ± 38	294 ± 69	70 ± 22	23 ± 9	0.07
Isengrain	591 ± 128	32 ± 6	225 ± 33	263 ± 67	53 ± 18	17 ± 7	0.12
CF99105	566 ± 93	27 ± 2	205 ± 17	260 ± 50	55 ± 17	19 ± 8	0.10
Campari	551 ± 101	23 ± 5	186 ± 36	263 ± 44	60 ± 12	19 ± 5	0.09
Maris-Huntsman	547 ± 111	19 ± 3	160 ± 20	291 ± 61	59 ± 19	19 ± 9	0.07
Tiger	544 ± 103	25 ± 4	189 ± 25	255 ± 53	54 ± 14	21 ± 6	0.10
Crousty	541 ± 92	24 ± 9	198 ± 55	248 ± 73	53 ± 16	18 ± 7	0,10
San-Pastore	427 ± 89	18 ± 4	154 ± 29	202 ± 40	41 ± 12	13 ± 5	0.09
Obriy	405 ± 61	16 ± 3	143 ± 20	189 ± 27	41 ± 8	16 ± 4	0.08
Gloria	402 ± 87	23 ± 5	163 ± 30	170 ± 36	33 ± 12	13 ± 6	0.14
MV-Emese	379 ± 49	24 ± 4	163 ± 26	150 ± 19	30 ± 5	12 ± 3	0.16
Spartanka	343 ± 79	21 ± 3	142 ± 23	138 ± 35	30 ± 12	12 ± 7	0.15
av	593	25	200	282	63	23	0.09
MSD[b]	184	8	54	94	26	12	

[a] Spring wheat. All others are winter wheat. [b] MSD, minimum significant difference.

MATERIALS AND METHODS

Samples. All wheat kernel samples were supplied by Dr. Zoltan Bedő at the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary. The field experiments included 26 different wheat varieties (24 winter and 2 spring varieties), which were grown in Hungary (Martonvásár) over three successive seasons (2004−2005, 2005−2006, 2006−2007) and in France (Enchantillon), Poland (Choryn), and the United Kingdom (Woolpit) in 2006−2007 only. The two spring wheat varieties from Poland were not analyzed, due to very low yields. The four sites were selected to give a wide range of climatic conditions, and the varieties were selected to give a wide range of alkylresorcinols and other phytochemical and dietary fiber components (*12*, *22*). The field experiments were a part of the integrated project HEALTHGRAIN and are described in more detail by Shewry et al. (*23*) with precipitation and temperature data, soil characteristics, fertilization, treatment with pesticides, and heading and harvest dates. The total precipitation from planting to harvest was higher in 2005 (318 mm) than in 2006 and 2007 (242 and 244 mm, respectively). The precipitation during grain-filling and harvest was also higher in 2005 than in 2006 and 2007. The average temperature from heading to harvest in Hungary was 19.4 °C in 2005 and 2006 and 20.5 °C in 2007. Both spring and harvest periods were hotter and drier in 2007 compared to 2005 and 2006. When the four locations in 2007 were compared, the average

Table 4. Average Content of Total Alkylresorcinols (AR) and AR Homologues in Different Varieties of Wheat Cultivated 2007 in Hungary, France, the United Kingdom, and Poland (n = 4 Locations)

