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EXPLOITING NATURAL VARIATION TO IMPROVE THE CONTENT AND COMPOSITION OF DIETARY FIBRE IN WHEAT GRAIN: A REVIEW

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Wheat is the major staple food in most temperate countries, including Europe and North America. In addition to providing energy and protein it is a significant source of a number of essential or beneficial components, including B vitamins, minerals, and dietary fibre. Cereal fibre has established benefits in reducing the risk of several chronic diseases but the consumption of fibre is below the recommended daily intake in the typical "Western diet". Improving the content and composition of wheat fibre is therefore an attractive strategy to improve the health of large populations at low cost. The major dietary fibre components of wheat grain are arabinoxylan and β-glucan. Both vary in their amount, composition, and properties in different grain fractions, with white flour being lower in total fibre than bran but having a higher proportion of soluble fibre. There is significant variation in the amounts and structures of arabinoxylan and β-glucan in bread wheat genotypes, including commercial cultivars from different regions of the world, old landraces and exotic lines. This variation is also highly heritable, which should allow plant breeders to develop improved cultivars. Gradients in fibre composition and content within the grain may also allow millers to produce specialist high fibre flours.

Keywords: wheat grain, dietary fibre, arabinoxylan, β-glucan, diet and health

1. Wheat in human diet and health

The annual global wheat production averages over 600 million tonnes per year, of which about 100 million tonnes are traded internationally (McFall & Fowler, 2009). It is the major staple food crop in the temperate countries where it can be cultivated, extending from Argentina to Scandinavia. However, there is also increasing demand for wheat in Asian and African countries where it cannot be cultivated, with consumption being associated with urbanisation and the adoption of a western diet. One reason for the success of wheat is its diversity, with the existence of over 25 000 genotypes (Feldman, 1995), which are adapted to a wide range of temperate conditions. This variation is striking in view of the fact that hexaploid bread wheat is a relatively young crop, originating little more than 10 000 years ago, and results from high genome plasticity (Dubcovsky & Dvorak, 2007).

Wheat makes a significant contribution to the human diet, even in countries such as the UK in which the diet is very varied. For example, Steer and co-workers (2008) calculated that UK adults consumed an average of 90 g of bread a day, which contributed 13% of their energy intake, 12% of protein, 21% of carbohydrate, 20% of dietary fibre (DF), 11% of

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folate, and 16% of iron. However, consumption of wheat in most countries is dominated by products made from white flour, for example, white bread accounts for about 67% and 61% of the bread consumed by men and women, respectively, in the UK (Steer et al., 2008).

Although white flour products are not intrinsically unhealthy, they may form part of the "Western diet" with other highly refined processed foods that are rich in fat, starch, and sugars and associated with the adoption of a more sedentary life style. This combination of diet and lifestyle is associated with a group of symptoms called the "metabolic syndrome" (Reaven, 1988). These symptoms include abdominal obesity, insulin resistance, hyperglycaemia (high blood sugars), dyslipidaemia (abnormal blood lipids) and hypertension (high blood pressure) and are associated with increased risk of diseases, such as type 2 diabetes, atherosclerosis, and cardio-vascular disease (Ford, 2005; Shaw et al., 2005). The metabolic syndrome and associated diseases are also increasing dramatically in developing economies, particularly in China and India (Finucane et al., 2011; Hu, 2011), and in children and adolescents in developed countries (Hannon et al., 2005; Ogden et al., 2010).

It is clear that dietary intervention can reverse this trend, with the consumption of foods rich in fibre, vitamins, minerals, and bioactive phytochemicals being particularly beneficial. In fact, the strongest evidence for health benefits of food is for wholegrain cereals, and particularly for the DF fraction. Benefits associated with increased consumption of cereal DF include lowering blood pressure and serum cholesterol, improving insulin sensitivity, and reducing the incidence of bowel and breast cancers (Richardson, 2000; Cade et al., 2007; Topping, 2007; Buttriss & Stokes, 2008; Anderson et al., 2009; Tighe et al., 2010; Wolever et al., 2010; Aune et al., 2011; EFSA Panel on Dietetic Products, Nutrition and Allergy, 2011). Soluble forms of DF may also be especially beneficial, conferring viscosity, which contributes to reduced serum cholesterol and slowing the digestion and absorption of food, reducing the glycaemic load (Topping, 2007; Tighe et al., 2010). Whole grain cereals also contain many other components that may have beneficial properties, particularly phenolic and terpenoid compounds. However, their precise biological activities are more elusive, as are those of many other food components that may have beneficial properties (for example, phytochemicals in fruits and vegetables).

