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Characterization of Variation in the Lignan Content and Composition of Winter Rye, Spring Wheat, and Spring Oat

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To characterize the range of variation in lignan content and composition caused by genotype and environment, seven dietary lignans, i.e., 7-hydroxymatairesinol, secoisolariciresinol, matairesinol, lariciresinol, pinoresinol, medioresinol, and syringaresinol, were analyzed by high-performance liquid chromatography−tandem mass spectrometry in whole-grain extracts of cereal samples collected at eight locations in Finland. In all, 28 winter rye, 73 spring wheat, and 55 spring oat samples were analyzed, representing 6, 9, and 5 cultivars, respectively. The total lignan content showed huge variations within the same cereal species: the range was 2500−6700 μg/100 g in the rye samples, 340−2270 μg/100 g in the wheat samples, and 820−2550 μg/100 g in the oat samples. The variations seemed to depend largely upon genetic differences. In rye, also environmental conditions affected the lignan content through grain size; smaller grains had significantly lower total lignan, syringaresinol, and lariciresinol content than larger grains. This study shows that varying cereal lignan concentrations reported in different studies may be, besides differences in analytical methods, largely dependent upon natural variations.

KEYWORDS: Lignans; cereals; HPLC−MS/MS; grain size

INTRODUCTION

Lignans constitute a group of natural polyphenols widely distributed in the plant kingdom. In numerous studies, they have been shown to possess a diverse spectrum of biological properties, showing, e.g., antioxidant, antitumor, antiviral, antibacterial, and antifungal effects (*1*−*3*). In 1998, the lignans secoisolariciresinol and matairesinol were reported to occur in various vegetables and cereals, present in our diet (*4*). A total of 7 years later, four other lignans, i.e., lariciresinol, pinoresinol, medioresinol, and syringaresinol, were identified in cereals and various kinds of foods (*5*, *6*). Recently, the list of known cereal lignans was further extended by 14 more lignans (*7*), including 7-hydroxymatairesinol, abundant in Norway spruce (*Picea abies*) knots (*8*). 7-Hydroxymatairesinol has been reported to be present also in sesame seeds (*9*).

The lignan content and composition of cereal samples have been determined in only a few studies (*4*−*7*). The concentrations are differing in the different studies. First, the obtained concentration depends upon the part of the grain analyzed; the lignans are concentrated in the bran part of the grain (*4*, *7*). Second, the obtained concentration depends upon the analytical method applied. Strong alkaline or acid extraction destroys some lignans, but on the other hand, alkaline hydrolysis increases the yield of most of the lignans because they seem to be largely in esterified forms, bound to the cereal matrix (*7*, *10*). Third, even if the same analytical method is applied, the lignan content may vary largely in different samples of the same species. For example, in one study, it was shown that the concentration of the lignan sesamin in sesame seeds shows a huge variation, ranging between 7 and 712 mg/100 g in 65 different samples (*11*). Moreover, the lignan content and composition were shown to vary in Norway spruce samples collected from different geographical locations (*12*). Consequently, it seems that differences in reported concentrations of a certain lignan may result, besides from differences in the analytical method, also from natural variations caused by genotype, growing, and climatic conditions, as well as their interaction.

In general, northern growing conditions typical for Finland differ markedly from those in more southern production regions (*13*) and may per se cause differences in lignan content and composition of cereals. However, variation of lignan content may also occur within the growing region, and this may result from differences in growing conditions at different locations and during different years. One of the potential mechanisms for such growing condition-induced differences in lignan content and composition is grain size variation.