cultivar	total AR (mean ± SD, μg/g of dm)	AR homologue content (mean ± SD, μg/g of dm) C17:0	C19:0	C21:0	C23:0	C25:0	ratio C17:0/C21:0
Tremie	825 ± 118	31 ± 4	258 ± 34	414 ± 61	88 ± 17	33 ± 8	0.07
Valoris	755 ± 58	29 ± 3	242 ± 17	373 ± 29	82 ± 7	29 ± 3	0.08
Avalon	743 ± 42	30 ± 2	240 ± 14	367 ± 21	78 ± 10	29 ± 4	0.08
Estica	728 ± 30	37 ± 2	262 ± 13	332 ± 14	71 ± 6	26 ± 2	0.11
Claire	714 ± 111	28 ± 5	235 ± 40	347 ± 51	76 ± 12	28 ± 5	0.08
Malacca	712 ± 88	35 ± 5	272 ± 37	312 ± 35	68 ± 12	25 ± 5	0.11
Riband	709 ± 134	25 ± 6	208 ± 43	372 ± 67	77 ± 18	27 ± 7	0.07
Cadenza[a]	680 ± 41	24 ± 2	198 ± 13	352 ± 22	76 ± 4	30 ± 2	0.07
Disponent	674 ± 84	23 ± 4	209 ± 31	336 ± 38	78 ± 12	28 ± 4	0.07
Rialto	669 ± 97	29 ± 4	234 ± 29	316 ± 52	68 ± 12	22 ± 3	0.09
Atlas-66	655 ± 41	33 ± 4	234 ± 11	301 ± 30	63 ± 8	24 ± 4	0.11
Chinese-Spring[a]	645 ± 62	31 ± 3	214 ± 17	286 ± 27	76 ± 10	38 ± 5	0.11
Tommi	636 ± 56	29 ± 3	219 ± 17	305 ± 33	62 ± 9	21 ± 2	0.10
Crousty	623 ± 85	29 ± 6	228 ± 31	286 ± 33	60 ± 11	21 ± 6	0.10
Lynx	612 ± 66	24 ± 4	190 ± 29	304 ± 25	71 ± 7	23 ± 3	0.08
Isengrain	607 ± 58	34 ± 2	237 ± 20	268 ± 32	51 ± 6	17 ± 2	0.13
Herzog	602 ± 78	24 ± 3	195 ± 26	296 ± 39	64 ± 11	24 ± 3	0.08
CF99105	594 ± 20	30 ± 3	217 ± 13	272 ± 10	56 ± 4	20 ± 1	0.11
Tiger	579 ± 48	27 ± 2	199 ± 11	272 ± 27	59 ± 7	23 ± 3	0.10
Maris-Huntsman	567 ± 44	21 ± 2	171 ± 17	302 ± 19	56 ± 8	18 ± 3	0.07
Campari	535 ± 70	25 ± 3	193 ± 23	245 ± 33	54 ± 9	17 ± 3	0.10
San-Pastore	495 ± 76	21 ± 3	178 ± 26	235 ± 39	47 ± 8	14 ± 3	0.09
Gloria	483 ± 21	28 ± 2	196 ± 11	204 ± 13	40 ± 2	16 ± 1	0.14
MV-Emese	406 ± 18	24 ± 2	169 ± 10	164 ± 9	34 ± 1	15 ± 1	0.15
Obriy	402 ± 41	17 ± 2	140 ± 14	188 ± 21	41 ± 3	16 ± 2	0.09
Spartanka	395 ± 23	22 ± 1	159 ± 10	164 ± 15	35 ± 2	15 ± 1	0.13
av	616	27	211	293	63	23	0.10
MSD[b]	134	7	46	69	16	7	

[a] Spring wheat. All others are winter wheat. [b] MSD, minimum significant difference.

temperature from heading to harvest (August in the United Kingdom and July in the other locations) was highest in Hungary at 20.5 °C and lowest in the United Kingdom at 14 °C, whereas it was about 18 °C in France and Poland. The winter before harvest was also warmer in Hungary than at the other locations. Furthermore, the total precipitation from heading to harvest was highest in the United Kingdom (233 mm) and lowest in France (101 mm), whereas it was 126 mm in Hungary and 204 mm in Poland. Fertilization was different at different sites, with a total of about 200 kg/ha nitrogen (N) in France and the United Kingdom, 110 kg/ha N in Poland, and 140 kg/ha N in Hungary each year (*23*).

Analysis of Alkylresorcinols. AR were extracted with ethyl acetate from intact cereal grains and analyzed by gas chromatography (GC) essentially according to the method of Ross et al. (*15*). Briefly, 200 μL of 0.5 mg/mL methyl behenate (C22:0, fatty acid methyl ester, Larodan Fine Chemicals AB, Malmö, Sweden) was added as an internal standard to whole grain samples (1 g) that were extracted with 40 mL of ethyl acetate for 24 h with continuous shaking at 20 °C. Portions of the extracts (4 mL) were then evaporated to dryness in vacuum using a centrifuge evaporator (Speedvac concentrator, Savant Instruments Inc., Farmingdale, NY). Ethyl acetate (200 μL) was added, and samples were analyzed by GC as described previously (*16*) but with a modified temperature program for accelerating the analysis (250 °C (0 min), 320 °C (20 min), 320 °C (22 min), 330 °C (30 min)). Results were reported on a dry matter basis. Dry matter content of whole grains was determined by oven-drying of crushed grains (coffee-type mill, Janke and Kunkel, IKA-WERK, Germany) at 105 °C for 16 h. All samples were analyzed in duplicate. Samples were reanalyzed if the coefficient of variation between two replicates was > 5%.

