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Spectroscopic Analysis of Diversity of Arabinoxylan Structures in Endosperm Cell Walls of Wheat Cultivars (*Triticum aestivum*) in the HEALTHGRAIN Diversity Collection

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Supporting Information

ABSTRACT: Fifty bread wheat (*Triticum aestivum* L.) cultivars were selected from the HEALTHGRAIN germplasm collection based on variation in their contents of total and water-extractable arabinoxylan. FT-IR spectroscopic mapping of thin transverse sections of grain showed variation in cell wall arabinoxylan composition between the cultivars, from consisting almost entirely of low-substituted arabinoxylan (e.g., *T.aestivum* 'Claire') to almost entirely of highly substituted arabinoxylan (e.g., *T.aestivum* 'Manital') and a mixture of the two forms (e.g., *T.aestivum* 'Hereward'). Complementary data were obtained using endoxylanase digestion of flour followed by HP-AEC analysis of the arabinoxylan oligosaccharides. This allowed the selection of six cultivars for more detailed analysis using FT-IR and ^{1}H NMR spectroscopy to determine the proportions of mono-, di-, and unsubstituted xylose residues. The results of the two analyses were consistent, showing that variation in the composition and structure of the endosperm cell wall arabinoxylan is present between bread wheat cultivars. The heterogeneity and spatial distribution of the arabinoxylan in endosperm cell walls may be exploited in wheat processing as it may allow the production of mill streams enriched in various arabinoxylan fractions which have beneficial effects on health.

KEYWORDS: Arabinoxylan, endosperm cell walls, FT-IR, HEALTHGRAIN, ^{1}H NMR, wheat

INTRODUCTION

Arabinoxylan comprises about 70% of the endosperm cell walls in bread wheat[1] and can have beneficial effects on human health. These include generating viscosity which may slow the rate of gastric emptying, reducing mobility in the small intestine, and lowering postprandial glucose and insulin responses in humans.[2] Arabinoxylan may also dilute the energy density of the diet, therefore prolonging intestinal digestive processes, which in turn aids the control of satiety.[3] Soluble fiber (such as water-extractable arabinoxylan) is also more readily fermentable in the human intestine and therefore has greater prebiotic potential.[4] Although arabinoxylan may be beneficial to human nutrition, it can also have detrimental effects in livestock feed due to its high viscosity.[5] The arabinoxylan content of wheat grain and flour also affects their functionality during processing, such as brewing and biofuel production,[6] breadmaking,[7,8] and gluten–starch separation.[9,10] Given the importance of arabinoxylan in the utilization of wheat, and that variation in arabinoxylan structure influences end use properties, there is a compelling need to understand the extent of variation and to develop genetic and biochemical markers to enable breeders to select wheat cultivars on the basis of arabinoxylan content, composition, and properties.[11]

In common with many other polysaccharides, cereal arabinoxylan exhibits a high degree of endogenous microheterogeneity, and it is therefore not possible to describe it using a single structure. A model was proposed[12] whereby arabinoxylan consists of highly substituted regions, mostly consisting of tetrameric repeating units of an unsubstituted and a double arabinofuranosylated (Ara*f*) xylose (Xyl*p*) residue. These units are interspersed with less-substituted regions, which include subregions containing up to seven contiguous unsubstituted xylose residues. The highly substituted regions are enriched in both *O*-2 and *O*-3 disubstituted, as well as *O*-2 monosubstituted, xylose residues, the latter being absent from the less-substituted regions. Variation in the arabinose/xylose (A/X) ratio between different arabinoxylan polymers is therefore due to variation in the relative proportions of these regions, as well as to variation in the composition of the less-substituted regions.

The extent of variation in the contents of phytochemicals and dietary fiber available to plant breeders within the gene pool of wheat has been assessed through a diversity screen of 150 bread wheat cultivars selected on the basis of their geographical origin, genetic background, and release date. These cultivars were grown on a single site in Hungary in 2004/05,[13] harvested, milled, and analyzed for dietary fiber[14] and a range of bioactive compounds

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which are considered to have health benefits. The study revealed substantial variation in the contents of dietary fiber and constituents thereof between different cultivars.

In addition to variation in gross composition, it is becoming evident that there is significant spatial heterogeneity in arabinoxylan composition in wheat grain.[15−17] Using FT-IR spectroscopic methods,[16] it was shown that the structure of the arabinoxylan in the endosperm cell walls changed during the later stages of grain development from a highly substituted form to a low substituted form, the transition being initiated in the outer (prismatic) endosperm cells and then spreading into the central (round) endosperm cells. The rate of the transition was also faster in material grown under hot/dry growing conditions and differed between cultivars. Further evidence for this transition has been provided[18,19] using monoclonal antibodies that differentiate between arabinoxylan of high and low Ara*f* substitution and using Raman microspectroscopy[20] and ^{1}H NMR.[20,21] The latter studies have given unique insight into the chemical structure of intact wheat endosperm cell walls, providing qualitative information on the proportions of mono-, di-, and unsubstituted arabinoxylan and the levels of substitution of adjacent units.