Recently, Peltonen-Sainio et al. (*14*) showed, on the basis of data recorded during 30 years of multilocation trials carried out in Finland, that a large variation occurs in the two major

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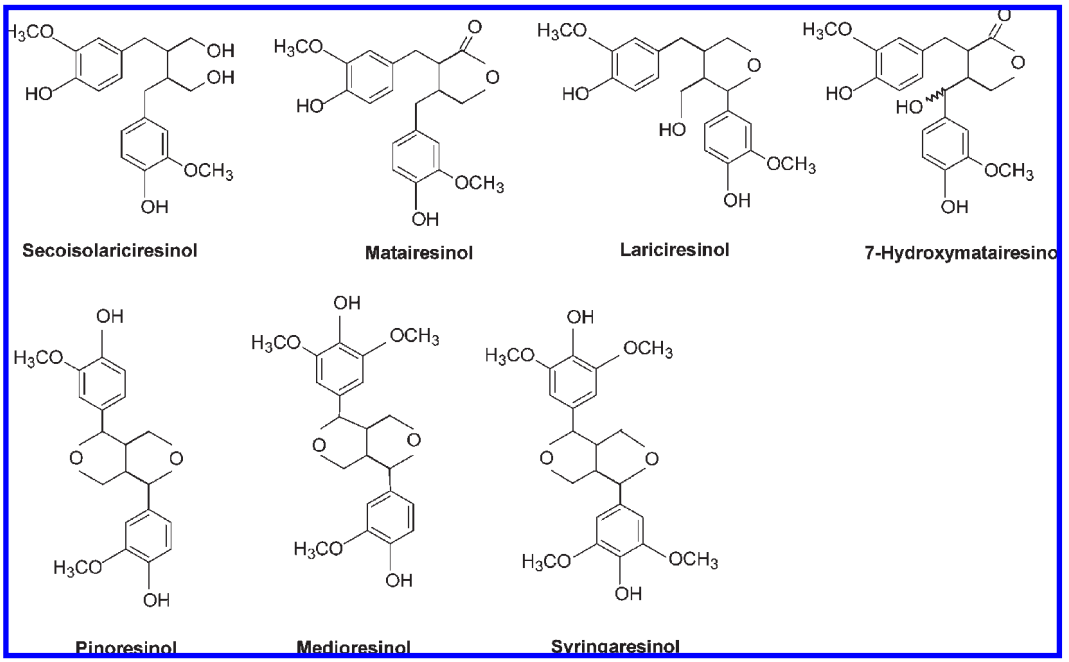

Figure 1. Molecular structures of the lignans determined in the present study.

yield-determining components, i.e., grain weight and grain number per square meter, and this is true for all cereal species. The grain number and the grain weight are determined at different times during the growing season, although grain set and grain fill also interact. Namely, as the potential grain number in cereals is determined prior to heading, the capacity of the pollinated grains to reach their potential size is dependent upon the prevailing growing conditions during the grain-filling phase but not exclusively. The final grain size is also dependent upon the number of set florets and developing grains per head and the sufficiency of the plant stand to support grain growth (*14*). Hence, the grain size is determined by variations in growing conditions during floret and grain set (before heading) but also during grain fill (after heading). Also, genotypes may play a role, because they may differ in their potential grain size and their sensitivity to respond to varying conditions; this is called genotype × environment interaction. Such interactions cause variations in final grain size. Furthermore, dependent upon how well the potential grain size is realized, differences are likely to occur in the cell wall content of the grain compared to, e.g., grain carbohydrate and protein storages. This may at least partly explain the large variation in lignan content of cereal grains of the same species.

In the present study, seven lignans, i.e., 7-hydroxymatairesinol, secoisolariciresinol, matairesinol, lariciresinol, pinoresinol, medioresinol, and syringaresinol (**Figure 1**), were quantified in 156 alkaline extracted and enzymatically hydrolyzed whole-grain cereal samples using high-performance liquid chromatography−tandem mass spectrometry (HPLC−MS/MS). The work was a first step in the characterization of variations of lignan content and composition in cereal grains aiming at (1) describing differences in lignan content and composition in grains of the cereal species winter rye (*Secale cereale* L.), spring wheat (*Triticum aestivum* L.), and spring oat (*Avena sativa* L.) grown at high latitudes and used for human consumption, (2) estimating the degree of variation in lignan content and composition caused by genetic differences (cultivars) and growth conditions, i.e., the impact of grain size, and thereby (3) giving focus for further studies that are required for, e.g., understanding the genetic control of the lignan content and composition of grains.

