

REVIEW

Exploiting genetic variation to improve wheat composition for the prevention of chronic diseases

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Introduction

The birth of the 7 billionth person on the planet (estimated to have occurred on October 31, 2011) received massive coverage in the national and international media, and followed a period of increasing, and increasingly volatile, prices of global food commodities, notably wheat, after a long period in which food had become progressively cheaper. Furthermore, global grain reserves are currently low and increasing volumes of “food crops” are being used for biofuel production. Consequently, the interest of governments, international bodies and the public in food security, and the potentially conflicting requirements of producing crops for food and energy, has never been greater. Against this background it is clear that increasing food production must be the first priority (Royal Society 2009). However, it is also important to consider the wider impacts of food on health, and the fact that the major impact of food on many individuals relates

Abstract

The increasing global population places a clear priority on increasing food production. However, the continued undernourishment of a significant proportion of the global population is accompanied by increases in developed and rapidly developing countries of over-consumption, particularly of highly refined processed foods which are rich in starch, sugars, and fats, including products made from white wheat flour. This diet combined with an increasingly sedentary lifestyle is associated with a cluster of symptoms which have been termed the metabolic syndrome and are associated with increased risk of type 2 diabetes, atherosclerosis, cardiovascular disease, and forms of cancer. There is clear evidence that the consumption of either wholegrain cereals or components present in these (notably dietary fiber) has beneficial effects in reducing the risk of the metabolic syndrome and associated diseases. This article therefore reviews the major groups of bioactive components present in the wheat grain and discusses strategies for manipulating their amounts and compositions to increase the health benefits of both wholegrain and white flour products.

not to restricted access but to over-consumption, particularly of highly refined processed foods which are rich in fat, starch, and sugars, and the fact that this is associated with the adoption of a more sedentary life style.

This combination of diet and lifestyle is associated with a cluster of symptoms which have been termed the “metabolic syndrome” (Reaven 1988). The major symptoms are obesity, particularly abdominal obesity, insulin resistance, hyperglycemia (high blood sugars), dyslipidemia (abnormal blood lipids), and hypertension (high blood pressure), and are associated with increased risk of type 2 diabetes, atherosclerosis, and cardiovascular disease (CVD) (Ford 2005; Shaw et al. 2005). Furthermore, although the metabolic syndrome and associated diseases have long been a problem in highly developed countries, it is clear that their increase is now a global phenomenon, including countries where other sectors of the population may be under-nourished. For example, Hu (2011) reported the “globalisation of diabetes,” with an increase in type 2

diabetes in China from less than 1% of adults in 1980 to nearly 10% in 2008 and an occurrence of almost 20% in adults in urban areas of South India. Similarly, Finucane et al. (2011) analyzed data from 199 countries from all regions of the world showing global increases in mean body mass index (a measure of obesity) between 1980 and 2008 of 0.4 kg/m² per decade and 0.5 kg/m² for women. Furthermore, both obesity and type 2 diabetes are increasing in children and adolescents, with consequences for their health as adults (Hannon et al. 2005; Ogden et al. 2010).

These global changes in health will not only have impacts on quality of life but also on national economies, with increasing proportions of the population requiring expensive health treatments. It is currently not possible to “cure” type 2 diabetes, although a recent report has suggested that a degree of reversal is possible (Lim et al. 2011). However, it is clear that the global epidemic could be combatted by reducing the risk factors which contribute to the metabolic syndrome, in particular the adoption of more healthy lifestyles, including increased consumption of healthy foods, including whole grain cereals instead of refined white wheat flour or polished white rice.

The role of food in the prevention of chronic diseases has long been recognized (WHO 2003) with a particular emphasis on consumption of fruit and vegetables. However, wholegrain cereals have a range of health benefits, some of which are shared with other food groups, and have the advantage that the wide consumption of cereals in a range of staple foods provides opportunities to deliver health benefits to large populations at low cost.

In particular, the consumption of either wholegrain cereals or components present in these (notably dietary fiber [DF]) has been reported to have beneficial effects in reducing the risk of a range of conditions including CVD and forms of cancer (summarized in Fig. 1).

The present paper will therefore review the range of bioactive components present in cereal grain, focusing on bread wheat, and discuss how genetic variation in their amount and composition can be exploited to maximize health benefits. This is based largely on studies carried out in the EU FP6 HEALTHGRAIN Programme (2005–2010) (Poutanen et al. 2008, 2010), which focused on two types of bioactive component – DF and phytochemicals (including vitamins). The individual components were selected due to their abundance in cereal grains (e.g. phenolic components) or because cereals form important dietary sources (e.g. DF, B vitamins). However, with the exception of DF and vitamins, little information is available on the precise levels of these components that are required in the diet, and in many cases their biological activities are poorly understood. Guidelines for the intake of DF vary considerably, for example, 18 g/day for adults in the UK (FSA 2006) and 25 g per adult per day for adults in the European Union by the European Food Standards Authority (EFSA 2010). Few, if any, countries meet these targets; for example, the average daily intake of fiber by UK adults is about 13 g, and there is no doubt that cereal fiber could make a significant contribution to improved compliance.

The quality of starch and protein is not considered as there is limited genetic variation in the quality of these components in bread wheat. Variation in starch composition is restricted to mutant genes that affect the proportions of amylose and amylopectin and related work in the HEALTHGRAIN project has developed wheat in which the proportion of amylose in starch is increased to 38% (from about 25% to 30% in “normal” cereal starches) and shown that this can be baked into bread with a high content of resistant starch (Hallström et al. 2011). Protein quality is also not a concern for consumers in developed countries as diets include a range of protein sources and