Statistical Analyses. Statistical analyses to study the effect of (1) year and variety and (2) location and variety on AR content and relative homologue composition were performed by analysis of variance (ANOVA) using Proc GLM in SAS ver. 9.1 (SAS Institute, Cary, NC). Minimum significant difference (MSD) was calculated with Tukey's distance estimate of varieties test to investigate differences between varieties. The MSD shows how much two varieties have to differ to be significantly different from each other. Because two varieties were missing at one location, these values were estimated by taking a mean of the same varieties at the other locations to obtain a balanced design. p values of < 0.05 were considered to be significant, and values reported are mean ± SD, unless otherwise stated. Correlation coefficients are reported as squared Pearson correlation coefficients.

RESULTS AND DISCUSSION

Twenty-six wheat varieties were grown during three years at one location, and at four different locations one year, and the total AR contents as well as the content of AR homologues C17:0, C19:0, C21:0, C23:0, and C25:0 were determined.

Effects of Year. The average AR contents in wheat varieties (n = 26) grown in Hungary 2005, 2006, and 2007 were about 460, 640, and 680 μg/g of dm, respectively (**Table 1**). In 2005 the AR content was significantly lower (p < 0.001) than in 2006 and 2007. In 2005 the precipitation during plant development, grain-filling, and harvest was much higher than in 2006 and 2007, resulting in larger grains with higher proportions of the endosperm. There was also a significant difference between years for the thousand-kernel weight (TKW), with significantly higher TKW in 2005 and 2006 (39−41 g/1000 kernels) than in 2007 (35 g/1000 kernels) (p < 0.001) (*23*). This is one possible explanation for the lower AR content in 2005. Zarnowski et al. (*2*) also reported different AR contents in barley grown at the same location during two different years and concluded that this was an effect of environmental factors such as climate, weather, and fertilization. Wierenga (*24*) reported that cereals grown under the same agronomic and climatic conditions showed only a small variation from year to year.

The contents (μg/g of dm) of all AR homologues for the wheat varieties grown in Hungary 2005−2007 except C21:0