MATERIALS AND METHODS

Chemicals and Reference Compounds. All solvents were of analytical grade and purchased from commercial sources. β-Glucuronidase/sulfatase (type H-1, from *Helix pomatia*) (Sigma-Aldrich Co., St. Louis, MO) containing 367 units/mg of β-glucuronidase and 10 units/mg of sulfatase was used for the enzymatic hydrolysis.

All of the analyzed compounds, except matairesinol-d_6, were prepared as described previously by Smeds et al. (*7*): 7-hydroxymatairesinol, secoisolariciresinol, lariciresinol, and pinoresinol were isolated from softwood species; medioresinol and syringaresinol were isolated from herbal species; matairesinol was prepared from 7-hydroxymatairesinol; and the internal standard 3,3′-di(trideuteromethoxy)-pinoresinol was prepared from pinoresinol. Matairesinol-d_6 was prepared from matairesinol as described by Adlercreutz et al. (*15*).

Samples. All samples were collected in Finland during the years 2003−2007. Dried grains of winter rye, spring wheat, and spring oat were sampled at the regional research stations of MTT Agrifood Research Finland in southern Ostrobothnia (Ylistaro 62° 57′ N, 22° 30′ E), in southwestern Finland (Mietoinen 60° 38′ N, 21° 55′ E), and in eastern Finland (Mikkeli 61° 42′ N, 27° 17′ E) and at the research sites of Boreal Plant Breeding Ltd. in Jokioinen (60° 48′ N, 23° 30′ E), Laihia (62° 59′ N, 22° 00′ E), Inkoo (60° 07′ N, 24° 05′ E), Forssa (60° 82′ N, 23° 67′ E), and Laukaa (62° 45′ N, 25° 97′ E). The locations well-represent the major cereal production region in Finland. In all, 28 winter rye samples of 6 different cultivars, 73 spring wheat samples of 9 different cultivars, and 55 spring oat samples of 5 different cultivars were analyzed. In all of these experiments, fertilizers and pesticides were used as is presently a common practice in Finnish farms.

Equipment. The dried grains of rye, wheat, and oat were finely ground to whole-grain flour using a Fidibus 21 stone mill (Komo GmbH, Germany). Lignans were separated on a 100 × 2.1 mm inner diameter, 3.5 μm, Zorbax SB-C8 column (Agilent Technologies, Inc.) and quantified in the cereal extracts by HPLC−electrospray ionization−MS/MS using an Agilent 1100 series HPLC (Agilent Technologies, Inc., Palo Alto, CA) system coupled to a Quattro Micro triple quadrupole instrument (Micromass Ltd., Manchester, U.K.). The analysis data were collected in the multiple reaction monitoring (MRM) mode using MassLynx V4.0 software, and the quantification was performed using QuanLynx V4.0 software (Micromass Ltd., Manchester, U.K.).

Analytical Method. The alkaline extraction was performed according to a modification of previously described procedures (*6*, *16*). Approximately 200 mg of the whole-grain flour was treated with 5.0 mL of 70% methanol containing 0.3 M NaOH for 1 h at 60 °C in an ultrasonic bath.

The samples were manually shaken every 5 min. The pH was adjusted to 5−6 with some drops of concentrated hydrochloric and acetic acid. The samples were then centrifuged, and the extracted solid was washed with methanol (0.5 mL) as described previously (*6*, *16*). The volume of the supernatant was adjusted to 5.5 mL, and an aliquot of 0.5 mL of this solution was withdrawn for the enzymatic hydrolysis. The enzymatic hydrolysis with a subsequent addition of internal standard (100 μL of a 5 μg/mL solution of matairesinol-d_6 and 100 μL of a 4.3 μg/mL solution of 3,3′-di(trideuteromethoxy)-pinoresinol), extraction with ethyl acetate, and reconstitution was performed as described previously (*7*).