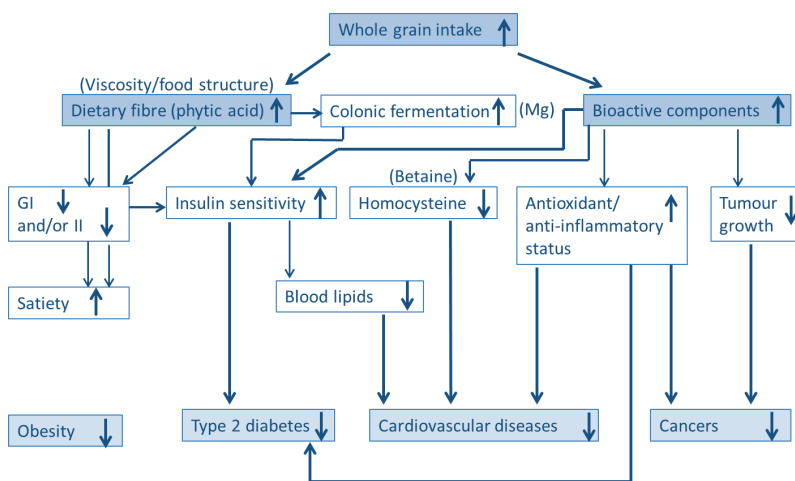


Figure 1. Current accepted mechanisms for how whole grain protects against major chronic diseases (used with permission from Anthony Fardet, INRA and Clermont Universite, UFR Medecine, France). GI, glycemic index; II, insulinemic index (taken from Björck et al. 2012 with permission).

hence individuals do not usually suffer from deficiencies of essential amino acids.

Similarly, we will not consider the application of genetic engineering technology (GM) as this is not accepted by consumers in many countries.

The HEALTHGRAIN diversity screen

The HEALTHGRAIN project included a major “diversity screen,” in which two detailed series of analyses were carried out. The first was on 150 wheat lines selected on the basis of diverse ancestry, wide geographical origin and a range of release dates (dating back to the mid-C19, but with most from the second half of the C20). They comprised 130 winter types and 20 spring types and were grown on a single site in Hungary in 2005 (Ward et al. 2008). Twenty-three of these lines and three additional lines were then grown on the same site in 2006 and 2007 and on three additional sites (in Poland, France, and the UK) in 2007 only (Shewry et al. 2010a). These two studies therefore provided data on samples grown in six (or for three lines only five) environments (site \times year combinations) which allowed the variation in composition to be partitioned between the effects of genotype (broad sense heritability), environment, and genotype \times environment interactions (G \times E).

Dietary Fiber

Dietary fiber, including wheat fiber, has been proposed to have a number of health benefits, including lowering blood pressure and serum cholesterol, improving insulin sensitivity and reducing the incidence of bowel and breast cancers (Bingham et al. 1985; Richardson 2000; Slavin 2004; Cade et al. 2007; Topping 2007; Buttriss and Stokes 2008; Anderson et al. 2009; Tighe et al. 2010; Wolever et al. 2010; Aune et al. 2011).

Wheat is an important source of DF in the diet of many countries, contributing about 40% of the total daily intake of fiber in the UK (Buttriss and Stokes 2008) with bread products accounting for about 20% (Steer et al. 2008). The major DF components in wheat grain are non-starch polysaccharides (NSP), which are the major components of the cell walls. Analyses of the 26 HEALTHGRAIN lines grown in multisite trials showed that NSP accounted for between 7.7% and 11.4% of the grain dry weight (average 9.5% dry weight) while the total DF content of the same material, including lignin, was determined using the widely accepted Uppsala method as 9.6–14.4% (average 11.7%) (Gebruers et al. 2010). A wider study (Fig. 2A) of 150 wheats grown on a single site showed higher values of total DF, from 11.5% to 18.3%, which was probably related to the

determination using an indirect method (Gebruers et al. 2008).

The cell wall polysaccharides comprise two major polymers, arabinoxylan (AX) and (1 \rightarrow 3,1 \rightarrow 4)- β -D-glucan (β -glucan), which account for about 70% and 20% of the total cell wall polysaccharides, respectively, in white flour, and about 65% and 30%, respectively, of the total in the aleurone layer. However, whereas cell wall polysaccharides account for only 2–3% of the dry weight of white flour derived from the starchy endosperm cells (Gebruers et al. 2008), they account for 35–40% of the aleurone layer (Barron et al. 2007). The outer layers of the grain (hyaline layer, testa, inner and outer pericarp) comprise about 45–50% of total cell wall material which contains about 10% lignin as well as polysaccharides (Barron et al. 2007; Stone and Morell 2009).

β -Glucan has been widely studied for its health benefits in barley and oats where it is the major cell wall component and has accepted health benefits in reducing coronary heart disease (Anonymous 2008). The extent to which these benefits are shared by wheat β -glucan is not known but differences in the distributions of 1,3 and 1,4 linkages occur between β -glucan in different cereals which may affect the solubility and viscosity (Lazaridou and Biliaderis 2007) – properties which are considered to relate to health benefits (Wood 2007). Gebruers et al. (2008) showed that the mean content of β -glucan in wholemeal of the 150 HEALTHGRAIN wheat lines ranged from 0.5% to 0.9%, with a mean of 7.2% (Fig. 2B), while contents in the 26 lines (Fig. 3) grown in multiple environments ranged from 0.46% to 0.95% (mean 0.66%) (Gebruers et al. 2010). Data from the latter study were used to calculate the broad sense heritability, which indicated that about half of the variation in content between the samples was due to genotype (Shewry et al. 2010a). No detailed studies of β -glucan from wheat flour have been reported, but analysis of whole grain shows that less than 20% of the total fraction is extracted in boiling water (Nemeth et al. 2010).

The content of AX fiber also varied in the 150 HEALTHGRAIN wheat lines, in both bran and white flour. Gebruers et al. (2008) showed that total AX (TOT-AX) in bran varied from 12.7% to 22.1% dry weight (mean 17.8%) but that water-extractable (WE-AX) accounted for only 0.3–0.85% dry weight (mean 0.4%) (Fig. 2C). Thus, WE-AX accounted for only 1.54–4.67% of TOT-AX (mean 2.38%). Similar values were reported for the 26 HEALTHGRAIN lines grown in multiple environments (12.1–22.6% TOT-AX, 0.27–0.92% WE-AX, Figure 3) (Gebruers et al. 2010) with WE-AX accounting for 1.87–3.41% of TOT-AX (mean 2.55%).