2. Components of DF in wheat grain tissues

The wheat grain comprises three major groups of tissues: the embryo (germ), endosperm, and outer layers, which account for about 3%, 80–85%, and 13–14% of the dry weight, respectively (Fig. 1) (Barron et al., 2007). The endosperm in turn comprises two distinct tissues, with the outer layer of cells forming the aleurone layer surrounding starchy endosperm cells. When wheat is milled, the starchy endosperm cells give rise to white flour, while the other tissues (including the aleurone) collectively form the bran fraction. The grain tissues differ significantly in their contents and compositions of bioactive components (see Fig. 1). In particular, the composition of the starchy endosperm cells is dominated by starch, which comprises about 90% of the dry weight, with about 10% protein, 2–3% cell wall polysaccharides (the major components of DF), and low contents of minerals, vitamins, and phytochemicals. By contrast, the bran is particularly rich in these components, with fibre in particular accounting for about 35–40% of the dry weight of the aleurone cells and 45–50% of the outer bran layers (Barron et al., 2007).

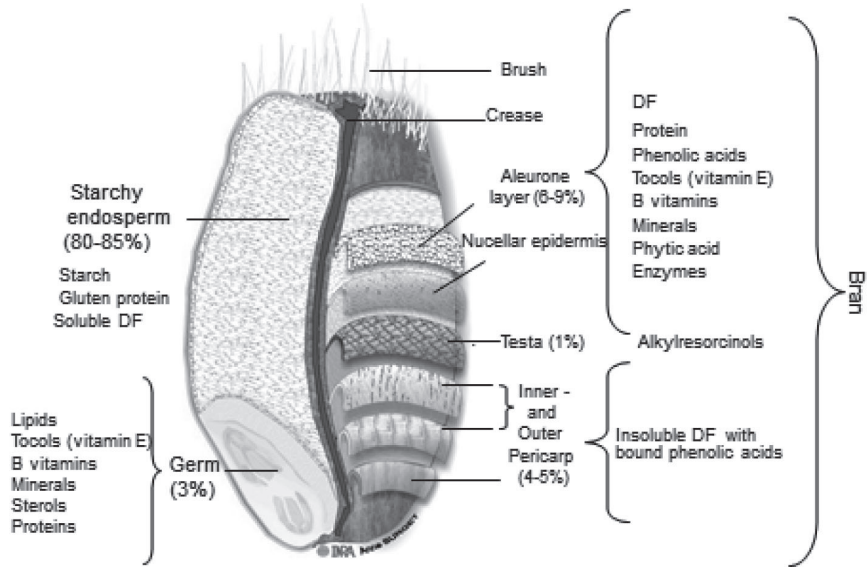

Fig. 1. The component tissues of wheat grain.
Taken from Surget and Barron (2005), with permission. Modified by Van Der Kamp (2012)

The total DF (TDF) fraction of whole wheat grain comprises three types of components: cell wall polysaccharides, lignin, and oligosaccharides (mostly fructo-oligosaccharides, usually called fructans). In a recent survey of 129 winter wheat lines, these components accounted for means of 10.8%, 1.33%, and 1.28% of the grain dry weight, respectively (Andersson et al., 2013). However, there are clear differences between the compositions of the major grain tissues (Table 1). The endosperm tissues (aleurone and starchy endosperm) have high proportions of arabinoxylan (60–70%), lower amounts of (1,3:1,4) β-D-glucan (β-glucan) (20–30%), small amounts of cellulose and glucomannan, and no lignin. By contrast, the cell wall composition of the outer pericarp tissue is more similar to that of the vegetative tissues of the plant (including straw), with high cellulose (30%) and lignin (12%) as well as high xylan (60%) contents, but lacking β-glucan.

Table 1. Compositions of cell wall in wheat grain tissues (% dry weight)

	Cell walls (% dry weight)	Cell wall components (% total polysaccharide)					
		Cellulose	Lignin	Pectin	Xylan	β-glucan	Gluco-mannan
Starchy endosperm	2–3	2	0	–	70	20	7
Total bran (pericarp, testa, aleurone)		29	8	–	64	6	–
Aleurone	40	2–4	0	–	62–65	29–34	–
Outer pericarp (beeswing)		30	12	–	60	–	–
Straw	85	37–40	14–17	0.5	39	–	–

Taken from Shewry and co-workers (2010a).

DF is also often divided into soluble and insoluble forms, which may differ in their physiological effects. In wheat the DF in bran is largely insoluble, whereas the DF in white flour is more soluble. This will be discussed in more detail later.

Consequently, the content and composition of DF in white flour fractions will depend on the extent to which the aleurone and other bran tissues are present (i.e. the flour extraction rate) as well as the intrinsic fibre content of the starchy endosperm cells.

3. Structure of wheat DF

Wheat AX consists of a backbone of (1→4)-linked β-D-xylopyranosyl residues to which terminal α-l-arabinofuranose substituents are linked as side branches, attached at the *O*-3 (monosubstituted) or both the *O*-2 and *O*-3 (disubstituted) positions of some xylose residues (Fig. 2).