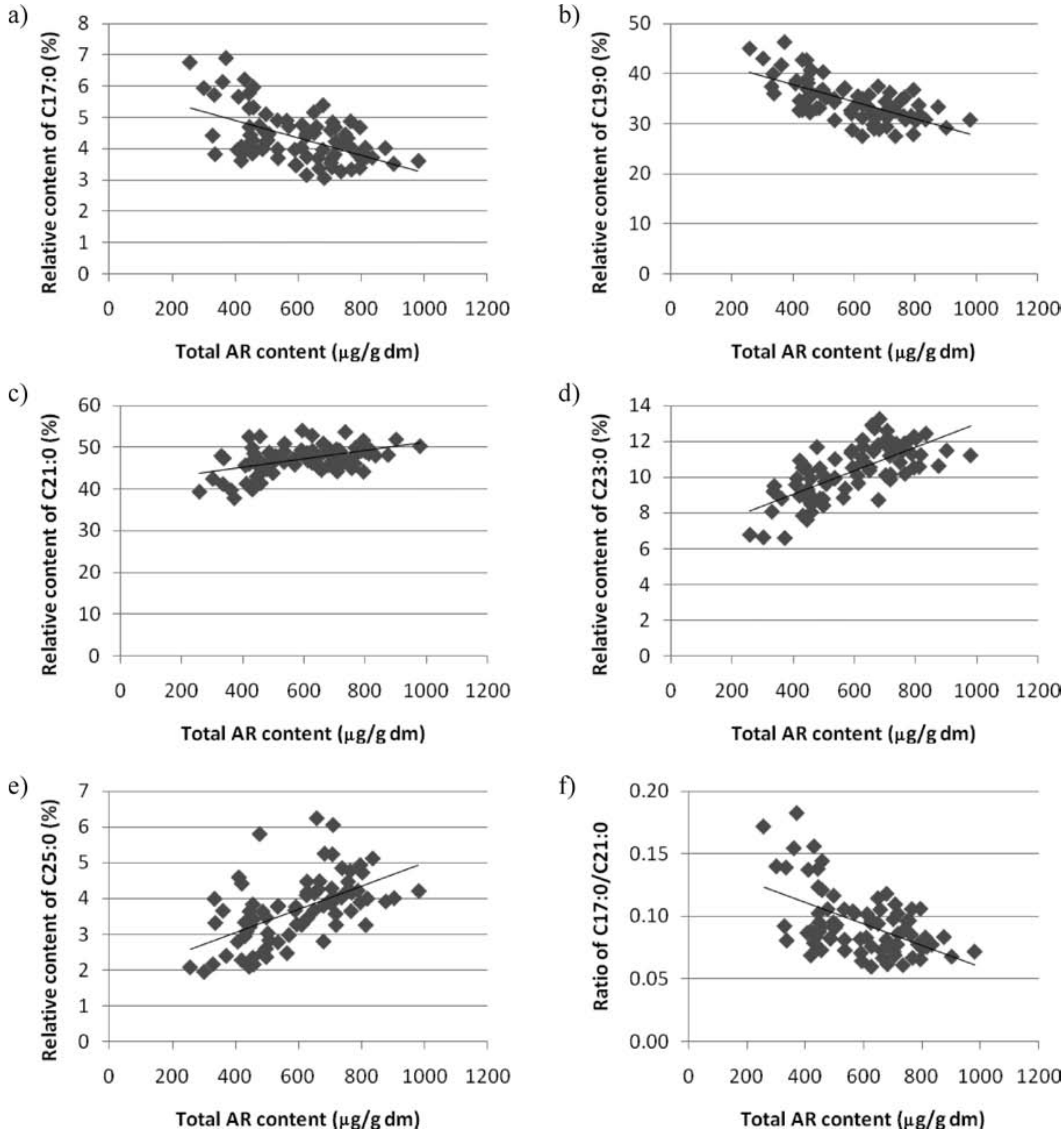

Figure 1. Correlation between total AR content (μg/g of dm) and relative proportion of AR homologue C17:0 (**a**) (R^2 = 0.28), C19:0 (**b**) (R^2 = 0.44), C21:0 (**c**) (R^2 = 0.23), C23:0 (**d**) (R^2 = 0.49), and C25:0 (**e**) (R^2 = 0.31), as well as the ratio of C17:0/C21:0 (**f**) (R^2 = 0.28), in all cultivars grown in Hungary in 2005−2007. $p < 0.001$ for all correlations.

were significantly different between years ($p < 0.001$), with the lowest content in 2005 for all AR homologues (**Table 1**). The relative homologue composition (in %) was, however, similar between years for all homologues with relative proportions of 4−5% C17:0, 33−37% C19:0, 47% C21:0, 9−11% C23:0, and 3−4% C25:0. Despite this, there was a significant difference between years for relative homologue composition except for C21:0 (results not shown). All values were in the range of what had been reported earlier in different wheat samples (see, e.g., refs *7* and *12*).

Effects of Location. The average total AR contents in different wheat varieties (n = 24−26) grown in Hungary, the United Kingdom, France, and Poland 2007 were about 680, 600, 570, and 610 μg/g of dm, respectively (**Table 2**). In Hungary, the average AR content for all cultivars was significantly higher ($p < 0.001$) than in the other locations. The spring and summer in Hungary were extremely dry and hot in 2007, with higher temperature than the other locations and low precipitation, which may explain the high AR content. The precipitation during grain-filling was also very low, resulting in smaller grains. There was a significant difference between all sites for TKW, with lowest TKW in Hungary (35 g/1000 kernels), intermediate TKW in Poland (39 g/1000 kernels) and the United Kingdom (42 g/1000 kernels), and highest TKW in France (50 g/1000 kernels) (*23*). The results show that AR content is affected by the weather conditions, with high contents during dry and warm summers and low contents during wet summers, especially during grain-filling. Ross et al. (*11*) found a large variation in AR content for two wheat cultivars grown at different locations in Europe (700−1100 μg/g for the cultivar Hereward and 600−1100 μg/g for the cultivar Rialto). They also found that AR content varied between different locations within the same field, showing that factors other than cultivar are important for determining AR content. Such factors could, for example, be soil composition, fertilization, and treatment with