WE-AX ranged from 0.30% to 1.4% dry weight (mean 0.51%) and TOT-AX from 1.35% to 2.75% dry

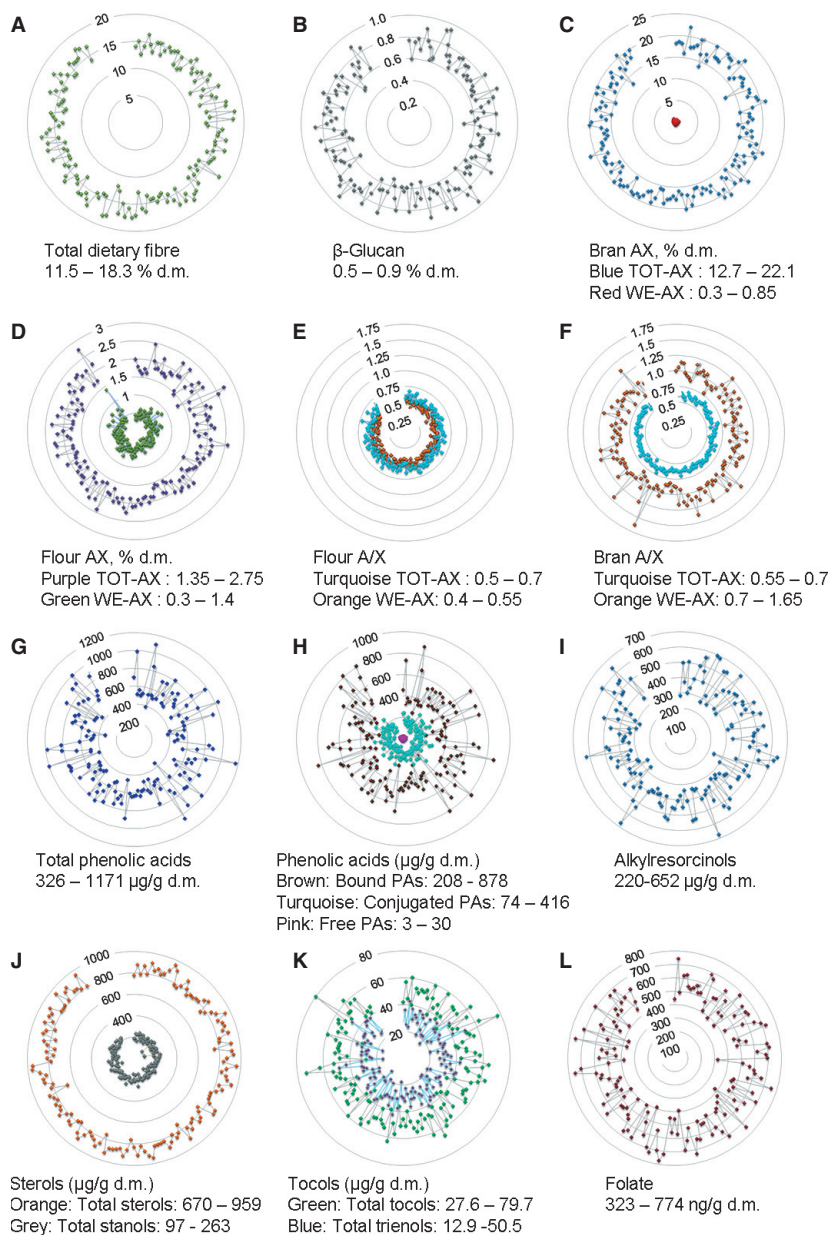


Figure 2. Radar plots showing levels of dietary fiber (% dry matter) and phytochemical components ($\mu\text{g/g}$ dry matter unless otherwise stated) in 150 wheat genotypes grown on a single site. (A) Total dietary fiber in wholemeal; (B) β -glucan in wholemeal; (C) bran AX (blue, TOT-AX; red, WE-AX); (D) flour AX (purple, TOT-AX; green, WE-AX); (E) ratio of A/X in flour (turquoise, TOT-AX; orange, WE-AX); (F) ratio of A/X in bran (turquoise, TOT-AX; orange, WE-AX); (G) total phenolic acids; (H) phenolic acid fractions (pink, free; turquoise, conjugated; brown, bound); (I) alkylresorcinols; (J) sterols (orange, total sterols; gray, total stanols); (K) tocots (green, total tocots; blue, total trienols); (L) folates (ng/g d.m.).

weight (mean 1.93%) in white flour of the 150 wheat lines (Gebruers et al. 2008) (Fig 2D). The proportion of WE-AX to TOT-AX in white flour of the 150 genotypes was therefore higher than that observed in the bran fraction, with WE-AX accounting for 16.08–50.51% (mean 26.59%) of TOT-AX. The corresponding values for the 26 lines grown in multiple environments were 0.24–1.03% dry weight (mean 0.53%) of WE-AX and 1.31–2.73% (mean 1.99%) TOT-AX (Gebruers et al. 2010), with WE-AX accounting for between 19.92% and 35.76% of TOT-AX (mean 26.75%). Ordaz-Ortiz et al. (2005) reported similar contents of WE-AX in flour of 20 wheat varieties (0.26–0.75% dry weight, mean 0.51%)

while the water-unextractable DF in the same samples varied from 0.88% to 1.52% dry weight (mean 1.15% dry weight).

Analysis of the data from the HEALTHGRAIN multi-site trials showed that the heritability was greater for flour AX than for bran AX, with genotype accounting for about 72% and 60% of the variation in TOT-AX and WE-AX, respectively, in flour and for 39% and 48% of the variation in TOT-AX and WE-AX, respectively, in bran (Shewry et al. 2010a) (Fig. 4).

Variation in the fine structure of AX also occurs, most notably in the degree of substitution with arabinose. AX comprises a backbone of β -D-xylopyranosyl residues

