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Defining the basis for variation in water absorption of UK wheat flours

Peter R Shewry[1], Abigail J Wood,[1] Kirsty Hassall,[1] Liz Howes[2], Mervin Poole[2], Paola Tosi[3] and Alison Lovegrove[1]

[1]Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK
[2]Heygates Ltd., Bugbrooke Mills, Northampton NN7 3QH, UK
[3]School of Agriculture, Policy and Development, University of Reading, Whiteknights Campus, Early Gate, RG6 7AR, UK.

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CONTENTS

1. Abstract

The water absorption (WA) of wheat flour is a major factor that affects bread-making performance. The milling procedure is modified to achieve the required WA level. However, it is difficult to achieve this level with UK wheat in some years.

The study aimed to:

1. Identify factors that affect the WA of UK-grown wheat. This included a comparison of wheat grown in years with typical (2016 and 2018) and atypically low (2013 and 2017) WA levels.
2. Determine whether variation in fibre composition and properties contributed to variation in WA.
3. Determine whether variation in nitrogen fertilisation contributed to differences in WA.

The amounts and compositions of a range of components, including starch, protein and fibre components (including pentosan fractions), in white flour were determined. Cultivars/lines in the study included those used in the Defra-funded Wheat Genetic Improvement Network (WGIN) and lines developed specifically to contain different amounts of pentosan (arabinoxylan) fibre in a common genetic background.

The Farrand Equation (based on protein, starch damage and moisture content) is widely used to predict WA. This project used statistical analysis to determine whether the addition of specific traits could improve the predictions, compared to the baseline model.

Analysis of wheat datasets from 2013, 2016, 2017 and 2018 showed that the baseline model predicted 74.9% of the variance in WA. By adding Principal Components that reflected variation in grain fibre content, the prediction increased to 90.0%. The fibre fraction that gave the greatest improvement (from 74.9% to 84.2%) was β-glucan. Analysis of the lines that varied in pentosan content showed that the prediction of WA by the baseline model (86%) was increased by the addition of data for traits related to water-soluble pentosan: to 94.2% by Relative Viscosity of aqueous extracts and to 96.7% by water-extractable arabinoxylan.

Comparison of lines grown with 100kg N/Ha and 200kg N/Ha showed no effects beyond those related to grain protein content (and, therefore, already allowed for in the baseline equation).

The results show that variation in the content of fibre components, particularly soluble fibre, may account for variation in WA between cultivars and between samples grown in different environments.

2. Introduction

Low water absorption (WA) of wheat flour sourced from UK crops drives the miller to supplement their grist with foreign wheat because of the impacts on baking yield, energy usage, mill wear and customer satisfaction. Low WA was identified in 2010, 2013, 2015 and 2017 UK crops but had not been previously recorded. Irrespective of the cause, it poses a serious threat to the market for home grown wheat. Consequently, there is an urgent need to identify the factors that determine WA and how their impact relates to differences between cultivars and growth conditions.

The water absorption of wheat flour, determined using either a Farinograph or DoughLAB (CCAT method no. 04), is a crucial test which allows bakers to optimise the mixing conditions for baking. Our current understanding of the contributions of grain components to WA was established in the 1950s. WA is determined by the amounts and compositions of three major grain components: starch, protein and cell wall polysaccharides (fibre).

Starch accounts for about 80% of white flour and hence is considered to have the greatest impact on WA. Intact granules absorb about 0.5-times their dry weight of water, which increases to 3-4-fold when damaged (Kent and Evers, 1994). Starch comprises two polymers, amylose and amylopectin, which generally account for about 30% and 70% of the total., respectively. Amylose and amylopectin have different WA capacity, with the swelling power of starch decreasing with increasing amylose content (Sasaki and Matsuki, 1998). Wheat starch granules show a bimodal size distribution, with large (10-40 μm) lenticular A granules and small (<10μm) spherical B granules enriched in amylose and amylopectin, respectively. Higher temperatures during grain filling lead to an increase in the proportion of A granules, while water stress significantly decreases the number of B granules and reduces the size of the largest A granules (Viswanathan and Khanna-Chopra, 2001; Brooks et al., 1982). The WA of starch will, therefore, be a function of the granule size distribution and granule composition, which are in turn affected by cultivar and environment.