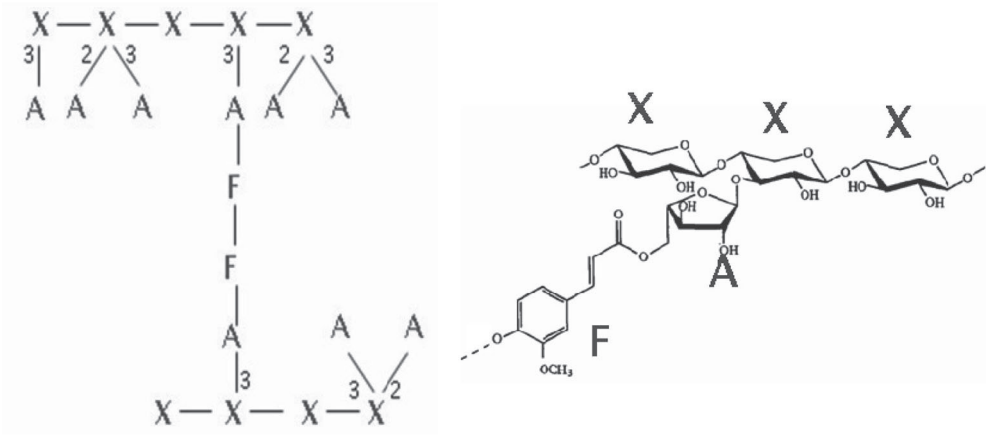

Fig. 2. Schematic (left) and detailed (right) structures of wheat starchy endosperm arabinoxylan. Taken from Shewry and co-workers (2010a) with permission.

Wheat AX is clearly highly heterogeneous and its precise structure has not been determined. However, variation in structure can be studied using a range of methods. The most fundamental difference is the ratio of arabinose to xylose, which will depend on the proportions of unsubstituted, monosubstituted, and disubstituted xylose residues. However, these ratios only provide an average for the whole population of AX molecules present in the tissue or tissues analysed, with no information on spatial or compositional variation.

Information on structural variation in AX can be obtained by using "enzyme mapping", in which fractions are digested with an endoxylanase to release oligosaccharide fragments (AXOS) that are separated by chromatography or electrophoresis. The structures of the individual AXOS have been determined (Ordaz-Ortiz et al., 2004), and their proportions therefore provide information on the fine structure of the AX as well as a convenient fingerprint to compare genetic, environmental, and developmental effects on composition (Toole et al., 2009b; 2010; 2011).

Spatial differences in composition can be determined by FT-IR microspectroscopy of thin sections of the grain, particularly the extent of arabinosylation of the xylan backbone. However, the requirement to remove starch before analysis means that the WE-AX is also removed, with the spectra corresponding to the WU-AX fraction. Nevertheless this approach has provided information on heterogeneity within the tissue as well as transitions related to development and the environment (Toole et al., 2007; 2009a; 2010; 2011; Saulnier et al., 2009). The proportions of monosubstituted and disubstituted xylose units can also be determined by NMR spectroscopy of WE-AX fractions (Toole et al., 2010; Anders et al., 2012).

The *O*-3 monosubstituted arabinose residues may also be substituted with ferulic acid at the 5 position, allowing the formation of cross-links (dimeric dehydrodiferulate or, more rarely, triferulate) by oxidation of ferulate groups present on adjacent AX chains. The extent of feruloylation also varies between tissues, with the AX in the starchy endosperm having a low level of feruloylation (about 0.3% of the total AX), only traces of dehydrodiferulate and no triferulate (Barron et al., 2007; Saulnier et al., 2012), while the aleurone cell walls contain about 3.2% ferulate, 0.45% dehydrodiferulate (Saulnier et al., 2012), and also *p*-coumaric acid (0.2–0.3 mg g^{-1} tissue) (Barron et al., 2007). The AX in the outer layers is generally more highly substituted than in the endosperm tissues, (with galactose and glucuronic acid substitutions) and is often termed glucuronoarabinoxylan. It is also acetylated and highly ferulylated, with triferulic acid being abundant in the outer pericarp (over 1 mg/ dry weight of tissue) (Hemery et al., 2009).

β-glucan comprises glucose residues joined by (1→3) and (1→4) linkages. Single (1→3) linkages are usually separated by three or four (1→4) linkages, resulting in the release of oligosaccharides, mainly trisaccharide (DP3) and tetrasaccharide (DP4) units, on digestion with a specific lichenase (endoglucanase) enzyme. However, stretches of (1→4) linked glucan of up to 14 units have been reported for wheat bran β-glucan (Liu et al., 2006).

4. Genetic variation in DF amount and structure

Crop improvement by plant breeding depends on the identification and exploitation of variation in traits that contribute to improved performance, including yield, resistance to biotic and abiotic stresses, and grain quality. It is therefore important to establish the extent of variation in the content and composition of AX in wheat and in closely related species that can be used to "introgress" traits into wheat cultivars.

Several surveys of DF content and composition of wheat have been reported. For example, Pritchard and co-workers (2011) analysed wholemeal flours of 338 hexaploid wheat lines from the Australian Winter Cereals Collection, showing variation in total non-starch polysaccharides from 3.18% to 9.14%, TOT-AX from 2.37% to 6.58%, and β-glucan from 2.0% to 14.3%. However, not all of the lines had been grown together and some of the variations may therefore have resulted from the effects of environment (as discussed below).