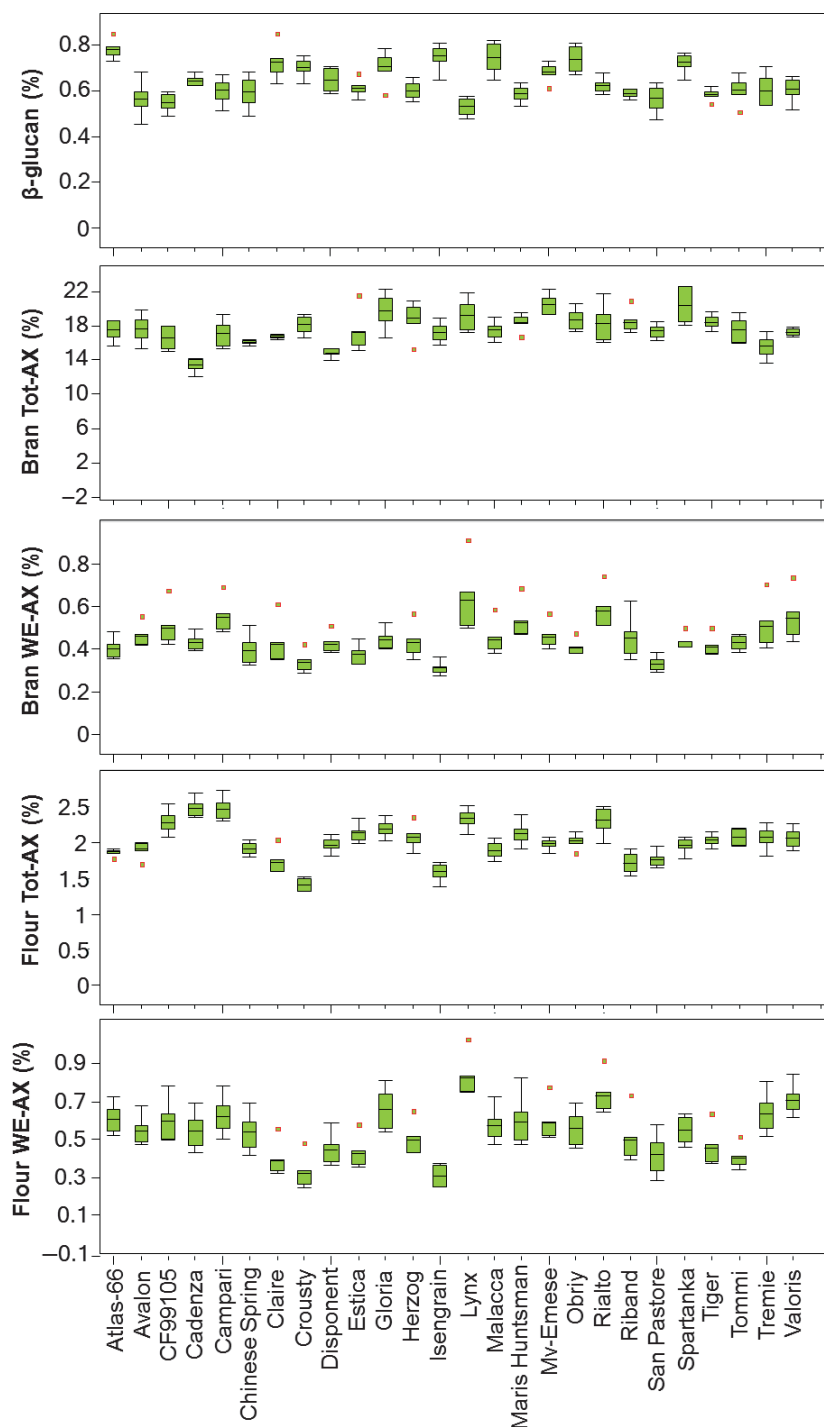


Figure 3. Box and whisker plots of contents of dietary fiber components in flour and bran of 26 wheat genotypes grown in six environments (Hungary, 2005–2007; France, Poland, UK in 2007). Boxes delineate the upper and lower quartile. Whiskers represent upper and lower values and means are represented by a solid line within boxes. Small squares represent statistical outliers.

linked through (1→4) glycosidic linkages with some residues being substituted with α -L-arabinofuranosyl residues at either position 3 or at positions 2 and 3. Substitution at position 2 only also occurs, but only rarely (Stone and Morell 2009). Variation in the overall degree of substitution can be determined as the ratio of arabinose to xylose released by hydrolysis. Analysis of flour from the 150

HEALTHGRAIN lines (Fig. 2E) showed that this ratio was higher in TOT-AX than in WE-AX, with ranges of 0.50–0.70 and 0.40–0.55 for TOT-AX and WE-AX, respectively. The A:X ratios were higher in bran fractions than in flour, but in this tissue the ratio in WE-AX was substantially higher than in TOT-AX (0.55–0.70 and 0.70–1.65, respectively) (Gebruers et al. 2008) (Fig. 2F).

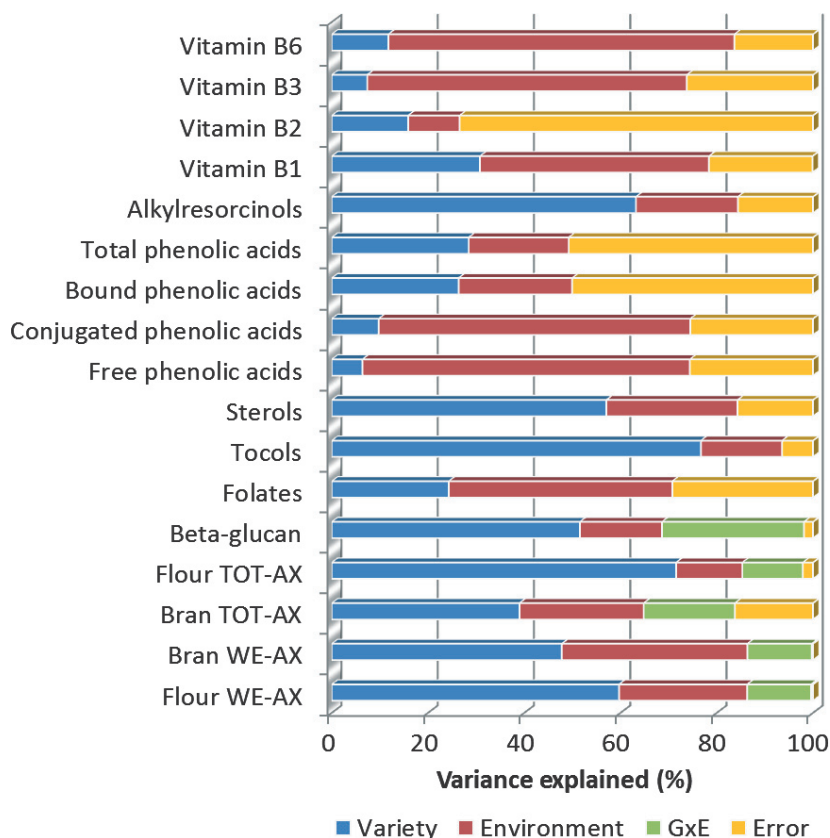


Figure 4. Variance components from heritability calculations of dietary fiber and phytochemical data. Blue, variety; red, environment; green, G × E (fiber components only); orange, error.