The aim of this study was to use FT-IR spectroscopic mapping, enzymatic fingerprinting, and ^{1}H NMR analysis to determine the extent of variation in the composition, structure, and spatial distribution of arabinoxylan in the endosperm cell wall network in 50 wheat cultivars selected from the samples analyzed for dietary fiber components in the HEALTHGRAIN diversity screen.[14]

MATERIALS AND METHODS

Growth of Cereal Cultivars. One hundred and fifty wheat cultivars, comprising 130 winter and 20 spring types, were selected on the basis of their geographical origin, genetic background, and date of origin (i.e., modern varieties, old and transitional varieties, and landraces).[22] All cultivars were grown at the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary (latitude, 47° 21′ N; longitude, 18° 49′ E; altitude, 150 m) in 2004/5 in two plots as described previously.[13]

Preparation of Cell Wall-Only Thin Sections. Ten transverse cell wall-only thin sections were cut from the central region of a grain from each of the selected cultivars. The sections were prepared using a Vibratome (Intracel, Royston, U.K.) and their cell contents washed out according to the method described previously.[15,16]

Images were collected for 10 sections from each grain, using light microscopy as described previously.[16] From these images, the best sections (those with intact cell wall networks with the least remaining starch and protein) were selected and allowed to dry at room temperature onto a barium fluoride disk (13 mm diameter × 1 mm thick) (Crystran Ltd., Dorset, U.K.), for FT-IR spectroscopic imaging.

FT-IR Spectroscopic Imaging. Four regions of each transverse section were selected to be mapped spectroscopically using FT-IR imaging, as follows: region a, the top of a section, between the pericarp and the cavity; b, the upper corner of a section, between the pericarp and the cavity; c, the side of a section, from the pericarp at the side to the center of the cheek region; and d, the center of a section, from the cavity to the center of the cheek. The selected regions of the endosperm cell wall network in each transverse section, for all 50 selected wheat cultivars were imaged using a Digilab Stingray imaging spectrometer (Digilab, Cambridge, MA, US) according to the method described previously.[16]

Data Analysis. All data analyses were performed using the image analysis software ENVI 4.0 (Research Systems Inc., Boulder, CO, USA). In order to avoid the influence of variation in sample thickness, and therefore spectroscopic intensity, all spectra were baseline corrected and normalized. This was done by converting all spectra to transmittance units, correcting the baseline to 1 (using the continuum−removal function), returning the spectra to absorbance units, and finally applying an area-normalization to the maximum peak at 1041 cm^{-1} (dividing by the height of the band then multiplying by a factor).

Because of the size of the grain, it was not possible to scan a whole section using FT-IR imaging. Images were therefore collected for four areas, 640 × 640 μm, selected to represent all cell types within the endosperm (subaleurone, prismatic, and central round cells). Using ENVI 4.0, an average spectrum was determined for each cell wall only thin section, by combining all spectra from all four regions within a section (i.e., the average of 4 × 16,384 = 65,536 spectra). Principal components analysis (PCA) was then applied to the spectra.

Because of the preponderance of arabinoxylan in the endosperm cell walls of wheat (approximately 70% of the cell wall polysaccharides), the features of these components dominate the spectra, with little detectable apparent contribution from other cell wall polysaccharides. Clear differences were identified in the height of the shoulder at 1075 cm^{-1}, which may be assigned to the level of substitution present in the arabinoxylan polymer.[15] In order to compare the spectroscopic images from different thin sections, a classification method was devised by creating an arbitrary boundary between two regions.[16] This criterion was transferable between all data sets for all sections. A false-color-coded image was produced from the individual spectra by assigning a color to each pixel, with each of the four regions in each section comprising 16,384 pixels. If the shoulder at 1075 cm^{-1} was above 66% of the height of the peak at 1041 cm^{-1} (representing highly substituted arabinoxylan), then the pixel corresponding to the spectrum was color coded blue; if the shoulder was below 66% (representing low-substituted arabinoxylan), then it was colored green. It was colored black if the absorbance of the spectrum was too low or too noisy, which was indicative of holes in the cell wall section and was colored white if the spectroscopic features were similar those of starch (rather than arabinoxylan). The 66% ratio was chosen because it was half way between the two average shoulder heights in a PCA plot produced for a typical endosperm cell wall spectroscopic image.[16] The numbers of pixels for each color could be calculated, allowing the proportions of highly substituted arabinoxylan and low-substituted arabinoxylan present in each of the selected regions of the cell wall sections to be determined. It should be noted that the difference between the blue and green cell wall types is signified by a lowering of the spectroscopic shoulder at 1075 cm^{-1}, which is indicative of a change in the substitution pattern of the arabinoxylan, by lowering the level of substitution present in the polymer.