A smaller number of lines (130 winter and 20 spring types) were compared as part of the EU HEALTHGRAIN project, after being grown together in single plots in 2005. Andersson and co-workers (2013) reported detailed analysis of DF components in wholemeal flours of 129 of the winter lines, as shown in Table 2. Total dietary fibre (TDF) varied from 11.5 to 15.5%, and TOT-AX from 5.53 to 7.42%. Substantial variation also occurred in other components, notably lignin, which is restricted to the pericarp and other outer layers (0.74 to

2.03%), cellulose, which is present throughout the grain but also concentrated in the outer layers (1.67 to 3.05%), and fructan (0.84 to 1.85%), which is present in flour and bran, but over two-fold higher in the latter (3.7% compared with 1.5%) (Haskå et al., 2008). The differences in the contents and compositions of DF components in the bran and flour mean that the composition of the whole grain is influenced by grain size, as smaller grains have a higher proportion of bran (discussed by Ward et al., 2008).

Table 2. Content of dietary fibre, dietary fibre polysaccharide residues, Klason lignin, arabinoxylan, cellulose, and fructan in 129 winter wheat varieties from the HEALTHGRAIN diversity screen

	Range	Mean
Total dietary fibre (%)[a]	11.5–15.5	13.4
Arabinose residues (%)[b]	2.11–2.95	2.52
Xylose residues (%)[b]	3.58–4.88	4.20
Mannose residues (%)[b]	0.31–0.56	0.39
Galactose residues (%)[b]	0.28–0.40	0.33
Glucose residues (%)[b]	2.39–3.71	2.85
Klason lignin (%)[b]	0.74–2.03	1.33
Uronic acids (%)[b]	0.41–0.61	0.50
Arabinose/Xylose	0.54–0.70	0.60
Arabinoxylan (%)[c]	5.53–7.42	6.49
Cellulose (%)[d]	1.67–3.05	2.11
Cellulose/Aarabinoxylan	0.26–0.42	0.33
Fructan (%)	0.84–1.85	1.28

[a]:Total dietary fibre calculated as the sum of DF analysed by the Uppsala method and fructans; [b]:analysed with the Uppsala method; [c]:arabinoxylan was: calculated by subtracting the arabinose fraction that might be part of arabinogalactan and considering that the arabinose/galactose ratio in extractable arabinogalactan is 0.69; [d]:cellulose was calculated by subtracting the β-glucan (analysed by Gebruers and co-workers, 2008) from total content of glucose residues analyzed by the Uppsala method.
Taken from Andersson and co-workers (2013) with permission

All 150 HEALTHGRAIN lines were also milled and the bran and flour fractions analysed, with both TOT-AX and WE-AX being determined. White flour contained between 1.35% and 2.75% TOT-AX (dry weight basis) (mean 1.93%) and between 0.30% and 1.40% WE-AX (mean 0.51%), with the corresponding values for bran being 12.7% to 22.1% (mean 17.8%) and 0.30% to 1.40% (mean 0.42%). Thus, a much greater proportion of the TOT-AX was soluble in flour, between about 20% and 50%, compared with between 2% and 5% of the TOT-AX in bran. Analysis of wholemeal samples of the HEALTHGRAIN lines showed that the content of β-glucan ranged from 0.5% to 0.95%, with a mean of 0.72% (Gebruers et al., 2008). Similar variation in AX content has been reported by others, for example, from 0.26–0.75% (mean 0.51%) WE-AX and from 0.88–1.52% (mean 1.15%) WU-AX in 20 wheat varieties (Ortiz-Ordaz et al., 2005).

There is also variation in the fine structure of AX between genotypes, including the ratio of arabinose to xylose residues and proportions of single and double arabinose substitutions

on the xylose backbone. For example, Gebruers and co-workers (2008) analysed AX fractions of the 150 HEALTHGRAIN lines by monosaccharide analysis, showing differences between WE-AX and TOT-AX fractions, and between bran and flour. The flour fractions showed A:X ratios ranging from 0.40 to 0.55 (mean 0.48) in WE-AX and from 0.55 to 0.70 (mean 0.58) in TOT-AX, whereas the ratios were higher in the bran fractions: 0.7 to 1.65 (mean 1.01) in WE-AX and 0.55 to 0.70 (mean 0.62) in TOT-AX. Toole and co-workers (2011) reported micro-spectroscopic analyses and enzyme fingerprinting of a sub-sample of the HEALTHGRAIN lines. NMR spectroscopy and FT-IR micro-spectroscopic imaging showed variation in the overall proportions of monosubstituted and disubstituted xylose residues, while analysis of endoxylanase digests showed variation in the proportions of AXOS containing mono- and disubstituted xylose.