The HPLC−MS/MS methods and quantification procedures have been described previously (*7*). All lignans except syringaresinol were analyzed in the negative mode. All lignans analyzed in negative mode were quantified against matairesinol-d_6; syringaresinol was quantified against 3,3′-di(trideuteromethoxy)-pinoresinol. The calibration standard solutions of 7-hydroxymatairesinol, matairesinol, secoisolariciresinol, lariciresinol, pinoresinol, and medioresinol contained six different concentrations of the compounds in the range of approximately 9 ng/mL to 1.5 μg/mL; the standard solutions of syringaresinol contained the compound in the range of 30 ng/mL to 5 μg/mL. Intra-assay variation of the method was determined by analyzing five extracts of the same cereal sample prepared in parallel. All samples of the same cereal species were analyzed in one single batch. The cultivar 'Amilo' was used for determination of intra-assay variation of the rye samples; 'Amaretto' was used for the wheat samples; and 'Roope' was used for the oat samples. The ion suppression or enhancement effect on the detector response because of matrix effects was determined by comparing the analysis results from a standard solution (a) dissolved in pure mobile phase [20:80 methanol/0.1% acetic acid (v/v)] and (b) spiked into an 'Amaretto' wheat cultivar extract. The amount of analytes known to be present in the wheat sample was subtracted. Each sample was prepared as three parallels.

Statistical Analyses. The set of cultivars varied between years, locations, and trials. The structure of the data has to be taken into account when the data are modeled, because the lignan concentrations are supposed to depend upon the environment. The used statistical analyses were based on the following statistical model:

$$y_{ijkl} = \mu + Y_k + E_l + T_{kl} + S_i + C_j + \varepsilon_{ijkl}$$

where μ is the intercept and Y_k, E_l, and T_{kl} are random effects of the environment (year, experimental site, and year-by-site interaction). S_i and C_j are fixed effects of species and cultivars, respectively. ε_{ijkl} stands for residuals. All random effects are assumed to be mutually independent and normally distributed. Assumption of normality was checked by plotting residuals against fitted values. Plots showed that log transformation was required in the analysis of medioresinol and lariciresinol. All statistical analyses were performed using SAS software (version 9.1) and MIXED procedures using the REML estimation method.

Regression analysis was applied for estimating the relationship between grain size and lignan content.

RESULTS AND DISCUSSION

Analytical Method. The method was selective, because no overlapping peaks were seen in the MRM chromatograms. The method also showed acceptable intra-assay variation for all of the lignans in all cereal species ($n = 5$, CV ≤ 10%). Very slight ion suppression because of matrix components was shown by 7-hydroxymatairesinol, secoisolariciresinol, and syringaresinol. The recovery of the detector response was in the range of 93.6−99.7%. Lariciresinol showed a larger ion suppression (recovery 85.4%), whereas matairesinol and pinoresinol showed a slight ion enhancement (1.1%).

Variation of Lignan Content and Composition in the Cereal Grain Extracts. A large variation in the grain lignan content and composition was observed in the 156 cereal samples analyzed. The total lignan content ranged from 820 to 2550 μg/100 g in the 55 oat samples, from 340 to 2270 μg/100 g in the 73 wheat samples, and from 2500 to 6700 μg/100 g in the 28 rye samples. When the concentrations of individual lignans in whole-grain cereal extracts obtained in different studies (*4*−*6*) were compared to the range obtained in the present study, the values generally fall within this range. However, the range is very broad in some cases; e.g., in oat, the concentration range of matairesinol is 0.3−125 μg/100 g and the concentration range of pinoresinol is 95−938 μg/100 g. This indicates that varying concentrations obtained in different studies may largely depend upon natural variations and that different analytical methods applied may play a minor role.

Matairesinol and secoisolariciresinol, for a long time the only known dietary (and cereal) lignans, were minor contributors to the total lignan content in all three cereal species. No matairesinol or only traces of matairesinol could be detected in wheat samples. Traces of 7-hydroxymatairesinol could be detected in the rye and wheat samples, which indicates that these cereals contain larger amounts of 7-hydroxymatairesinol, which, however, has been degraded during the alkaline treatment. Previously, higher amounts of 7-hydroxymatairesinol have been detected in rye and wheat bran than in oat bran (*7*).