^{1}H NMR Analysis. Endosperm cell wall network samples were prepared by hand dissection from approximately 200 transverse cell wall-only thin sections and allowed to dry. These were then mixed with 700 μL of a solution of D_2O containing as reference TSP (sodium 3-(trimethylsilyl)-propionate-d_4) and stirred using a spatula at room temperature for ∼10 s. Five hundred and fifty microliters of the whole mixture was then transferred to a 5 mm NMR tube for analysis. ^{1}H NMR spectra were recorded at 70 °C on a 600 MHz Bruker Avance spectrometer (Bruker Biospin, Rheinstetten, Germany) according to the method described previously.[20,21]

While only about 25−35% of the wheat endosperm arabinoxylan are extractable in water,[23] hydration of the samples was sufficient for the signals from the arabinose side chain and xylan backbone sugars to be observed at high temperature (70 °C) by high resolution ^{1}H NMR. Signals may originate from both water-extractable and water-unextractable components of arabinoxylan since it is the local mobility of the hydrated sugar units that determines whether NMR signals are observable. However, the low amount of material available under the preparation conditions used meant that only semiquantitative data could be collected. Despite this shortcoming, the ^{1}H NMR spectra give a

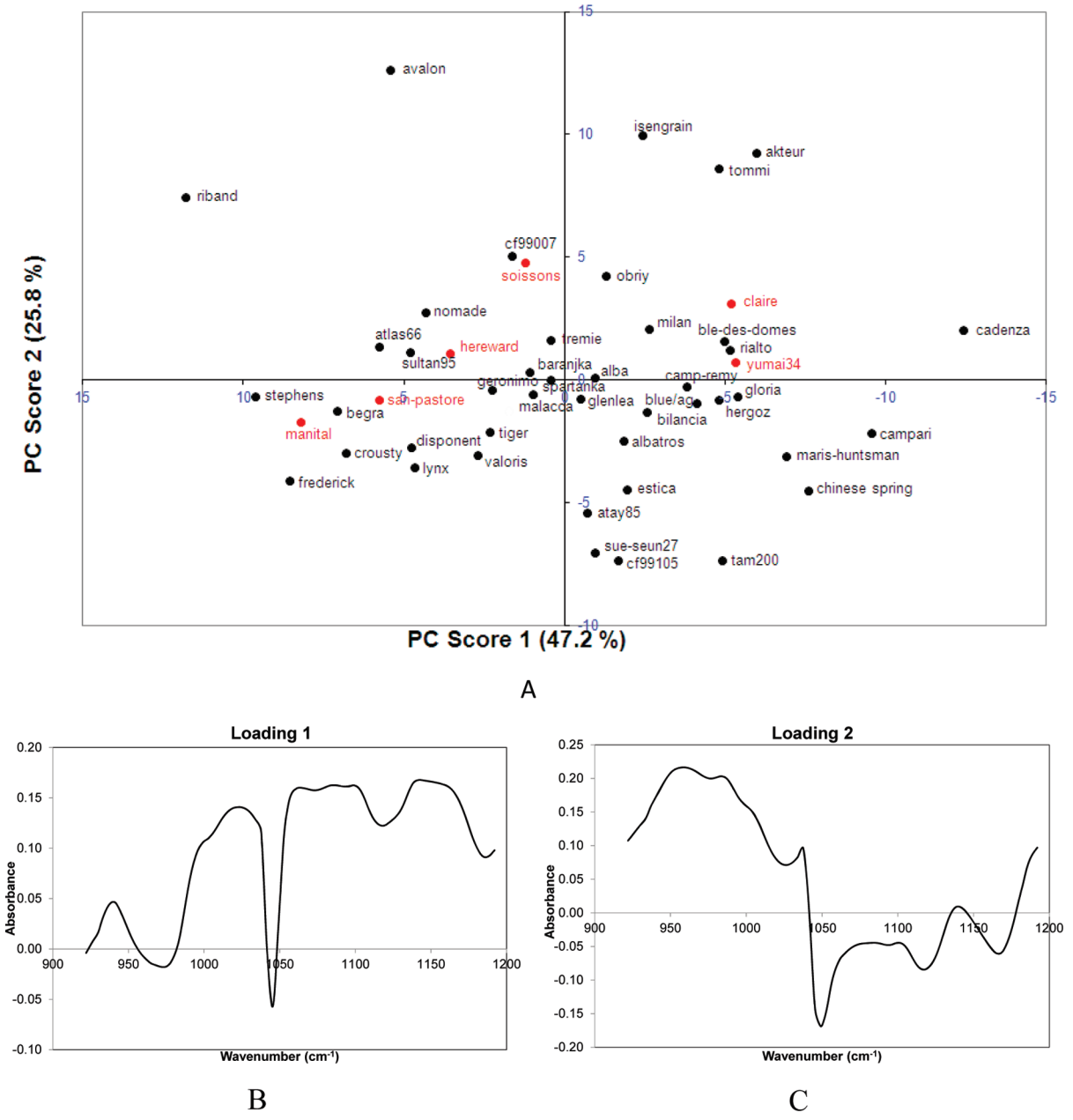

Figure 1. PCA plot (A), plus loadings (B and C), for the averaged FT-IR spectra for the spectroscopic images for each of the 50 wheat cultivars analyzed in the HEALTHGRAIN diversity screen. Red spots indicate the six cultivars selected for further analysis.

unique insight into the structure of intact arabinoxylan in wheat endosperm cell walls.

The A/X ratio, the ratio of mono- to disubstituted Xyl*p* residues and the proportions of mono-, di-, and unsubstituted Xyl*p* residues were determined.[20,21] The signal assignments for the integrals were based on detailed ^{1}H NMR data for arabinoxylan oligosaccharides.[24,25] The six wheat cultivars were analyzed twice.