Variation in the proportions of xylose residues which are monosubstituted (at position 3) and disubstituted (at positions 2 and 3) with arabinose can also be determined by enzyme mapping or NMR spectroscopy. Digestion with a specific endoxylanase enzyme releases arabinoxylan oligosaccharides (AXOS) which range in size from xylose (X) and xylobiose (XX) to larger segments of the xylan backbone with arabinose substitution. These fragments can be separated by chromatography and analyzed by mass spectroscopy to determine their structures. Differences in the proportions of AXOS released from different lines can then be used as fingerprints to compare the structures of AX in the different lines. The analysis of the 150 HEALTHGRAIN wheat lines by enzyme mapping showed clear variation in the proportions of mono-substituted and disubstituted AXOS (Shewry et al. 2010b; Toole et al. 2011), but this variation was not related to the differences in the A:X ratios determined for the same grain samples by Gebruers et al. (2008).

Genetic analyses of WE-AX have been carried out, measuring either WE-AX or the viscosity of aqueous extracts of flour (which is largely determined by WE-AX). Martinant et al. (1998) determined WE-AX, extract viscosity and the ratio of arabinose:xylose in WE-AX in two

mapping populations of wheat and identified a major quantitative trait locus (QTL) for all three traits on chromosome 1B. This QTL explained 32–37% of the variation in extract viscosity and 35–42% of the variation in the A:X ratio. Quraishi et al. (2010) studied five additional crosses and identified 12 QTL. However, combining the data from the various crosses led to the identification of three meta-QTL for WE-AX viscosity located on chromosomes 1B, 3D, and 6B. The 1B meta-QTL corresponded to the QTL on 1B identified by Martinant et al. (1998) while Charmet et al. (2009) reported that the meta-QTL on 6B accounted for up to 59% of the variation in two of the populations.

Quraishi et al. (2010) also combined data from the crosses with association genetics analysis of the HEALTHGRAIN lines. This identified seven loci involved in WE-AX viscosity; three of which co-located with the meta-QTL located on chromosomes 1B, 3D, and 6B, and four additional loci on chromosomes 3A, 5B, 7A, and 7B.

The most significant locus identified by these studies was that on chromosome 1B and Quraishi et al. (2010) have shown that this region of the chromosome contains four genes which may contribute to the trait. They have also established molecular markers which are linked to

the loci determining WE-AX viscosity and hence potentially exploited in plant breeding programs.

AX fiber is probably the most important component of the wheat grain in terms of its contribution to human health. The wide genetic variation in amount and solubility and the high heritability, particularly in white flour, suggest it as an attractive target for breeders and the identification of major QTL is leading to the development of markers for use in plant breeding programs as well as identifying the genes that determine AX content. However, although the benefits of AX fiber are now widely accepted, we still know little about the precise relationships between AX structure and the various impacts on human health (which are enumerated above). These relationships will undoubtedly take some time to unravel, but such studies are necessary to identify precise targets for improving the health benefits of AX in the future.

Phenolic Compounds

Phenolic compounds are the most abundant phytochemicals in wheat grain, and the most structurally diverse. They have also been widely studied as the major group of compounds with antioxidant activity, although the biological significance of this activity in human health remains to be established. They include phenolic acids (PAs), alkylresorcinols (ARs), lignans, and flavonoids. Like DF, they are concentrated in the outer layers of the grain.

Phenolic acids

The most abundant are the PAs, which are aromatic rings with one or more hydroxyl groups. They are divided into two groups based on hydroxybenzoic acid (notably syringic and vanillic acids) and hydroxycinnamic acids (notably *p*-coumaric acid, ferulic acid, and sinapic acid). Furthermore, they occur in three forms within the grain: as free acids, as soluble conjugates with sugars, sterols, or terpenes, and bound to insoluble polymeric components (arabinoxylan, lignin, proteins). PAs exhibit antioxidant activity with strong correlations being reported between the content of total PAs and total antioxidant activity (So et al. 2002; Beta et al. 2005; Mpofu et al. 2006) and hence have been proposed to contribute to the health benefits of whole grain cereals.

Analysis of total PAs in wholemeal flours of the 150 HEALTHGRAIN lines showed wide variation, from 326 to 1171 $\mu\text{g/g}$ dry weight (mean 657 $\mu\text{g/g}$ dry weight) (Fig. 2G). Free PAs accounted for only 0.5–1% of the total, and soluble conjugated PAs for about 20%, but both varied widely in amount between lines: from 3 to 30 $\mu\text{g/g}$ dry weight for free and 76–416 $\mu\text{g/g}$ dry weight for conjugated components (Li et al. 2008) (Fig. 2H).

The major PA in the grain is ferulic acid, which accounted for about 80% of the bound PAs. The contents and composition of PAs determined in the HEALTHGRAIN study are consistent with those reported in other studies (Pussayanawin and Wetzel 1987; Rybka et al. 1993; Hatcher and Kruger 1997; Lempereur et al. 1997).

Ferulic acid is largely bound to the cell wall AX, to the 5 position of monosubstituted arabinose residues at position 3 of the xylan backbone. The extent of feruloylation of AX is low in the starchy endosperm cell walls, with ferulic acid estimated to account for 0.2–0.4% (w/w) of WE-AX and 0.6–0.9% (w/w) of WU-AX of wheat flour (Bonnin et al. 1998). The aleurone AX are more highly esterified and cross-linked than the AX in the starchy endosperm cell walls, with about 3.2% of the AX dry weight being ferulic acid and 0.45% diferulic acid (Antoine et al. 2003; Parker et al. 2005) with additional esterification with *p*-coumaric acid and acetyl groups also occurring (Rhodes et al. 2002; Antoine et al. 2004). The presence of bound PAs may well contribute to some of the reported health benefits. For example, it has been suggested that ferulic acid bound to AX is released by fermentation in the colon leading to specific health benefits (Vitaglione et al. 2008).