The between-species variation is evident because of large genetic differences, but within-species and especially within-cultivar variations may largely depend upon environmental conditions. Within-cultivar variation may also arise from differences in grain set and filling, which are both dependent upon the position in the ear or panicle (*17*), although this reason for lignan content variation is probably modest compared to environment-induced variation.

Samples of the same cultivar were collected from different locations and during different years; however, we could not find significant within-cultivar variations between different locations and years (year × location), probably because the number of samples was too limited in this preliminary survey to enable detection of such variations. Consequently, the assessment of environmental conditions is possible on the basis of study only through indirect measurement, i.e., by comparing the lignan content of samples of different grain size of the same cultivar. A factor potentially associated with variations in the grain lignan content is the single grain weight; however, we could not find such significant correlation in comparisons between or within species.

Between-Species Variation. As expected, the grain lignan content and composition was significantly different for different cereal species, with the highest total content in winter rye, the second highest in spring oat, and the lowest content in spring wheat (**Figure 2**). This order is similar to that obtained previously in whole grain (*6*) but different from that obtained for total lignan content in bran (*7*).

Syringaresinol was the dominating lignan in all three species, especially dominating in wheat and rye, in which the contribution to the total lignan content was approximately 80% (**Figures 2** and **3**). In oat, the contribution was only 42%, with larger contributions of lariciresinol and pinoresinol than the other species (**Figure 2**).

Within-Species (Between-Cultivar) Variation. When comparing the average total lignan content of different cultivars of the same species, we found significant variations in cultivars of all three species (**Figure 3**). In rye, the cultivars 'Elvi' and 'Picasso' had the highest total lignan content, and cultivar 'Bor 9415' had the lowest. In oat, the cultivar 'Roope' had the lowest total lignan content, and in wheat, the cultivar 'Picolo' had the highest total lignan content. Significant variations were also found in the syringaresinol content in cultivars of all three species (**Figure 3**), and the trend was the same as with the total lignan content. With regard to the content of other lignans, significant differences were generally found between cultivars, with the exception of the medioresinol and 7-hydroxymatairesinol content in rye cultivars