Protein may affect WA due to differences in amount, composition and packing in the grain. Bread making flours all contain between 11-12% protein; therefore, variation in protein amount is unlikely to account for differences in WA. In general., proteins absorb approximately 1.8 times their dry weight of water, but this may vary between proteins and differences in flour protein composition. In particular, the ratio of the gluten to soluble proteins, could potentially affect WA capacity (Rakszegi et al., 2014). Differences may also occur in the physical packing of the protein around the starch granules, giving rise to a vitreous or floury texture. Vitreousness is greater with high grain nitrogen and high growth temperatures (Kindred et al., 2008), but the physical chemical basis for this trait is not understood.

Finally, cell wall polysaccharides account for only 2-3% of flour, with arabinoxylans (AX, often called pentosans) accounting for about 70% of this total (Mares and Stone, 1973). However, the total

amount of AX varies from 1.35% to 2.75% (dry weight) between cultivars, and the water-soluble fraction from 0.3% to 1.4% (dry weight) (Gebruers et al., 2008)). Pentosans have a very high water-holding capacity, about 10-times their dry weight for the water-insoluble fraction and 11-times their dry weight for the water-soluble faction (Guzmán et al., 2015; Finnie and Atwell, 2016). About 70% of the variation in total pentosans and 60% of the variation in soluble pentosans is determined by the cultivar, with smaller effects of the environment. In particular, the proportion of soluble pentosans is higher under cool wet conditions (Shewry et al., 2010). Several early studies examined the prediction of WA by protein and starch damage and concluded that pentosans did not contribute to its variation (Dodds, 1972; Belderok, 1973; Greer & Stewart, 1959; Tipples et al., 1978). This is reflected in the widely used Farrand equation (Farrand, 1969) which predicts WA based on protein, starch damage and moisture content:

WA = 68.26 + (0.878 x protein) + (0.334 x damaged starch) - (1.97 x moisture).

By contrast, Stevens and Stewart (1982) concluded that 14% of the 48% of unaccounted variability could be explained by soluble pentosans. In a separate study, the addition of pentosanase (purified 1-4, β-xylanase) had a negative impact on both dough rheology and baking quality (McCleary et al., 1986). The second major type of fibre in wheat flour is β-glucan, which accounts for about 20% of the total cell wall polysaccharides (Mares and Stone, 1973). B-glucan has not been studied in detail from wheat flour but has been studied in barley and oats where it is the major cell wall polysaccharide and forms highly viscous solutions (Lazaridou and Biliaderis, 2007). Finally, white flour contains up to 0.4 % dry weight of arabinogalactan-peptide (AGP) (Loosveld et al.,1997;1998) which comprises a 15-residue amino acid peptide (Van den Bulck et al., 2002) including three hydroxyprolines which are *o*-glycosylated with branched arabinogalactan chains (Tryfona et al., 2010). AGP is not a cell wall component and most is extracted in water (Wilkinson et al., 2017). β-glucan and AGP could also contribute to WA and we therefore determined the amounts of both in the study.

For most grain samples the dominant role of starch damage in determining WA means that millers can readily achieve the degree of WA required by bakers. However, although the miller can grind the wheat from anomalous years (such as 2010, 2013 and 2015, 2017) harder to increase starch damage, and therefore increase water absorption, this approach is limited by the higher energy cost and damage to the surface of mill rolls. Because UK bakers are not limited to sourcing their flour from UK millers, the economics of milling means that millers will supplement their grist with wheat from beyond the domestic market to maintain flour production that meets customer costs and specifications. A better understanding of what contributes to WA will, therefore, provide routes to manage the domestic crop through improved agronomy, breeding or processing and consequently lead to a more consistent demand for the domestic crop by UK millers.