The high contents of PAs in wheat are clearly relevant to improving the health benefits. However, analysis of the 26 wheat lines grown on multiple sites (Fig. 5) (Fernandez-Orozco et al. 2010) showed that only a limited amount of this variation was heritable, about 28% for total PAs, and 6%, 10%, and 26% for the free, conjugated, and bound fractions, respectively (Fig. 4) (Shewry et al. 2010a). Hence it would be difficult to select for high contents of PAs in plant breeding programs. By contrast, the contents of free, conjugated, and total PAs showed strong correlations with the mean temperature between heading and grain harvest while the free and bound PAs showed negative correlations with total precipitation during the same period. Hence it may be possible to regulate the PA content by the selection of appropriate growth conditions.

Alkylresorcinols

Alkylresorcinols are phenolic lipids comprising a 1,3-dihydroxylated benzene ring with an alkane chain at position 5. A range of forms occur in wheat in which the alkane chain is usually saturated and comprises either 17, 19, 21, 23, or 25 carbons (Andersson et al. 2008). They are located in the outer layers of the grain (the nucellar epidermis, testa, and inner pericarp) (Landberg et al. 2008) and can be used as a biochemical marker for these tissues in milling fractions of wheat (Hemery et al. 2009). They have also been proposed as biomarkers for the consumption

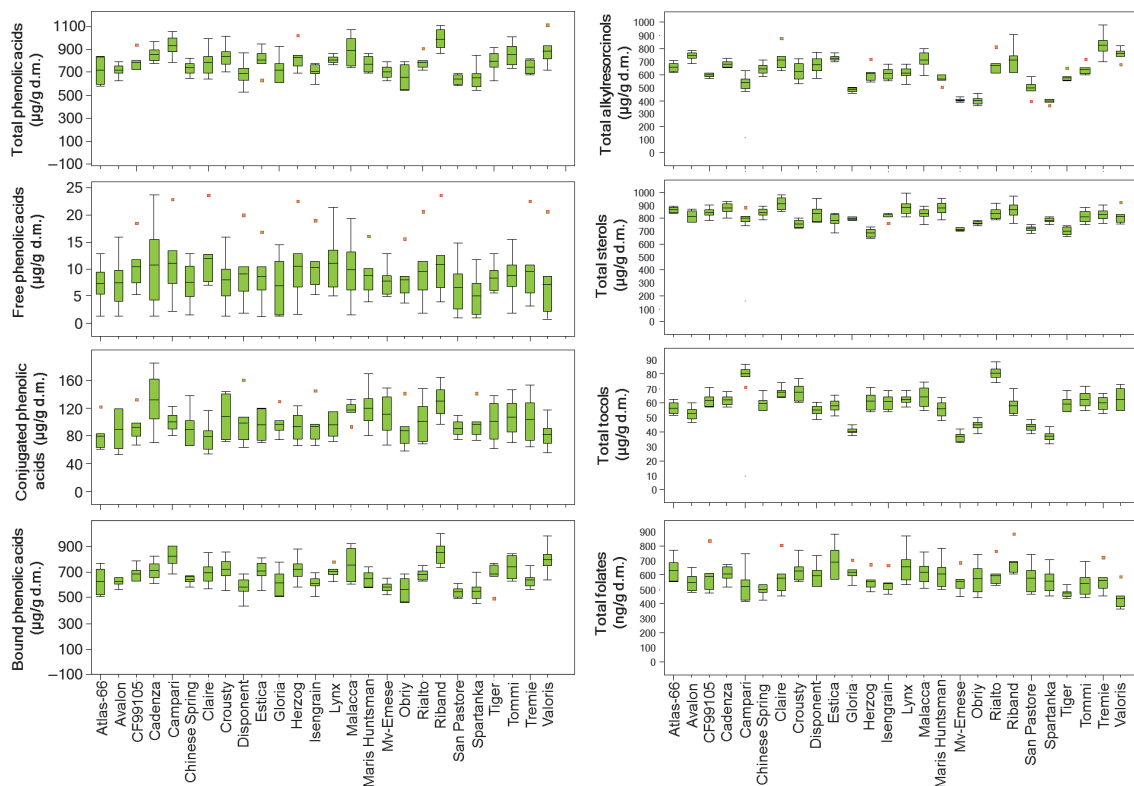


Figure 5. Box and whisker plots of contents of phytochemical components in wholemeals of 26 wheat genotypes grown in six environments (Hungary, 2005–2007; France, Poland, UK in 2007). Boxes delineate the upper and lower quartile. Whiskers represent upper and lower values and means are represented by a solid line within boxes. Small squares represent statistical outliers.

of wholegrain products in human nutrition studies and may also have specific health benefits (Ross et al. 2004). Andersson et al. (2008) showed that the total AR content in wholemeal flours of the 150 HEALTHGRAIN wheat lines ranged from 220 to 652 $\mu\text{g/g}$ dry weight (mean 431 $\mu\text{g/g}$ dry weight) (Fig. 2I). Furthermore, about 63% of the variation in the 26 lines grown on multiple sites was heritable (Fig. 4) (Andersson and Åman 2010; Shewry et al. 2010a), meaning that their amount should be amenable to selection by plant breeding.

The total contents of ARs determined in HEALTHGRAIN are very similar to those reported for Swedish (412 $\mu\text{g/g}$ dry weight) (Chen et al. 2004) and North American (300–700 $\mu\text{g/g}$ dry weight) wheats (Hengtrakul et al. 1990) but lower than reported in a previous study of Western European wheats (595–1429 $\mu\text{g/g}$ dry weight) (Ross et al. 2003).

Terpenoids

Terpenoids are derived from five carbon isoprene units and are the second most abundant class of phytochemicals in wheat grain. Two groups have been studied

in detail – the sterols and tocopherols. A third group, the carotenoids, are present in lower amounts but are of particular interest in durum wheat as lutein is the major determinant of the yellow color which is a quality trait for breeders and consumers (Troccoli et al. 2000).

Sterols

Plant sterols are steroid alcohols, comprising a tetracyclic cyclopenta[α]phenanthrene ring with a hydroxyl group at the C4 position and a flexible side chain at the C17 carbon position. They are divided into three types – the 4-desmethyl sterols which are the major components in plant tissues and the quantitatively minor 4α -monomethyl sterols and 4,4-dimethyl sterols which are precursors of the 4-desmethyl sterols. The major plant 4-desmethyl sterols have a Δ^5 double bond in the B ring and modifications at the C24 position in the side chain (Piironen et al. 2000). Cereals also contain significant amounts of saturated sterols, which are called stanols. Plant sterols and stanols have well-established cholesterol-lowering effects (Kritchevsky and Chen 2005) and are incorporated into many spreads and other food products.