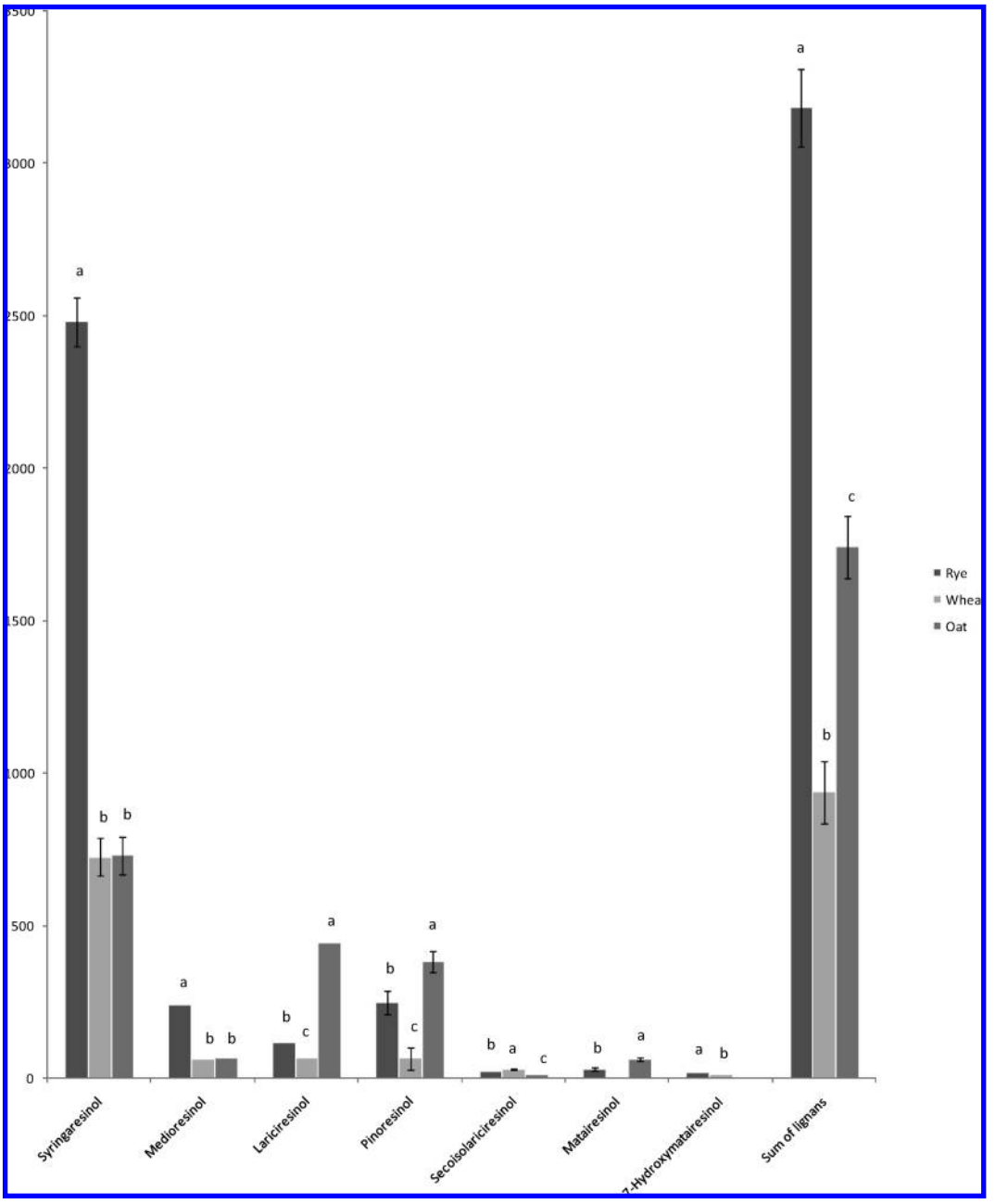

Figure 2. Concentrations of lignans (μg/100 g) in extracts of rye, wheat, and oat grains; values are expressed as mean values $\pm$ standard error. Rye, $n = 28$; wheat, $n = 73$; oat, $n = 55$. Concentrations of each lignan or total lignans within each species marked by different letters show a very significant difference ($p \leq 0.001$). Medioresinol, lariciresinol, and 7-hydroxymatairesinol are log-transformed.

or pinoresinol content in wheat cultivars (**Table 1**). Large variations in lignan composition cannot be seen, although the relative amounts may vary between cultivars. For example, the relative amount of syringaresinol of the total lignan content varies (**Figure 3**), and the wheat cultivar 'Picolo', which contained the highest amount of syringaresinol and total lignans, did not contain the highest amounts of lariciresinol, pinoresinol, secoisolariciresinol, or 7-hydroxymatairesinol (**Table 1**).

Within-Cultivar Variation: The Role of the Grain Size. According to the relative standard errors, there were large variations especially in pinoresinol content within the wheat cultivars (**Table 1**), which suggests an impact of environmental conditions. One hypothesis associating grain size with lignan content could be that the lignan content of smaller grains would be higher than that of larger grains because they contain a higher proportion of lignan-rich cell-wall components. To investigate the role of the grain size for the lignan content and composition, we collected samples of selected cultivars during the year 2007 in two or three different experiments and sorted them into different size groups by using sieves of 2.2, 2.5, and 2.8 mm. The selected cultivars were the rye cultivar 'Picasso', the wheat cultivars 'Amaretto', 'Kruunu', and 'Zebra', and the oat cultivars 'Belinda' and 'Roope'. Our results did not, however, at all support the hypothesis described above. On the contrary, for rye cultivars, larger grains contained significantly higher amounts of syringaresinol, lariciresinol, and total lignans (**Table 2**). For oat and wheat cultivars, the grain size had no significant effect on the lignan content.