We, therefore, determined the contributions of starch, protein, pentosans, β-glucan and AGP to differences in the WA of grain samples, including elite cultivars grown in years with typical (2016,

2018) and low (2013, 2017) WA. Furthermore, to explore the specific role of pentosans we compared a series of lines with genetically determined differences in pentosan content of white flour. These studies have provided new information on the role of pentosans and other components in determining WA of wheat flour, both in typical and low WA years.

3. Materials and methods

3.1. Field trials

Modern elite UK wheat cultivars were grown on the experimental farm at Rothamsted Research, Harpenden, UK in 2013, 2016, 2017 and 2018 as part of the Wheat Genetic Improvement Network (WGIN) (Barraclough et al., 2010.). Cultivars were grown in triplicate 9 x 3 m plots at two levels of nitrogen fertilization, 100 and 200 kg/Ha, using ammonium nitrate. Plots were randomized within main plots of the nitrogen treatments.

Thirteen Yumai 34 x Valoris DH lines and the parents were grown at Rothamsted in 2018, in 9 x 1.8 m plots with 200 kg N/ha.

3.2. Milling and determination of WA and starch damage

Milling was carried out using a Brabender MLU 202 test mill and MLU 302 impact finisher according to established UK-industry test milling requirements. WA was determined by Farinograph (CCAT method no.4). Starch damage was determined using the Chopin SDmatic (CCAT method no. 24) (which gives greater within-laboratory precision than Farrand methods) for the 2017 and 2018 samples and by NIRS for all samples. Protein was determined by Dumas combustion for the 2017 and 2018 samples (ISO/TS 16634-2:2009) and by NIRS for 2013 and 2016 samples.

For 2013 and 2016, grain from the triplicate plots was combined for each variety prior to milling. In 2017 and 2018, grain from individual plots was milled and analysed separately.

3.3. Analysis of arabinoxylans (pentosans) and β-glucan (dietary fibre components)

For determination of water-extractable (WE) AX and total (TOT) AX, monosaccharide analysis following mild acid hydrolysis was as described by Bromley et al., 2013 with samples analysed in triplicate. Relative viscosity of water extracts was determined using the method of Freeman et al. (2016). Analysis of arabinoxylan oligosaccharides (AXOS) and β-glucan was conducted as described by Freeman et al. (2017) with minor modifications, flours were digested with recombinant endo-1,4-xylanase (PRO-E0062) and lichenase (a glucan-hydrolase) (PRO-E0017) (Prozomix). The oligosaccharides were separated by HPAEC and peak areas were expressed relative to a Melibiose internal standard. Samples were analysed in duplicate.

3.4. Determination of grain hardness and vitreousness

Grain hardness was determined using the Perten Single Kernel Characterization System (SKCS) 4100 (Perten, IL, United States) on samples of 100 grains (for 2013 and 2016) and 300 grains (for 2017 and 2018 and for the Yumai 34 x Valoris DH lines) harvested from each plot. A total of 300 grains were analysed for each sample given in Appendix 1. Vitreousness was determined by visual examination and scoring and expressed as % vitreouness (ICC 129 Method, 1980). Grain was cut transversally using a single edge blade and placed in a 96-well plate for visual scoring (33 kernels were cut for each plot), a total 7722 kernels were scored.

3.5. Solvent retention capacity (SRC)

Solvent retention capacity (SRC) was determined according to AACC Method 56-11.02 (AACC International., 2009). SRC is the weight of solvent held by flour after centrifugation, expressed as percent of flour weight, on a 14% moisture basis. Four solvents: water, 50% sucrose, 5% sodium carbonate and 5% lactic acid, were independently used to produce four SRC values for samples from 2013 and 2016. Only 50% sucrose SRC was determined for 2017-2018 samples.