It is probable that the structure of wheat β-glucan also varies, but there is little information on this. The proportions of oligosaccharides of DP3, DP4, and DP5 released by lichenase digestion of single samples of β-glucan from wheat bran have been reported to be 70.4: 21.3:8.5 (Liu et al., 2006) and 67.1:24.2:8.7 (Lazaridou et al., 2004), with DP3:DP4 molar ratios of 4.38 and 3.66, respectively, but little is known about β-glucan in flour. Genetic variation has not been investigated systematically, but variation in the ratio of DP3:DP4 fragments probably contributed to the variation in enzyme fingerprinting of flour reported by Toole and co-workers (2011) and Shewry and co-workers (2010b), as a β-glucanase enzyme (lichenase) was included in the digests as well as endoxylanase and the DP3 and DP4 fragments were included in the multivariate data analysis.

5. Effects of genotype and environment on DF amount and composition

Several reports have also shown that the variation in DF content in wheat is highly heritable. For example, 26 of the HEALTHGRAIN lines discussed above were grown on multiple sites over three years, giving a total of six environments for most of the lines. This allowed the variation in the amount of DF (Gebruers et al., 2010b) to be partitioned between the effects of genotype (G), environment (E), and G × E interactions (Shewry et al., 2010) (Fig. 3). In white flour fractions about 60% of the variation in the content of WE-AX and 70% of the variation in TOT-AX was attributed to genotype, while the values for bran were lower (48% for WE-AX and 39% for TOT-AX). The heritability of β-glucan in wholemeal was about 51%.

However, Li and co-workers (2009) reported contrasting results on the heritability of AX. They compared two sets of spring and winter wheats (25 lines of each) grown at three locations each in the USA. They showed that environment had a much greater effect on WE-AX and TOT-AX than genotype in winter wheats, and on WE-AX but not TOT-AX in spring wheats and concluded that the relative effects of genotype and environment on AX amount and composition depended on the precise genotypes and environments that were studied.

Rakszegi and co-workers (2014) have also reported effects of heat and drought stresses on the proportions and composition of AX and β-glucan in wholemeal samples.

Environmental effects on AX, and in particular on the proportion and properties of WE-AX, may also result in part from the effects of endoxylanase activity, which varies between samples and shows high environmental dependence (Gebruers et al., 2010a).

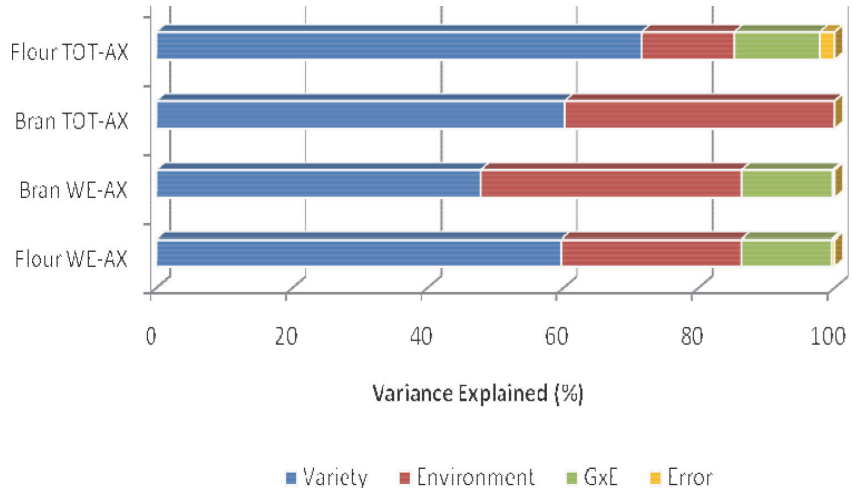

Fig. 3. Variance components from heritability calculations of dietary fibre and phytochemical data. Blue, variety; red, environment; green, G x E; orange, error. Taken from Shewry and Ward (2012) with permission.

6. New sources of variation in DF amount and composition

The variation in the contents of both WE-AX and TOT-AX observed in the HEALTHGRAIN diversity screen may be sufficient for many applications, particularly if high fibre content is to be combined with good bread making properties. However, higher contents may be advantageous, particularly for ingredients and health foods. In addition, variation in structure may be desirable, although the precise relationships between AX structure and health benefits remain to be defined. Three types of material can be used as sources of such variation.

6.1. Diverse lines of bread wheat

The HEALTHGRAIN diversity screen of 150 bread wheat cultivars identified one wheat cultivar, Yumai 34, which had the highest contents of both WE-AX (1.4% dry weight) and TOT-AX (2.7% dry weight) in flour, with the content of the former being over 50% greater than in the next highest cultivar (the French cultivar Ble des Domes, 0.9% dry weight) (Gebruers et al., 2008). Yumai 34 is a winter wheat that was released in the Henan province in Northern China in 1998. It has since been widely grown and remains a major cultivar in this region. It has been described as a "landmark cultivar since it combined high yield potential, broad adaptation, and excellent quality characteristics for pan bread and Chinese noodles" (Zhou et al., 2007). This demonstrates that significant diversity in fibre content is present in modern cultivars, and that high fibre content can be combined with good processing properties.