Analyses of wholemeal flours from the 150 HEALTHGRAIN lines showed a relatively narrow range of variation in total content of sterols (including stanols) from 670 to 959 $\mu\text{g/g}$ dry weight (mean 844 $\mu\text{g/g}$ dry weight) (Fig. 2J). Within the total sterol fraction a wider range was observed in the amount of stanols, from 97 to 263 $\mu\text{g/g}$ dry weight (2.71-fold) compared to the major sterol components sitosterol (342–530 $\mu\text{g/g}$ dry weight, 1.55-fold) and campesterol (95–169 $\mu\text{g/g}$ dry weight, 1.78-fold) (Nurmi et al. 2008). This high stability is consistent with the essential role of sterols in membrane structure. Similar contents of total phytosterols have also been reported in several previous studies of wheat (Weihrauch and Gardner 1978; Piironen et al. 2002; Ruibal-Mendieta et al. 2004; Nyström et al. 2007). Analysis of the 26 HEALTHGRAIN lines grown on multiple sites also showed high heritability of total sterol content, with genotype accounting for 57% of the variation (Fig. 4) (Nurmi et al. 2010; Shewry et al. 2010a).

Tocols

Tocols comprise a chromanol ring with a C16 phytol side chain and are classified into two types in which the side chain is either saturated (tocopherols, T) or contains three double bonds at carbons 3, 7, and 11 (tocotrienols, T-3). Each type exists in four forms, which differ in the positions of methyl groups on the chromanol ring and are called α (5,7,8-trimethyl), β (5,8-dimethyl), γ (7,8-dimethyl), and δ (8-methyl). Although the name “vitamin E” is often applied to all tocols, they differ in their biological activity, and current dietary recommendations for vitamin E intake in North America and Nordic countries recognize only forms of α -tocopherol. However, other forms of tocopherol and tocotrienols may have other biological activities, as reviewed by Piironen et al. (2009). The tocols have strong antioxidant properties which may be responsible for some of their health benefits (Aziz and Stocker 2000; Pfluger et al. 2004).

The total tocol content of wholemeal flours of the 150 HEALTHGRAIN lines ranged from 27.6 to 79.7 $\mu\text{g/g}$ dry weight, with a mean of 49.8 $\mu\text{g/g}$ dry weight (Lampi et al. 2008) (Fig. 2K). The major forms in the lines were α -tocopherol (9.1–19.9 $\mu\text{g/g}$ dry weight) and β -tocotrienol (10–44 $\mu\text{g/g}$ dry weight), with lower contents of α -tocopherol (3.3–13.3 $\mu\text{g/g}$ dry weight) and α -tocotrienol (2.5–7.6 $\mu\text{g/g}$ dry weight). The proportion of tocotrienols (saturated forms) ranged from 40.3% to 71.4% (12.9–50.5 $\mu\text{g/g}$ dry weight) of the total tocols. Analysis of the 26 lines (Fig. 5) grown in multisite trials showed that the content of tocol was the most highly heritable of all of the bioactive components that were studied, with genotype accounting for 76% of the total variation (Fig. 4)

(Lampi et al. 2010; Shewry et al. 2010a). The total contents and compositions of tocols determined in HEALTHGRAIN were consistent with those of previous studies (Panfili et al. 2003; Ruibal-Mendieta et al. 2005; Hidalgo et al. 2006).

B Vitamins

The B vitamin complex comprises eight water-soluble components which often occur together in the same foods and were initially considered to be a single component. Wheat, and in particular, wholegrain, is a source of several B vitamins, notably thiamine (B1), riboflavin (B2), niacin (B3), pyridoxine (B6), and folate (B9). Bread and bread products have been reported to account for about 17–18% of the total intake of thiamine, about 11% of the total intake of riboflavin, and 11% of the total intake of niacin in adults in the USA, with fortified ready-to-eat cereals providing an additional 10% in all three cases (National Research Council 1998). Fortified ready-to-eat cereals and other grain products also account for 15% of the dietary intake of pyridoxine (National Research Council 1998).

All B vitamins are concentrated in the bran and/or germ, and commercial milling removes about 68% of the total thiamine, 58–65% of the riboflavin, and 85% of the pyridoxine (Keagy et al. 1980). Earlier work reviewed by MacMasters et al. (1971) showed that 32% and 64% of thiamine is present in the aleurone and embryo (including scutellum), respectively, 37% and 26% of riboflavin, 82% and 2% of niacin, and 61% and 21% of pyridoxine. The contents of these four vitamins in the starchy endosperm were only about 3%, 32%, 12% and 6%, respectively, of those present in the wholegrain (MacMasters et al. 1971; Betschart 1988). The remaining 1% of total thiamine, 5% of riboflavin, 4% of niacin, and 12% of pyridoxine were present in the pericarp and testa. Consequently, the consumption of wholegrain cereal products as opposed to products made with white flour results in significant increases in the intakes of all of these important vitamins.

The beneficial effects of folates (vitamin B9) in the prevention of neural tube defects and several other diseases are well documented (Molloy 2002; Buttriss 2004). Folate occurs in a number of forms, called vitamers, and Piironen et al. (2008) showed that the combined contents of these in the 150 HEALTHGRAIN lines ranged from 323 to 774 ng/g dry weight (mean 560 ng/g dry weight) (Fig. 2L). However, the heritability in the 26 lines grown in multiple environments was low, just 24% (Fig. 4) (Shewry et al. 2010a). The total folate contents determined in HEALTHGRAIN were within the ranges reported in several previous studies (Davis et al. 1984; Håkansson et al. 1987; Müller 1993; Gujska and Kuncewicz 2005).