3.6. Determination of amylose in starch

Amylose content of flours (% total starch) was determined using the commercial Megazyme Amylose/Amylopectin K-AMYL kit (Megazyme Ltd, Bray, Ireland). Analyses were carried out on 25mg of flour, following manufacturer's specifications.

3.7. Determination of protein fractions

The ratio of gliadins to total gluten proteins in each flour sample was determined by extracting and quantifying the 50% (v/v) propan-1-ol-soluble protein under non-reducing and reducing conditions, respectively.

100mg aliquots of flour of each sample were extracted, independently, with 2ml 50% (v/v) propan-1-ol or 2ml 50% (v/v) propan-1-ol, 25mM Tris pH 6,8, 2% dithiothreitol (DTT). Extraction was carried out at room temperature for 20 min with constant shaking, following which samples were centrifuged at 6500x g for 10 min. The supernatant was retained and the protein content quantified using the Direct Detect® Infrared Spectrometer (Merk, Damstadt, Germany) following manufacturers' instructions.

3.8. Statistical analysis/modelling

3.8.1. For the WGIN samples, linear mixed models were fitted to explain variation in water absorption (response). A random effect for Year was included in all models. A baseline model was defined to include all variables of the Farrand equation (Starch damage, Protein and Flour moisture). This

baseline model was compared to models including these baseline variables and a single dietary fibre component (of which there were 13), and to a model incorporating all 13 dietary fibre components. To avoid issues of collinearity, the 13 dietary fibre components were first processed through a principal component analysis based on the correlation matrix generated, resulting in up to 10 Principle Components (PCs), accounting for 99% of the variation, to be included in the linear mixed model. Models were then compared through an approximation to the percentage variance accounted for, calculated as the percentage change in the sum of the variance components for each model in order to determine which components and which model provided the best fit for the differences observed in WA.

3.8.2. For the Yumai 34 x Valoris samples: Models were fitted in the same process as above but in a linear regression framework. Given the smaller sample size, only 7 PCs, accounting for 99% of the variation, were considered for the full fibre model. Models were compared using the adjusted R^2 value, a measure of the percentage variance accounted for by the model.

4. Results

4.1. Comparison of elite cultivars grown in years with typical (2016,2018) and atypical (low) (2013, 2017) water absorption (WA).

Grain samples grown in four years were compared, comprising two years in which WA was considered by the UK milling industry to be typical (2016, 2018) and two in which WA was considered to be atypically low (2013, 2017). Samples were obtained from the WGIN variety trials, from large plots with 200 kg N/Ha. A total of 16 cultivars of different nabim Groups were analysed (Table 1). Archived material was used from 2013 and 2016, with samples from three replicate plots combined for milling. Biological replicates were combined in these years for technical reasons as the equipment used for milling and WA measurements (Brabender and Farinograph, the widely recognised industry standard) required a minimum sample size of 300g. Fresh grain samples were used in 2017 and 2018 and replicate plots milled separately (Table 2).

The WA of the samples is shown in Figures 1 and 2. The mean WA in 2017 was clearly lower than that in the other years, confirming that this year was “atypical”. However, it is interesting to note that the range of WAs overlapped with the range for 2018. By contrast, the means for 2013 and 2016 were similar to that for 2018.

Table 1. WGIN cultivars used for analysis with nabim Group, endosperm texture classification and year of release.

Variety	nabim Group	Classification	Year of Release
Crusoe	1	Hard	2012
Gallant	1	Hard	2009
Hereward	1	Hard	1989
Malacca	1	Hard	1997
Solstice	1	Hard	2002
Xi19	1	Hard	2002
Cadenza	2	Hard	1992
Cordiale	2	Hard	2000
Soissons	2	Hard	1987
Riband	3	Soft	1987
Robigus	3	Soft	2003
Claire	3	Soft	1999
Conqueror	4	Hard	2009
Istabraq	4	Soft	2004
Hereford	4	Hard	2007
Mercia	1	Hard	1985

Table 2. Grain samples used for analysis, showing numbers of replicate samples milled each year.