Enzyme Mapping. Endoxylanase (EC 3.2.1.8) from *Trichoderma viride* (xylanase M1) was purchased from Megazyme (Bray, Ireland). The activity of the endoxylanase determined by the supplier on water-extractable arabinoxylan (at 40 °C, pH 4.5) was 1670 U/mL, and the pH optimum was 4.5−5.0.

Whole grain samples were ground in a rotor speed mill to pass a 0.1 mm screen. Samples (0.5 g) were treated with 5 mL of boiling 80% (w/v) aqueous ethanol for 10 min and the insoluble residue separated by centrifugation (6300*g*, 10 min). The procedure was repeated once and the residue finally washed with 5 mL of 95% (v/v) ethanol and again separated by centrifugation (6300*g*, 10 min). The supernatant was discarded and the residue dried overnight in an oven at 40 °C. Samples were then digested overnight (16 h) with 5 mL of purified water (Milli-Q, Millipore) containing 72 U endoxylanase.[17,26]

After centrifugation, the supernatants were diluted with water (1/40), and 10 μL aliquots were injected on a Carbopac PA-200 (3 × 250 mm) analytical column (Dionex, Sunnyvale, USA) run at 25 °C with a flow rate of 0.4 mL/min. Arabinoxylan oligosaccharides were separated using the following elution conditions with ultrapure water (A), 1 M NaOAc (B), and 0.5 M NaOH (C): 0 min (A:60%, C:40%), 30 min (A:43%, B:17%, C:40%); 35 min (A:35%, B:25%, C:40%); 36 min (A:20%, B:40%, C:40%), held up to 38 min; and 39 min (A:60%, C:40%) held up to 65 min.

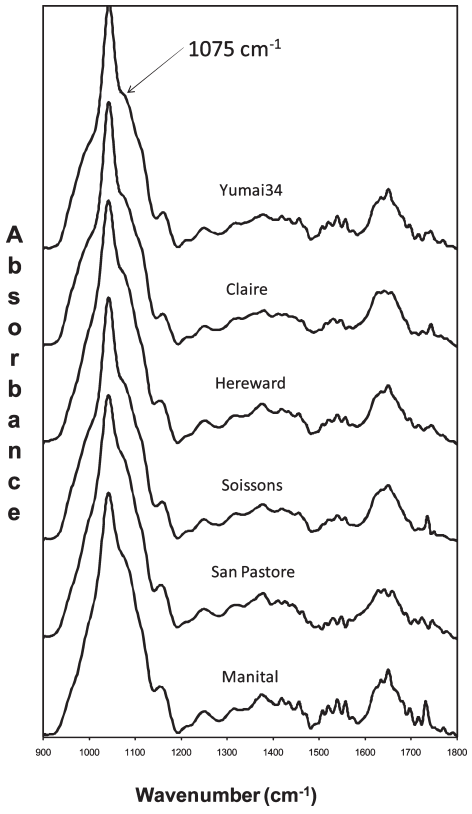

Figure 2. Average FT-IR spectra for the endosperm cell walls of the six wheat cultivars.

Detection was with a TSP EC2000 pulse amperometric detector (PAD, Thermo Separation Products, Piscataway, NJ) using the pulse potentials E1 = +0.05 V, E2 = +0.6 V, and E3 = −0.6 V. Peak identification was based on the retention times of reference compounds previously isolated.[17,26]

For principal component analysis (Figure 2), peak areas were normalized for each chromatogram as follows: $S_N Peak_i = (SPeak_i/\sum_{i=1}^{i=n} SPeak_i) \times 100$, with $S_N Peak_i$ being the normalized area of peak i and $SPeak_i$ the area of peak i.[17,26]

RESULTS AND DISCUSSION

Fifty bread wheat (*T. aestivum*) cultivars were selected from the samples analyzed previously[14] to provide a range of high (>2.2%), low (<2.0%), and medium (2.0−2.2%) contents of total arabinoxylan and high (>0.4%), low (<0.7%), and medium (0.4−0.7%) contents of water extractable arabinoxylan. Thin transverse sections comprising endosperm cell walls were prepared from mature grains of the 50 cultivars and analyzed using FT-IR spectroscopic mapping. PCA was then carried out on the average spectra to provide a broad visual comparison of arabinoxylan structure (Figure 1).

Although many of the cultivars are clustered around the central part of the plot, others clearly varied in composition, with no apparent clustering of winter or spring types. PC1 accounted for almost half (47.2%) of the variance, and the plot for loading 1 (Figure 1) showed a major feature which corresponded to the main carbohydrate (arabinoxylan) peak at 1047 cm^{-1}. Examination of the spectra (Figure 2) showed that changes in the ratio between this peak and the shoulder at 1075 cm^{-1} were responsible for loading 1. Loading 1, therefore,