The cultivars and lines analysed in HEALTHGRAIN were selected to represent a range of types, geographical origins, and release dates. However, they only represent a small fraction of the 25 000+ lines present in wheat gene banks (Feldman, 1995). Hence, screening of further lines in germplasm collections and cultivars from different geographical origins may lead to the identification of wider variation.

6.2. Old varieties of bread wheat

It has been suggested that intensive plant breeding has resulted in reduced contents of beneficial components in modern cultivars of wheat, due to an emphasis on increasing the contents of starch (to give higher yields) and protein (for improved bread making) (Morris & Sands, 2003; Sands & Morris, 2009). Analysis of the HEALTHGRAIN dataset, which included land races and lines dating back over 100 years, showed no evidence for this effect on either fibre components (Fig. 4), vitamins, or phytochemicals (Shewry et al., 2011). We have since screened a core set of over 100 lines from the Watkins collection of wheat land races. These were collected from 32 European, Asian, and North African countries in the 1920s and 1930s (Miller et al., 2001; Wingen et al., 2014) and are held at the John Innes Centre, Norwich, UK. Analysis of wholemeal samples grown on the same site for total and water-extractable pentosans (i.e. AX) showed substantial variation in both fractions, which extended beyond that found in current commercial varieties (unpublished results of Reynolds, Shewry & Lovegrove).

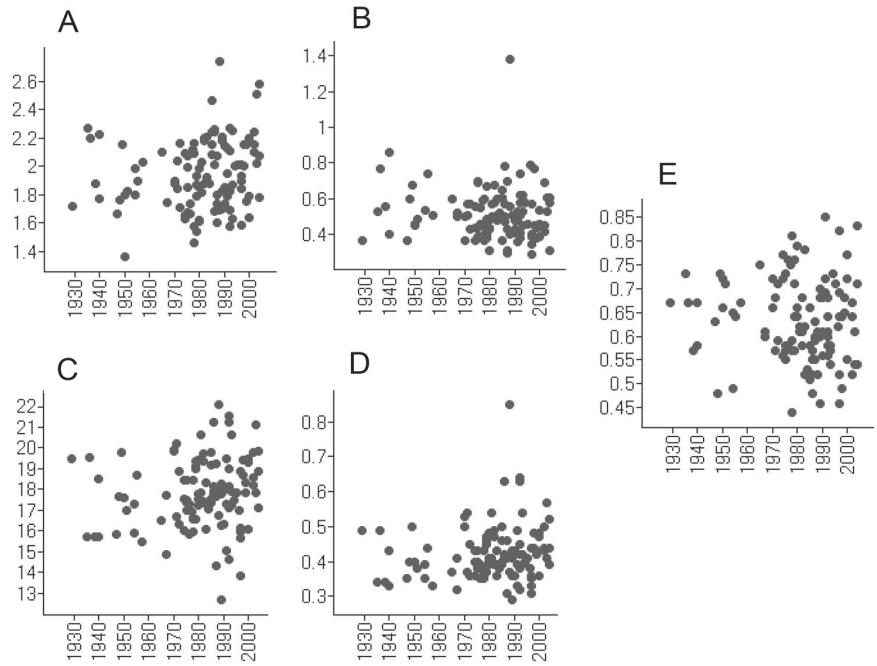

*Fig. 4***.** Relationship between release date and the contents of DF components in 150 wheat lines grown in 2005 as part of the HEALTHGRAIN diversity screen. A: flour TOT-AX ; B: flour WE-AX; C: bran TOT-AX; D: bran WE-AX; E: wholemeal β-glucan

6.3.. Ancient wheat species

Hexaploid bread wheat (genomes AABBDD) is a young species, having arisen in cultivation about 10 000 years ago, probably by spontaneous hybridization of cultivated tetraploid wheat (genomes AABB) with the wild grass *T. tauschii* (DD) (Dubcovsky & Dvorak, 2007).

However, cultivated forms of diploid and tetraploid species also occur and are cultivated to varying extents. The most widely cultivated is durum (pasta) wheat, a cultivated form of tetraploid *T. turgidum* var. *durum*. Between about 35 and 40 million tonnes are produced

annually, out of total wheat production of over 600 million tonnes, particularly in the Mediterranean region and similar climates in other continents. Other wheat species were grown historically but are today only cultivated on small areas, either for cultural reasons or for the expanding market in health foods: einkorn (A genome diploid *T. monococcum* var. *monococcum)*, emmer (AABB tetraploid *T. turgidum* var. *dicoccum*) and spelt (*T. aestivum* var. *spelta*), which is a cultivated form of hexaploid wheat (AABBDD). Spelt, emmer, and most forms of einkorn differ from bread and durum wheats in being hulled (i.e. the glumes remain tightly closed over the grain and are not removed by threshing). These wheats are often proposed to be rich sources of bioactive components, either for producing healthy foods or as traits to transfer into higher yielding and more agronomically adapted cultivars of bread wheat (see, for example, Ruibal-Mendieta and co-workers, 2005; Lachman and co-workers, 2013).