	2013	2016	2017	2018
Cadenza	1	1	2	3
Claire	1	1	3	3
Conqueror	1	1	3	3
Cordiale	1	1	2	3
Crusoe	1	1	3	3
Gallant	1	1	3	0
Hereford	1	1	3	3
Hereward	1	1	3	3
Istabraq	1	1	3	3
Malacca	1	1	3	3
Mercia	1	1	3	3
Riband	1	1	3	3
Robigus	1	1	2	2
Soissons	1	1	3*	3
Solstice	1	1	3	3
Xi19	1	1	3	3

*Sample mixed with cv Solstice during harvest

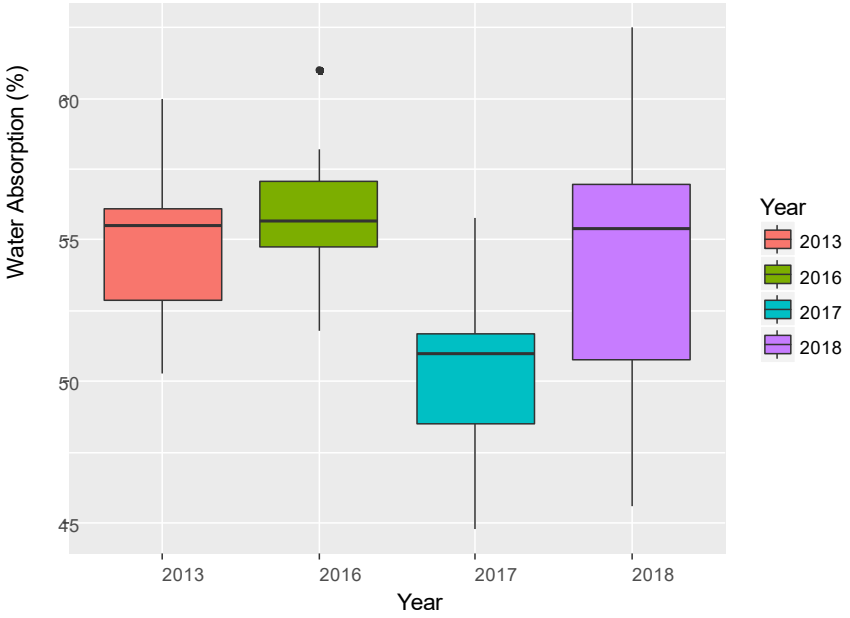

Figure 1. Boxplots for water absorption of each sample determined for flours from the four years**.** A reduced variance is seen in 2013 and 2016 due to the pooling of biological replicates.