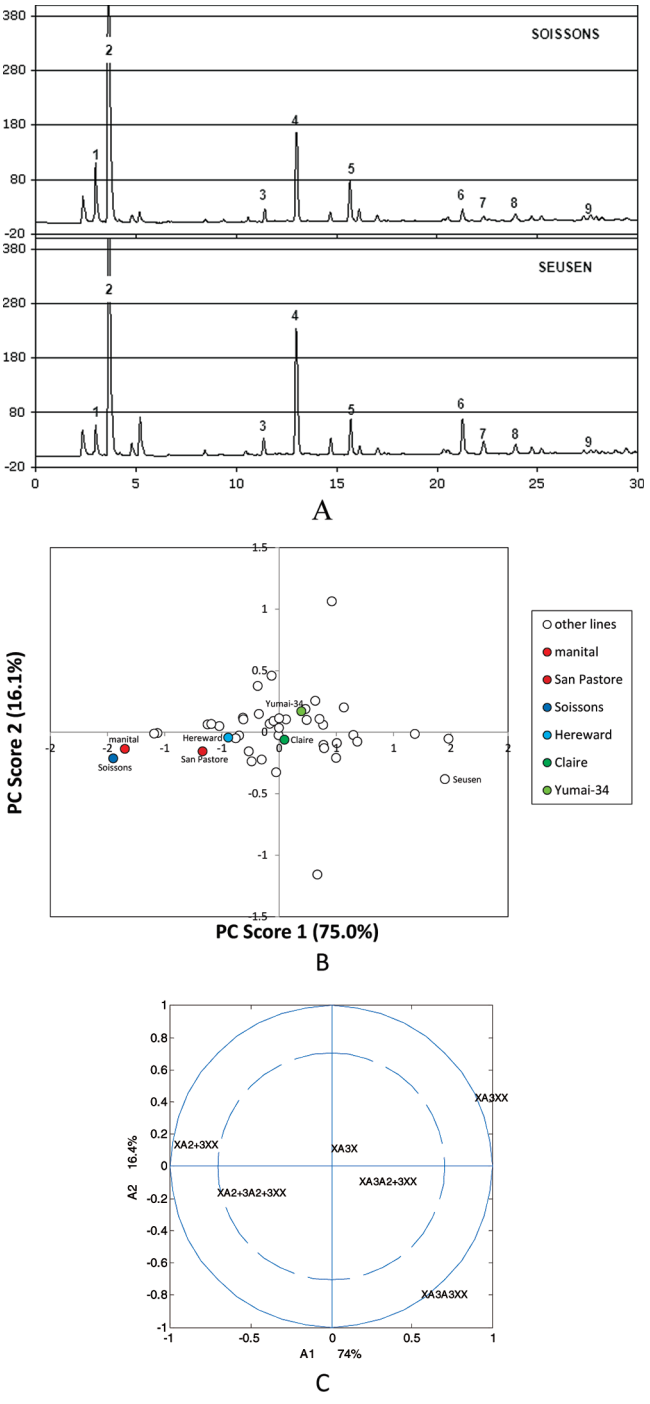

Figure 3. (A) HPAEC chromatograms obtained by treating contrasted cultivars Seusen and Soissons with xylanase. Peak identification: 1, X; 2, XX; 3, XA^3X; 4, XA^3XX; 5, $XA^{2+3}XX$; 6, XA^3A^3XX; 7, XA^3XA^3XX; 8, $XA^3A^{2+3}XX$; 9, $XA^{2+3}A^{2+3}XX$. (B) PCA analysis of the profiles obtained by treating samples with xylanase and separating the oligosaccharides released by HPAEC. (C) Loading plot for the PCA plot shown in B.

highlighted differences between the wheat cultivars whereby the relative height of the main arabinoxylan peak at 1047 cm^{-1} in relation to the shoulder at 1075 cm^{-1} was higher for cultivars falling to the right-hand side of the PCA plot (i.e., PC1 was −ve), and it was lower for the cultivars falling to the left-hand side of the plot (i.e., PC1 was +ve). PC2 showed differences due to cell-wall