More recently, the 26 HEATHGRAIN wheat lines grown on four sites in 2007 were analyzed for four other B vitamins: thiamine (vitamin B1), riboflavin (vitamin B2), pyridoxine (vitamin B6), and the biologically available forms of niacin (vitamin B3) (Fig. 6). The contents of thiamine (5.53–13.55 $\mu\text{g/g}$ dry weight), riboflavin (0.77–1.40 $\mu\text{g/g}$ dry weight), and pyridoxine (1.27–2.97 $\mu\text{g/g}$ dry weight) were within the ranges reported previously. The content of the bioavailable forms of vitamin B3 (0.16–1.74 $\mu\text{g/g}$ dry weight) was about 10–15% of the total contents of vitamin niacin reported in previous studies. Strong correlations were observed between the contents of thiamine, niacine, and pyridoxine, and partitioning of the variance in the contents of these three B vitamins showed that between 48% and 70% was accounted for by the

environment and only 30%, 7%, and 12%, respectively, by the genotype (Shewry et al. 2011). By contrast, the content of vitamin riboflavin was not correlated with the contents of other B vitamins and 73% of the variance was ascribed to the error term (which includes genotype \times environment interactions) with 11% ascribed to environment and 16% to genotype.

These studies indicate that it may not be realistic to use plant breeding to increase the contents of B vitamins in wheat grain. Genetic engineering has been used to increase the contents of folates (vitamin B9) in tomato fruit (De la Garza et al. 2004) and rice (Storozhenko et al. 2007), and a similar approach could be used for wheat. However, this approach is unlikely to be used for the other B vitamins, at least in the short term, as their

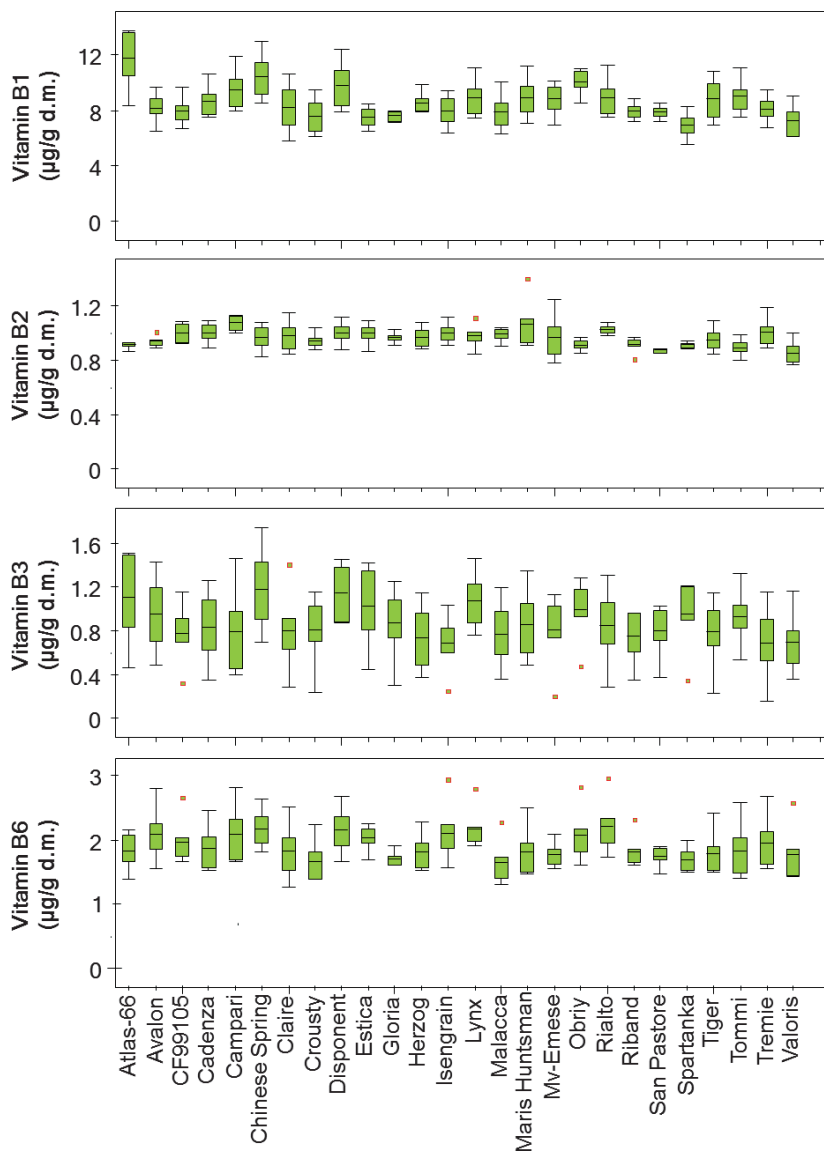


Figure 6. Box and whisker plots of contents of B vitamins in wholemeals of 26 wheat genotypes grown in four environments (Hungary, France, Poland, UK in 2007). Boxes delineate the upper and lower quartile. Whiskers represent upper and lower values and means are represented by a solid line within boxes. Small squares represent statistical outliers.

biosynthetic pathways are complex and their genetic control and regulation are not well understood (Webb et al. 2007; Begley et al. 2008).

Discussion

Diet and lifestyle are important determinants of health and quality of life in all countries, and are particularly important in relation to the epidemic increases in the incidence of the metabolic syndrome and related diseases that are occurring in many developed and rapidly developing countries. Health agencies in many countries promote the benefits of a balanced diet, including a significant proportion of fruit and vegetables. For example, the “Eatwell Plate” promoted by the UK Food Standards Agency suggests that fruit and vegetables should account for about a third of the total dietary intake with starchy foods (bread, rice, potatoes, and pasta) accounting for about another third (www.food.gov.uk/multimedia/pdfs/publication/eatwellplate0210.pdf). However, such representations frequently under-emphasize the role of cereals in providing health benefits as well as calories, and in particular the importance of whole grain products.

The fact that wheat, rice, and other cereals are staple foods means that they are ideal vehicles to deliver health benefits to large populations at relatively low cost. A major limitation at present is that the beneficial components are concentrated in the bran and germ which are removed by milling, and that whole grain products are less acceptable to many consumers and may have higher production costs. However, these do not need to be major barriers: increased consumer awareness and greater availability are already leading to increased consumption of whole grain products in many countries, while innovative processing can be used to improve the acceptability and reduce the production costs. The studies discussed here show that the health benefits of wheat can also be increased by genetic improvement of the wheat grain, by exploiting natural variation or by non-traditional approaches such as mutagenesis or transgenesis. This combination of genetic improvement, increased consumer education and awareness, and increased availability of low cost products should therefore contribute to improved health outcomes.

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