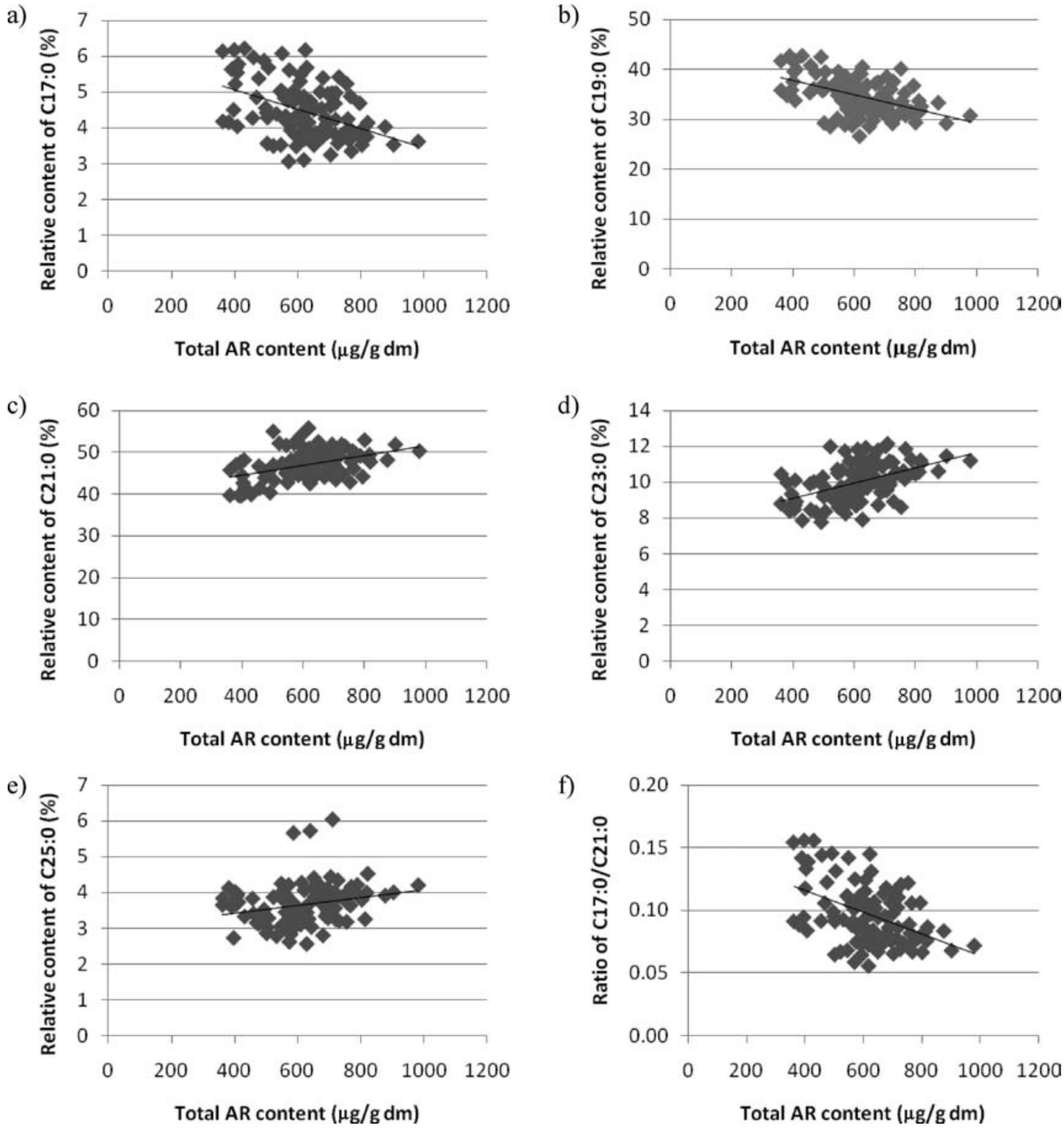

Figure 2. Correlation between total AR content (μg/g of dm) and relative proportion of AR homologue C17:0 (**a**) (R^2 = 0.19), C19:0 (**b**) (R^2 = 0.25), C21:0 (**c**) (R^2 = 0.18), C23:0 (**d**) (R^2 = 0.25), and C25:0 (**e**) (R^2 = 0.05), as well as the ratio of C17:0/C21:0 (**f**) (R^2 = 0.2), for all cultvars grown in 2007 at four different locations. $p < 0.001$ for all correlations except C25:0, where $p = 0.019$.

pesticides. In the present study soil composition, fertilization, and pesticide treatment differed between different locations, which may explain some of the variation. Treatment with pesticides has earlier been shown to be of importance for the biosynthesis of AR in seedlings of rye (*25*, *26*).

The content (μg/g of dm) of all AR homologues for the wheat varieties grown in 2007 was significantly different between locations ($p < 0.001$), with the highest content found in Hungary (**Table 2**). The relative homologue composition (in %) was, however, similar between locations for all homologues with relative proportions of 4−5% C17:0, 34−36% C19:0, 46−48% C21:0, 9−10% C23:0, and 3−4% C25:0. Despite this, there was a significant difference ($p = 0.001$) between the four locations for relative homologue composition (results not shown). The proportions were also similar to the homologue composition in the wheat varieties grown in Hungary in 2005−2007.