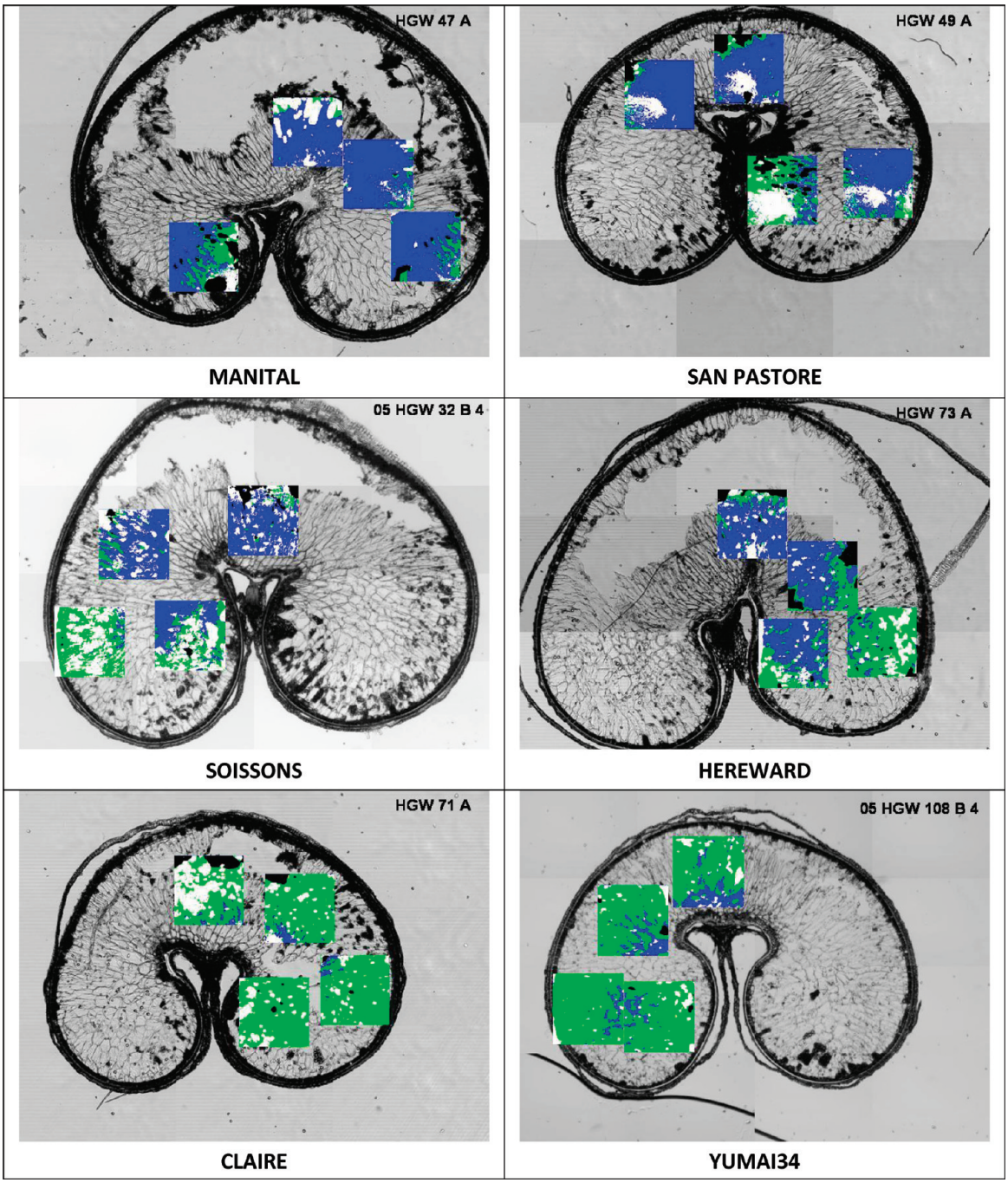

Figure 4. Images obtained by FT-IR microscopy overlaid onto the corresponding visible microscope images, for transverse cell wall-only sections for the six wheat cultivars, selected to provide a range of AX arabinose substitutions from low-substituted (LS-AX) to highly substituted (HS-AX).

carbohydrates such as β-glucan, and it accounted for only 25.8% of the variance and did not show any significant differences between the selected cultivars.

The structure of arabinoxylan in the 50 cultivars was also compared using a complementary approach, by determining the patterns of arabinoxylan oligosaccharides released after digestion with an endoxylanase (enzyme mapping). The arabinoxylan oligosaccharides were separated by HP-AEC, and previous studies have shown that they comprise xylose (X), xylobiose (XX), and arabinoxylan oligosaccharides of DP 4−9 (XA^3X, XA^3XX, $XA^{2+3}XX$, XA^3A^3XX, $XA^3A^{2+3}XX$ XA^3XA^3XX, and $XA^{2+3}XA^{2+3}XX$) (Figure 3A),[17,26] according to the nomenclature previously developed.[27] PCA of the profiles of the arabinoxylan oligosaccharides revealed clear differences, with PC1 and PC2 together accounting for 91.1% of the total variance (Figure 3B). PC1 accounted for 75% of the total variance and the loading plot (Figure 3C) showed that this related mainly to the degree of arabinoxylan substitution, with cultivars on the left-hand side being rich in disubstituted arabinoxylan oligosaccharides ($XA^{2+3}XX$ and $XA^{2+3}A^{2+3}XX$) and cultivars to the right in monosubstituted arabinoxylan oligosaccharides (XA^3XX). PC2 accounted for 16.1% of the variance with the main difference