In conclusion, genetic differences seem to affect both the lignan content and composition of cultivars of wheat, oat, and rye. In the case of rye, the lignan content was associated with differences in the grain size, suggesting the importance of environmental

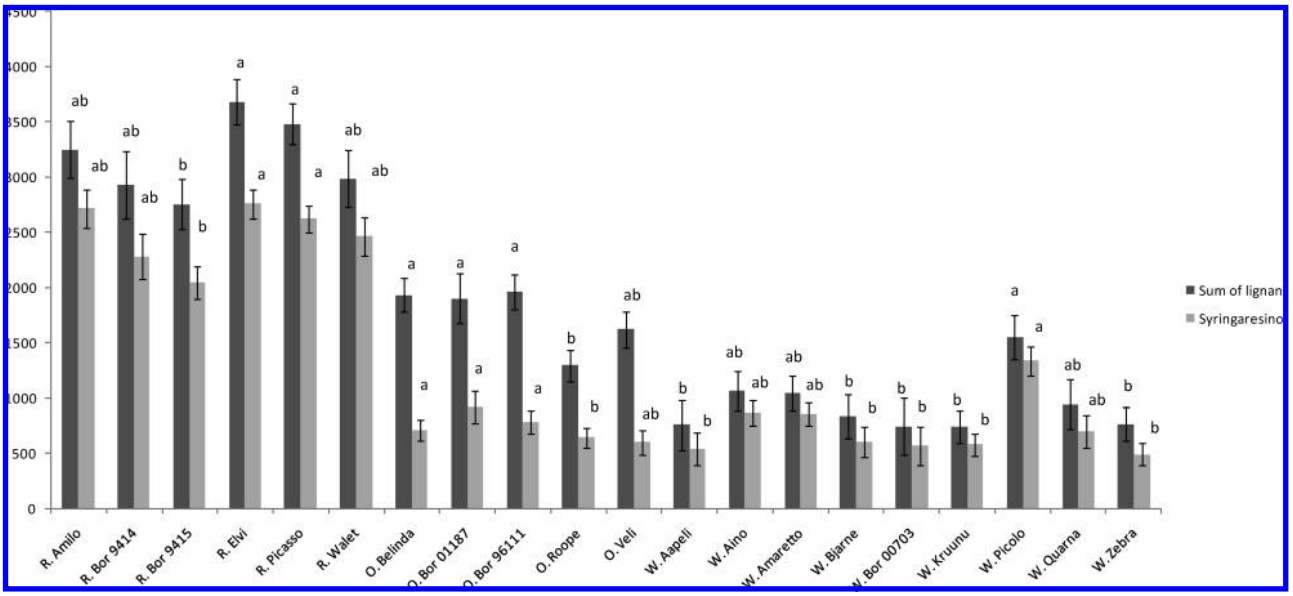

Figure 3. Concentrations of syringaresinol and sum of lignans (μg/100 g) in grain extracts of different cultivars of rye, wheat, and oat; values are expressed as mean values ± standard error. n = 2−16 (see **Table 2**). R = rye; O = oat; W = wheat. Concentrations of syringaresinol or total lignans within cultivars of the same species marked by different letters show a very significant difference ($p \leq 0.001$ or $p \leq 0.01$).