Effects of Varieties. The average contents of AR in different varieties in Hungary in 2005−2007 and at the four locations 2007 are shown in **Tables 3** and **4**, respectively. The AR content varied between 340 and 810 μg/g of dm in the different years (**Table 3**) and between 400 and 830 μg/g of dm at the different locations (**Table 4**). There was a significant difference between varieties in both cases ($p < 0.001$). To show the varieties that were significantly different from each other, the minimum significant difference (MSD) was calculated. For varieties grown in Hungary in 2005−2007 the MSD was 184 μg/g of dm for total AR (**Table 3**), and for varieties grown in 2007 at different locations it was 134 μg/g of dm (**Table 4**). The variety with the highest AR content both in Hungary in 2005−2007 and in the four locations in 2007 was Tremie, whereas the variety with the lowest AR content was Spartanka. Of the eight varieties with highest AR contents, all except one were similar in Hungary in 2005−2007 and at the four locations in 2007. The same was true for the eight varieties with the lowest AR contents. The results show that AR content is a highly heritable phytochemical component even though environment affects the levels.

The content (μg/g of dm) of all AR homologues was also significantly different between varieties both at different locations and in different years ($p < 0.001$) (**Tables 3** and **4**). The MSDs were 8, 54, 94, 26, and 12 μg/g of dm for C17:0, C19:0, C21:0,

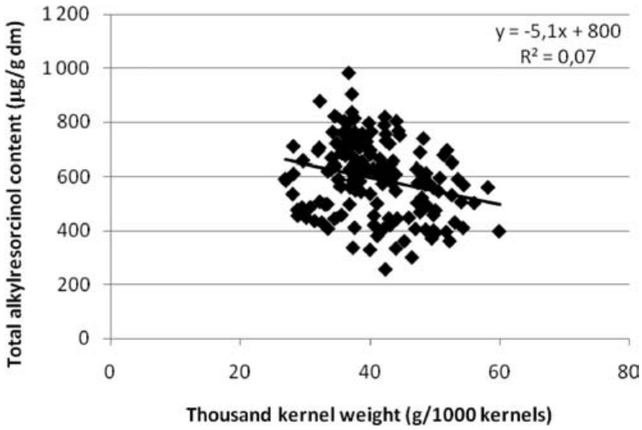

Figure 3. Correlation (p < 0.001) between thousand-kernel weight and alkylresorcinol content in wheat varieties grown in Hungary in 2005−2007 and in France, Poland, and the United Kingdom in 2007.

C23:0, and C25:0 contents, respectively, for varieties grown in Hungary in 2005−2007 (**Table 3**) and 7, 46, 29, 16, and 7 μg/g of dm for C17:0, C19:0, C21:0, C23:0, and C25:0 contents, respectively, for varieties grown in 2007 at different locations (**Table 4**). The relative homologue composition was also significantly different between locations for all homologues (results not shown). The relative proportions varied more between varieties (3−6%, C17:0; 29−43%, C19:0; 40−53%, C21:0; 8−12%, C23:0; and 3−6%, C25:0) than between years and locations. The ratio between C17:0 and C21:0 varied continuously between 0.07 and 0.16 (**Tables 3** and **4**). These variations are slightly larger than what has been reported earlier in wheat (*7*), but still in the same range. For a linear regression there was a significant correlation between total content of AR and relative proportion of AR homologues both at different locations (p < 0.001 for all homologues except C25:0 for which p = 0.019) and in different years (p < 0.001 for all homologues). C17:0 and C19:0 were lower at higher AR content, whereas C21:0, C23:0, and C25:0 were higher (**Figures 1** and **2**). There was also a significant correlation between total AR content and the ratio of C17:0/C21:0 (p < 0.001), which was higher at higher AR content (**Figures 1** and **2**).

Correlation with Thousand-Kernel Weight. The TKW varied between 27 and 60 mg for all samples (*22*). A weak, but highly significant, negative correlation (p < 0.001, R^2 = 0.07) between TKW and AR content was found (**Figure 3**), which is in agreement with Wieringa (*24*) and Mejbaum-Katzenellenbogen et al. (*27*). This was also shown earlier in rye (*13*) and can be explained by the fact that small grains have a larger proportion of outer layers where AR are located. It has also been shown that the AR content decreases during maturation of the grains (*28*) due to dilution during deposition of starch and protein in the maturing grain. The results may explain the higher AR content during dry and warm summers, because these grains also have a lower TKW.

In conclusion, both total AR content and content of individual AR homologues were affected by year, location, and variety. An exceptionally dry and warm climate resulted in smaller grains with lower TKW and higher total AR content, whereas a high precipitation, especially during grain-filling and harvest, gave larger grains with lower total AR content. The results also showed that there was a significant correlation between total AR content and relative proportion of individual AR homologues, as evidenced by the observation that the relative proportion of C17:0 and C19:0 was higher at higher total AR content, whereas C21:0, C23:0, and C25:0 was lower. The ratio of C17:0/C21:0 was also lower at higher overall AR contents. These results might have implications, for example, when using AR as markers for the presence of wheat and rye brans in foods (*7*) or as biomarkers of wheat intake by humans (see, e.g., refs *29* and *30*). In fact, the variability in the level of AR and the C17:0/C21:0 ratio is very low compared to the general variability in plant secondary metabolites, which will support their use as biomarkers.