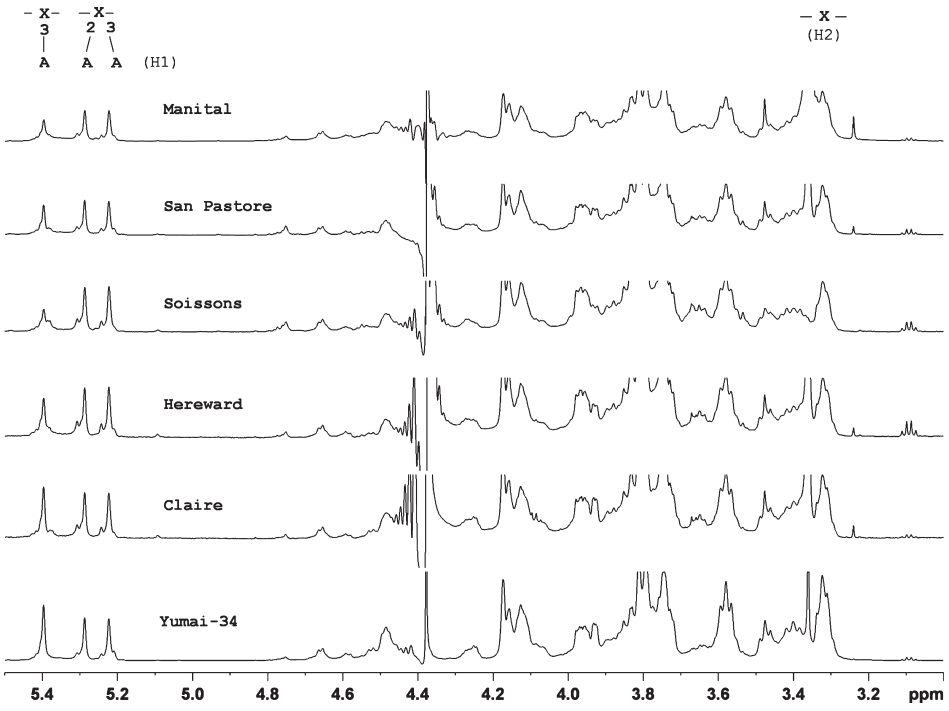

Figure 5. ^{1}H NMR spectra for the endosperm cell walls of the six wheat cultivars, selected to provide a range of AX arabinose substitution from low substituted (LS-AX) to highly substituted (HS-AX). The analyses were carried out on two occasions and were reproducible.

being in the relative proportions of $XA^{3}XX$ and $XA^{3}A^{3}XX$ arabinoxylan oligosaccharides.

On the basis of these analyses and previously published analyses of the water extractable arabinoxylan and total arabinoxylan contents, six cultivars were selected for more detailed study. These showed broadly similar distributions in the two PCA plots, with *T. aestivum* 'San Pastore' and 'Manital' lying toward the left-hand side, 'Yumai 34' and 'Claire' toward the center right, and 'Hereward' to the center left. However, the sixth cultivar 'Soissons' was located in the center of the PCA plot from the FT-IR analysis but to the left of the plot based on enzyme mapping; thus, the enzyme mapping showed a more highly substituted structure for arabinoxylan in 'Soissons' than the FT-IR analysis. However, it should be borne in mind that these two approaches measure arabinoxylan fractions which may not be identical. Thus, whereas the endoxylanase used for enzyme mapping digests about 75% of the arabinoxylan present in white flour,[26] the preparation procedure used for the FT-IR mapping may result in the loss of some water extractable arabinoxylan.

Further analysis of the six selected cultivars ('San Pastore', 'Manital', 'Claire', 'Yumai 34', 'Hereward' and 'Soissons') was carried out by both FT-IR and ^{1}H NMR. Figure 2 shows the averaged FT-IR spectra for each cultivar, on the basis of scanning six cell wall only thin sections of each. It shows that they differ in the height of the shoulder at 1075 cm^{-1}, which is related to the extent of arabinoxylan substitution.[16,21] However, comparison of the FT-IR spectra determined for four separate areas of the grain showed wide spatial variation in the extent of arabinoxylan substitution, as shown in Figure 4, which overlays the FT-IR spectroscopic images onto visual images. It may be noted that in reality there is not a clear division between the low-substituted arabinoxylan and highly substituted arabinoxylan structures but that there is a gradual transition between the two structures.[16] The cell walls of the two Italian cultivars 'San Pastore' and 'Manital' consisted almost entirely of highly substituted arabinoxylan (blue pixels), whereas those of 'Claire' (U.K.) and 'Yumai 34' (Chinese) consisted almost entirely of low substituted arabinoxylan (green pixels), and those of 'Hereward' (U.K.) and 'Soissons' (French) comprised varying mixtures of the two arabinoxylan types. Furthermore, the proportion of low substituted arabinoxylan was greater in the outer parts of the endosperm, which consists primarily of prismatic cells, in all cultivars, while the proportion of highly substituted arabinoxylan was greater in the central regions of the endosperms which consisted mainly of round cells. This spatial heterogeneity was a generic feature of all of the wheat cultivars analyzed and is consistent with previous studies [16,21] which showed that the proportion of low substituted arabinoxylan in the endosperm cell walls increased during the grain filling period, with the transition starting in the subaleurone layer and then extending to the center of the grain. The rate of this transition was also faster when plants were grown at a higher temperature with restricted water availability from 14 days after anthesis (daa), with differences in the rate occurring between different cultivars.[16]

Complementary ^{1}H NMR analysis (Figure 5) showed clear differences in the heights of the peaks for the mono-, di-, and unsubstituted xylose residues, demonstrating differences in the proportions of these structures between cultivars. The assignment of major signals in the ^{1}H NMR spectra of arabinoxylan have been described in detail previously[20] on the basis of analysis of the spectra of arabinoxylan oligosaccharides.[24,25] Briefly, integration of three regions of the spectrum is required to quantify the numbers of unsubstituted, monosubstituted, and disubstituted xylose residues in arabinoxylan. The integral (*I*) of the Ara*f* H-1 signal (δ 5.39) is related to the number of monosubstituted residues; the total integral (*II*) of Ara*f* H-1 signals in the range (δ = 5.20−5.32) is related to the number of disubstituted residues; and the integral (*III*) of Xyl*p* H-2 (δ 3.32) is a measure of the unsubstituted residues. These correspond to the integrals

Table 1. ^{1}H NMR Results for the Experiment Carried out with Two Repetitions (Rep 1 and Rep 2)