There has been a limited number of systematic studies of the content and composition of DF in ancient and durum wheats compared to bread wheat, but those that have been carried out have reported little difference. For example, Abdel-Aal and co-workers (1998) showed that the contents of TDF in white flour samples from single accessions of einkorn, spelt, and bread wheat were 4.6±0.3%, 4.3±0.1%, and 4.0±0.2% dry weight, respectively. Analyses of fibre in wholemeal flours from dehulled grains have also been reported. Abdel-Aal and co-workers (1995) compared five spelt lines with single samples of einkorn, durum, and bread wheats. All had similar contents of soluble fibre, from 1.6% to 1.9% dry weight, but the contents of insoluble fibre varied, being lowest in einkorn (6.9%) and highest in bread wheat (10.8%). The five spelt samples had similar contents of insoluble fibre, from 8.1% to 8.4%. The contents of TDF were 8.7% in einkorn, 11.1% in durum, 9.8% to 10.3% in spelt, and 12.5% in bread wheat. A similar trend for TDF was reported by Løje and co-workers (2003); 8.7±0.7% for einkorn (22 samples), 7.9% for emmer (single sample), 10.3% for spelt (single sample), and 12.3±0.7% for bread wheat (two samples); while Bonafaccia and co-workers (2000) showed little difference between the contents of TDF and soluble DF in three spelt lines, one durum wheat and one bread wheat. A more extensive study of three spelt lines grown on five sites (15 samples in total) gave means of 9.4±0.4% TDF and 8.1±0.4% soluble DF, compared with values of 12.1±0.6% and 10.5±0.6% for five bread wheat samples (Ranhotra et al., 1996). These studies therefore indicate that "ancient" wheats are not good sources of increased DF for improving bread wheat.

Little information is available on the detailed composition of DF in ancient wheats. Marconi and co-workers (1999) reported 0.92% to 1.27% dry weight of β-glucan in wholemeal samples of 5 spelts, compared with 10.5% to 14.9% TDF, while Escarnot and co-workers (2010) reported the contents of fibre components of wholegrain flours of four spelt and three wheat cultivars. These were means of 10.3% (spelt) and 11.7% (wheat) TDF; 1.4% and 1.6% soluble DF; 1.7% and 2.4% cellulose; 5.4% and 7.3% hemicellulose (presumably mainly AX and β-glucan); and 1.3% and 0.7% lignin.

Information is also available from the HEALTHGRAIN study, in which 10 durum wheats, five spelts, five emmers, and five einkorns were analysed in addition to 150 bread wheats, five oats, 10 barleys, and 10 ryes (Table 3). White flour and bran fraction were analysed for TOT-AX and WE-AX, the A:X ratios of these fractions, and wholemeals for β-glucan. Although the numbers of "ancient wheat" lines were small, there was no evidence for variation in these values beyond that present in bread wheat.

Table 3. Ranges of contents of TOT-AX and WE-AX in white flour and bran fractions and of β-glucan in wholemeal flours of wheat species. Taken from the HEALTHGRAIN study reported by Gebruers and co-workers (1978).

	Species	Genomes	Number of lines	Flour range TOT-AX (%)	Flour mean TOT-AX (%)	Flour range WE-AX (%)	Flour mean WE-AX (%)	Bran range TOT-AX (%)	Bran mean TOT-AX (%)	Bran range WE-AX (%)	Bran mean WE-AX (%)	Whole-meal range β-glucan (%)	Whole-meal mean β-glucan (%)
Bread wheat	*T. aestivum* var. *aestivum*	AABBDD	150	1.35–2.75	1.95	0.30–1.40	0.51	12.7–22.1	17.8	0.30–0.85	0.42	0.50–0.95	0.72
Spelt	*T. aestivum* var. *spelta*	AABBDD	5	1.60–2.15	1.75	0.30–0.45	0.35	11.1–13.9	12.7	0.30–0.35	0.30	0.55–0.70	0.65
Durum wheat	*T. turgidum* var. *durum*	AABB	10	1.70–2.35	1.95	0.25–0.55	0.40	10.9–13.7	12.0	0.30–0.55	0.40	0.25–0.45	0.35
Emmer	*T. turgidum* var. *dicoccum*	AABB	5	1.40–1.95	1.70	0.15–0.55	0.25	6.1–14.4	8.9	0.20–0.45	0.30	0.30–0.40	0.35
Einkorn	*T. aestivum* var. *monococcum*	AABB	5	1.45–2.35	1.95	0.50–0.65	0.60	9.5–10.4	10.0	0.45–0.65	0.55	0.25–0.35	0.30

7. Gradients in DF content and composition

The mature wheat starchy endosperm comprises three different cell types: the sub-aleurone (or peripheral) cells, the prismatic cells that radiate in columns below the sub-aleurone cells, and the central cells in the cheeks of the grain (Evers & Millar, 2002). These cell types differ in their composition, with the sub-aleurone cells being particularly rich in protein and the prismatic and central cells being rich in starch. FT-IR microspectroscopy and micro-scale enzyme fingerprinting have shown that differences in cell wall composition also occur across the grain, including the ratio of AX:β-glucan and the degree of substitution of the AX (Toole et al., 2007; 2009; 2011a, b; 2012; Saulnier et al., 2012). Grain development is associated with an increase in the ratio of monosubstituted to disubstituted AX, with this transition being initiated in the outer part of the starchy endosperm (Toole et al., 2007; 2009; 2010) and being accelerated under hot and dry growing conditions (Toole et al., 2007). In addition, differences in the extent of the gradients also occur between cultivars, as shown in Figure 5 (which is coloured to show regions enriched in monosubstituted xylose (green) and disubstituted xylose residues (blue)).