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LITERATURE CITED

(1) Ross, A. B.; Kamal-Eldin, A.; Åman, P. Dietary alkylresorcinols: absorption, bioactivities and possible use as biomarkers of whole grain wheat and rye rich foods. *Nutr. Rev.* **2004**, *62*, 81–95.
(2) Zarnowski, R.; Suzuki, Y.; Yamaguchi, I.; Pietr, S. J. Alkylresorcinols in barley (*Hordeum vulgare* L. *distichon*) grains. *Z. Naturforsch.* **2002**, *57C*, 57–62.
(3) Ross, A. B.; Kochhar, S. Rapid and sensitive analysis of alkylresorcinols from cereal grains and products using HPLC-coularray-based electrochemical detection. *J. Agric. Food Chem.* **2009**, *57*, 5187–5193.
(4) Knödler, M.; Most, M.; Schieber, A.; Carle, R. A novel approach to authenticity control of whole grain durum wheat (*Triticum durum* Desf.) flour and pasta, based on analysis of alkylresorcinol composition. *Food Chem.* **2010**, *118*, 177–181.
(5) Landberg, R.; Kamal-Eldin, A.; Salmenkallio-Marttila, M.; Åman, P. Localization of alkylresorcinols in wheat, rye and barley kernels. *J. Cereal Sci.* **2008**, *48*, 401–406.
(6) Mattila, P.; Pihlava, J. M.; Hellstrom, J. Contents of phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides in commercial grain products. *J. Agric. Food Chem.* **2005**, *53*, 8290–8295.
(7) Chen, Y.; Ross, A. B.; Åman, P.; Kamal-Eldin, A. Alkylresorcinols as markers of whole grain wheat and rye in cereal products. *J. Agric. Food Chem.* **2004**, *52*, 8242–8246.
(8) Andersson, A. A. M.; Åman, P.; Wandel, M.; Frølich, W. Alkylresorcinols in wheat and rye flours and breads. *J. Food Compos. Anal.* **2010**, in press.
(9) Hemery, Y.; Lullien-Pellerin, V.; Rouau, X.; Abecassis, J.; Samson, M.-F.; Åman, P.; von Reding, W.; Spoerndli, C.; Barron, C. Biochemical markers: efficient tools for the assessment of wheat grain tissue proportions in milling fractions. *J. Cereal Sci.* **2009**, *49*, 55–64.
(10) Landberg, R.; Kamal-Eldin, A.; Andersson, A.; Vessby, B.; Åman, P. Alkylresorcinols as biomarkers of whole-grain wheat and rye intake: plasma concentration and intake estimated from dietary records. *Am. J. Clin. Nutr.* **2008**, *87*, 832–838.
(11) Ross, A. B.; Shepherd, M. J.; Schüpphaus, M.; Sinclair, V.; Alfaro, B.; Kamal-Eldin, A.; Åman, P. Alkylresorcinols in cereals and cereal products. *J. Agric. Food Chem.* **2003**, *51*, 4111–4118.
(12) Andersson, A. A. M.; Kamal-Eldin, A.; Fraś, A.; Boros, D.; Åman, P. Alkylresorcinols in wheat varieties in the HEALTHGRAIN diversity screen. *J. Agric. Food Chem.* **2008**, *56*, 9722–9725.
(13) Evans, L. E.; Dedio, W.; Hill, R. D. Variability in the alkylresorcinol content of rye grains. *Can. J. Plant Sci.* **1973**, *53*, 485–488.
(14) Nyström, L.; Lampi, A.-M.; Andersson, A. A. M.; Kamal-Eldin, A.; Gebruers, K.; Courtin, C.; Delcour, J. A.; Li, L.; Ward, J. L.; Fraś, A.; Boros, D.; Rakzsegi, M.; Bedo, Z.; Shewry, P. Phytochemicals and dietary fiber components in rye varieties in the HEALTHGRAIN diversity screen. *J. Agric. Food Chem.* **2008**, *56*, 9758–9766.
(15) Ross, A. B.; Kamal-Eldin, A.; Jung, C.; Shepherd, M. J; Åman, P. Gas chromatographic analysis of alkylresorcinols in rye (*Secale cereale* L.) grains. *Sci. Food Agric.* **2001**, *81*, 1405–1411.
(16) Gohil, S.; Pettersson, D.; Salomonsson, A.-C. Analysis of alkylresorcinols and alkenylresorcinols in triticale, wheat and rye. *J. Sci. Food Agric.* **1988**, *45*, 43–52.
(17) Landberg, R.; Kamal-Eldin, A.; Andersson, R.; Åman, P. Alkylresorcinol content and homologue composition in durum wheat