Table 1. Concentrations of Lignans (μg/100 g) in Extracts of Rye, Wheat, and Oat Samples[a]

cultivars	n	medioresinol	lariciresinol	pinoresinol	secoisolariciresinol	matairesinol	7-hydroxymatairesinol
				Rye			
'Amilo'	3	220 a	75.5 b	176 ± 48.5 b	24.8 ± 2.36 ab	25.5 ± 9.05 ab	20.2 a
'Bor 9414'	2	228 a	145 ab	238 ± 53.8 ab	10.4 ± 2.81 d	24.6 ± 10.8 ab	18.1 a
'Bor 9415'	4	232 a	108 ab	310 ± 45.5 a	15.7 ± 2.09 d	29.7 ± 8.17 ab	20.9 a
'Elvi'	6	291 a	158 a	313 ± 43.5 a	22.1 ± 1.91 bc	36.1 ± 7.63 ab	17.3 a
'Picasso'	10	268 a	177 a	267 ± 42.1 ab	29.1 ± 1.77 a	44.7 ± 7.01 a	21.0 a
'Walet'	3	218 a	75.7 b	185 ± 48.5 b	15.8 ± 2.36 c	17.9 ± 9.05 b	17.7 a
				Wheat			
'Aapeli'	4	55.9 b	67.3 ab	75.0 ± 45.5 a	28.5 ± 2.10 cd	nd	10.8 ab
'Aino'	7	58.2 b	54.0 b	67.3 ± 41.3 a	21.7 ± 1.69 cde	nd	10.7 ab
'Amaretto'	13	63.1 ab	59.2 b	53.3 ± 39.8 a	30.4 ± 1.53 cd	nd	10.4 ab
'Bjarne'	5	61.5 ab	79.9 abc	63.2 ± 43.6 a	41.9 ± 1.91 ab	nd	10.6 ab
'Bor 00703'	3	46.3 bcd	54.6 ab	60.0 ± 48.5 a	22.0 ± 2.36 cde	nd	10.0 ab
'Kruunu'	16	56.4 bcd	45.0 bcd	56.9 ± 39.2 a	20.0 ± 1.47 cde	nd	8.47 b
'Picolo'	5	81.6 a	57.1 b	57.8 ± 43.5 a	28.8 ± 1.90 cd	nd	9.99 ab
'Quarna'	4	77.6 abc	76.8 abc	70.9 ± 45.5 a	42.5 ± 2.09 a	nd	10.4 ab
'Zebra'	16	72.5 abc	95.1 a	82.6 ± 39.2 a	36.9 ± 1.47 bc	nd	12.4 a
				Oat			
'Belinda'	16	79.7 a	340 b	683 ± 38.6 a	6.70 ± 1.42 b	63.4 ± 5.63 c	nd
'Bor 01187'	4	75.6 abc	573 a	319 ± 45.5 b	5.56 ± 2.09 b	0.00 d	nd
'Bor 96111'	9	64.3 ab	599 a	375 ± 39.2 b	10.3 ± 1.51 ab	104 ± 6.13 a	nd
'Roope'	16	57.0 b	285 b	214 ± 37.8 c	12.6 ± 1.34 a	65.2 ± 5.39 c	nd
'Veli'	8	50.3 bcd	518 a	319 ± 39.8 b	11.4 ± 1.56 a	82.6 ± 6.31 b	nd

[a] Values are expressed as mean values ± standard error. Concentrations of each lignan within cultivars of the same species marked by different letters show a very significant difference ($p \leq 0.0001$, $p \leq 0.001$, or $p \leq 0.01$). Medioresinol, lariciresinol, and 7-hydroxymatairesinol are log-transformed. nd = not detected.

Table 2. Impact of Grain Size on Average Lignan Content (μg/100 g) in Whole-Grain Extracts of the Winter Rye Cultivar 'Picasso'[a]

grain size (mm)	n	sum of lignans	lariciresinol	syringaresinol
2.2	3	4077 c	392 c	2864 c
2.5	2	4700 b	511 b	3152 b
2.8	3	5783 a	654 a	4038 a

[a] Only lignans showing significant differences between grain sizes are listed. The samples are from two different experiments conducted during the year 2007. Concentrations of each lignan or sum of lignans marked by different letters show a very significant difference ($p < 0.001$).

conditions. This study was a preliminary assessment that gives justification for further studies on the importance of genotype and environmental conditions for differences in lignan content and the applicability of this, e.g., in plant breeding, when targeting cultivars with high lignan content.

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