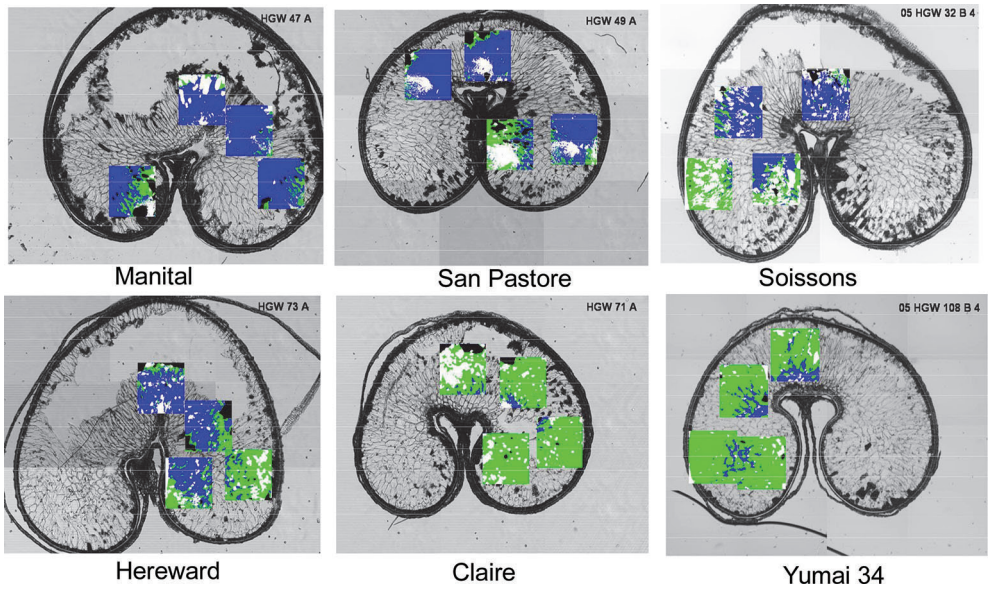

Fig. 5. FT-IR spectroscopic images overlaid onto the corresponding visible microscope images for transverse cell wall-only sections for the six wheat cultivars, selected to provide a range of AX arabinose substitution from low-substituted (LS-AX) to highly substituted (HS-AX).
A colour was assigned to each pixel (16,384 pixels per image) depending on the height of a shoulder at 1,075 cm^{-1} compared to that of the major AX peak at 1,041 cm^{-1}. If the shoulder was below 66% the pixel was coloured green to represent LS-AX and if it was above 66% it was coloured blue to represent HS-AX. Taken from Toole and co-workers (2010) with permission

We have confirmed the presence of these gradients at a larger scale by analysing fractions prepared by sequential abrasion (pearling) of the grain, with between 6% and 10% of the grain dry weight being removed in each of six sequential steps (He et al., 2013). Enzyme

mapping of fractions from a typical grain sample showed a decrease in the ratio of AXOS containing monosubstituted xylose to those containing disubstituted xylose from about 4 to 1.5, compared with a ratio of 2.13 for the whole grain. Similarly, the ratio of DP3:DP4 oligosaccharides released from β-glucan fell from 4.52 to 1.96, compared with 2.36 in whole grain (unpublished results of Lovegrove, He & Shewry).

Gradients in composition within the starchy endosperm may contribute to the differences in AX content, which have been reported for mill streams (Delcour et al., 1999; Wang et al., 2006; Ramseyer et al., 2011), although they may also result from increasing bran contamination as the extraction rate is increased. Nevertheless, the selection of specific mill streams to produce specialist high fibre flours is worth exploring.

8. Conclusions

Wheat is a major source of dietary fibre in the human diet, with well established benefits in reducing the risk of chronic diseases. Exploiting genetic variation in the amount and composition should allow the production of new types of wheat and food products, allowing health benefits to be delivered to consumers in acceptable products with low or zero added cost.

Abbreviations: A: arabinose; AX: arabinoxylan; WE-AX: water-extractable AX; WU-AX: water-unextractable AX; TOT-AX: total-AX; AXOS: arabinoxylan oligosaccharides; DF: dietary fibre; DP: degree of polymerisation; E: environment; FT-IR: Fourier-transform infra-red; G: genotype; NMR: nuclear magnetic resonance; TDF: total dietary fibre; X: xylose.

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