(*Triticum durum*) kernels and pasta products. *J. Agric. Food Chem.* **2006**, *54*, 3012–3014.
(18) Nurmi, T.; Nyström, L.; Edelmann, M.; Lampi, A.-M.; Piironen, V. Phytosterols in wheat varieties in the HEALTHGRAIN diversity screen. *J. Agric. Food Chem.* **2008**, *56*, 9710–9715.
(19) Lampi, A.-M.; Nurmi, T.; Ollilainen, V.; Piironen, V. Tocopherols and tocotrienols in wheat varieties in the HEALTHGRAIN diversity screen. *J. Agric. Food Chem.* **2008**, *56*, 9716–9721.
(20) Li, L.; Shewry, P.; Ward, J. L. Phenolic acids in wheat varieties in the HEALTHGRAIN diversity screen. *J. Agric. Food Chem.* **2008**, *56*, 9732–9739.
(21) Piironen, V.; Edelmann, M.; Kariluoto, S.; Bedo, Z. Folate in wheat varieties in the HEALTHGRAIN diversity screen. *J. Agric. Food Chem.* **2008**, *56*, 9726–9731.
(22) Ward, J. L.; Poutanen, K.; Gebruers, K.; Piironen, V.; Lampi, A.-M.; Nyström, L.; Andersson, A. A. M.; Åman, P.; Boros, D.; Rakszegi, M.; Bedo, Z.; Shewry, P. The HEALTHGRAIN cereal diversity screen: concept, results, and prospects. *J. Agric. Food Chem.* **2008**, *56*, 9699–9709.
(23) Shewry, P.; Piironen, V.; Lampi, A.-M.; Edelmann, M.; Kauriluoto, S.; Nurmi, T.; Nyström, L.; Ravel, C.; Charmet, G.; Andersson, A. A. M.; Åman, P.; Boros, D.; Gebruers, K.; Dornez, E.; Courtin, C.; Delcour, J. A.; Rakzsegi, M.; Bedo, Z.; Ward, J. L. The HEALTHGRAIN wheat diversity screen: genotype and environment effects on phytochemicals and dietary fiber components. *J. Agric. Food Chem.* **2010**, DOI: 10.1021/jf100039b.
(24) Wierenga, G. W. On the Occurrence of Growth Inhibiting Substances in Rye. Doctoral thesis, Institute for Research on Storage and Processing of Agricultural Procedure, Wageningen, The Netherlands, **1967**; pp 1−6.
(25) Magnucka, E. G.; Suzuki, Y.; Pietr, S. J.; Kozubek, A.; Zarnowski, R. Action of benzimidazole fungicides on resorcinolic lipid metabolism in rye seedlings depends on thermal and light growth conditions. *Pestic. Biochem. Physiol.* **2006**, *88*, 219–225.
(26) Magnucka, E. G.; Suzuki, Y.; Pietr, S. J.; Kozubek, A.; Zarnowski, R. Cycloate, an inhibitor of fatty acid elongase, modulates the metabolism of very-long-side-chain alkylreosrcinols in rye seedlings. *Pest Manag. Sci.* **2009**, *65*, 1065–1070.
(27) Mejbaum-Katzenellenbogen, W.; Sikorski, A.; Tluscik, F. Alkylresorcinols in rye (*Secale cereale* L.) grains. 2. Dependence of alkylresorcinol level on weight and specific weight of grains. *Acta Soc. Bot. Polon.* **1975**, *44*, 597–606.
(28) Verdeal, K.; Lorenz, K. Alkylresorcinols in wheat, rye, and triticale. *Cereal Chem.* **1975**, *54*, 475–483.
(29) Ross, A. B.; Kamal-Eldin, A.; Åman, P. Dietary alkylresorcinols: absorption, bioactivities and possible use as biomarkers of whole grain wheat and rye rich foods. *Nutr. Rev.* **2004**, *62*, 81–95.
(30) Landberg, R.; Åman, P.; Friberg, L.; Adlercreutz, H.; Kamal-Eldin, A. Dose-response of whole grain biomarkers - alkylresorcinol in plasma and their metabolites in urine in relation to intake. *Am. J. Clin. Nutr.* **2009**, *89*, 290–296.

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