	NMR integration				xylose residues[a]					
	ara:xyl ratio[b]		mono/disubstituted ratio[c]		unsubstituted[d]		monosubstituted		disubstituted	
wheat cultivar	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Manital	0.58	0.61	0.97	0.75	0.61	0.61	0.19	0.17	0.20	0.22
San Pastore	0.55	0.55	1.11	0.88	0.63	0.64	0.19	0.17	0.18	0.19
Soissons	0.65	0.65	0.80	0.78	0.58	0.59	0.19	0.18	0.23	0.23
Hereward	0.63	0.59	0.97	0.82	0.58	0.62	0.20	0.17	0.21	0.21
Claire	0.59	0.58	1.21	1.13	0.60	0.60	0.22	0.21	0.18	0.19
Yumai34	0.47	0.44	1.33	1.46	0.67	0.69	0.19	0.19	0.14	0.13

[a] Expressed as percentages. [b] Arabinose to xylose ratio. [c] Ratio of monosubstituted to disubstituted xylose residues. [d] Unsubstituted xylose.

I, *II*, and *III* in the formulas presented previously.[21] On the basis of these assignments, it was possible to calculate the arabinose to xylose and mono- to disubstituted ratios and the proportions of un-, mono- and disubstituted xylose residues as shown in Table 1. These results were consistent with data obtained by FT-IR microspectroscopy and enzymatic fingerprinting, indicating that the endosperm cell walls of 'Manital' and 'San Pastore' consist mainly of highly substituted arabinoxylan and that those of 'Claire' and 'Yumai 34' consist almost entirely of low substituted arabinoxylan. The cell walls of 'Soissons' also consisted mainly of highly substituted arabinoxylan, on the basis of ^{1}H NMR analysis and enzymatic fingerprinting

The grain samples analyzed here were from plots grown on the same site in 2005. Therefore, the environment is unlikely to have differed greatly between the plots. Furthermore, analyses of 26 selected cultivars (including 5 of the six cultivars analyzed here) grown on the same site in Hungary over three years (2005, 2006,2007) and on three additional sites (in the U.K., France and Poland) in 2007 only showed that the contents of total arabinoxylan and water extractable arabinoxylan in flour were highly heritable, with ratios of genetic variance to total variance of 0.71 and 0.59, respectively, and ratios of genotype × environment variance to total variance of about 13% in both cases.[28]

However, all six cultivars were grown outside their region of adaptation, with 'Soissons' (France) and 'Hereward' (U.K.) being modern breadmaking wheats and 'Claire' (U.K.) a modern biscuit wheat from the Western European germplasm (according to the European Adaptation Map[29]), while 'Manital' (Italy) and 'San Pastore' (Italy) are modern and transitional bread wheat varieties from the Southern European germplasm and 'Yumai 34' (China) a modern breadmaking wheat variety from the Chinese germplasm. It is known that the rate of transition from highly substituted arabinoxylan to low substituted arabinoxylan increases in grain grown under environmental stress of increased temperature and reduced water availability,[16,20] and the extent to which these changes occurred may therefore have differed between the six wheat cultivars depending on their degree of adaptation to the growth conditions.

The differences in arabinoxylan structure identified between the bread wheat cultivars may have implications for grain processing quality as the arabinoxylan content and composition strongly affect functionality during processing including distilling and brewing[6] and breadmaking.[7,8] They may also have implications for the effects of arabinoxylan as dietary fiber on human health. The level of arabinoxylan substitution may affect the solubility of the fiber,[2] with the extent and distribution of the side chains being important factors determining properties including viscosity which may affect its physiological activity. Because of these health benefits, the diversity in substitution levels identified during this study may provide a basis for wheat breeders to select new wheat cultivars with enhanced health benefits.

Spatial heterogeneity of arabinoxylan in endosperm cell walls was observed in all of the cultivars studied here. This nonuniform distribution of components within the wheat grain may be exploited in wheat processing as it may allow the production of mill streams enriched in various arabinoxylan fractions (total arabinoxylan, water extractable arabinoxylan, low substituted arabinoxylan, or highly substituted arabinoxylan) with specific health benefits. Such naturally obtained products may be more desirable as food ingredients for health-conscious consumers than products obtained through chemical processing.[30]

FT-IR, enzyme mapping and ^{1}H NMR have been used to characterize arabinoxylan in the endosperm cell walls of wheat, showing diversity in composition and structure. The diversity of cell wall composition between different cultivars broadly followed their genetic relatedness, which is consistent with the observation that fiber content and composition of cereals is genetically determined.[11] Such genetic control suggests that there is the potential to breed cultivars with contents and compositions of arabinoxylan that are optimized for different end use requirements, for example, increasing the viscosity of grain products for human nutrition and reducing the viscosity of grain for animal feed.

■ ASSOCIATED CONTENT

Supporting Information. Table showing the selection criterion for the 50 wheat cultivars. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS USED

Ara*f*, arabinofuranosyl units; Ara/Xyl, arabinose/xylose ratio; FPA, focal plane array; HP-AEC, high performance anion exchange chromatography; PCA, principal component analysis; TSP, sodium 3-(trimethylsilyl)-propionate-d_4; Xyl*p*, xylopyranosyl residues.

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