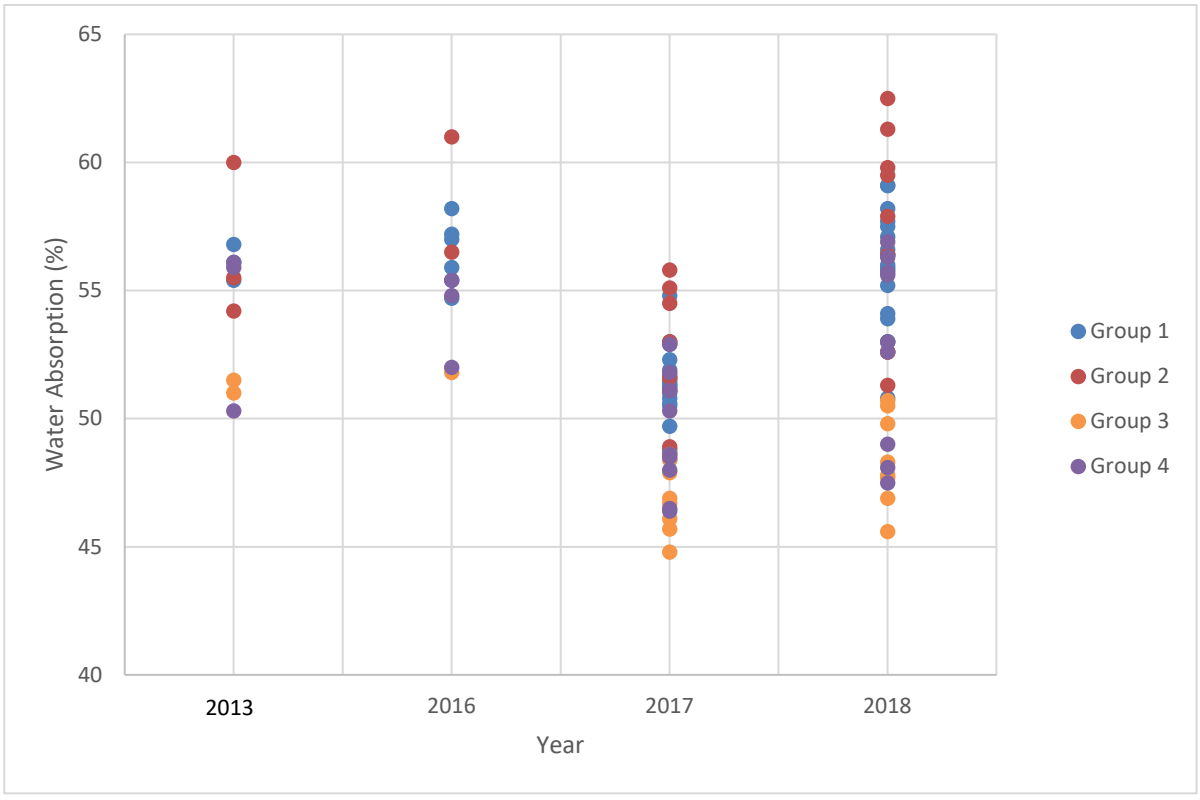

Figure 2. Water absorption of the individual flour samples from the four years. Coloured based on nabim group**.**

The flours were analysed for a range of components and properties, as summarised in Table 3. The components analysed were selected based on their potential (known or hypothesised) to influence water absorption. These included starch damage, protein content, flour moisture content (known WA components) and a number of measurements specifically related to fibre content and composition. Soluble (water-extractable) arabinoxylan (WE-AX) and total AX (TOT-AX) were determined by monosaccharide analysis and are expressed as xylose units (to avoid the requirement to correct arabinose values for other non-starch polysaccharide components that also contain arabinose). TOT-AX and β-glucan were also determined by enzyme fingerprinting (expressed in arbitrary units). This analysis also allowed the ratio of these two major fibre components to be calculated in addition to providing information on β-glucan structure (expressed as the ratio of oligosaccharides comprising three glucose (G3) and four glucose (G4) units released by digestion).

The SRC test (Slade and Levine, 1994) determines the ability of flour to retain a set of four solvents (water, 50% sucrose, 5% sodium carbonate, 5% lactic acid). These solvents are preferentially absorbed by one or more of the major grain components, with 50% sucrose being preferentially absorbed by pentosans (and thus provides a measure of arabinoxylan) (Gaines, 2004). The sucrose SRC was therefore determined for all samples. Finally, arabinogalactan peptide (AGP) was determined by monosaccharide analysis and expressed as galactose units.
The full datasets for the samples are given in Appendix 1.

Table 3. Traits measured in all flour samples.

Type of trait	Trait	Analytical method	Shorthand in Tables and Figures
Starch	% amylose	Megazyme K-AMYL kit	% amylose
	Starch damage (NIR)	NIR	Starch damage
Protein	% protein	NIR Protein 'as is' in 2013 and 2016 samples Dumas combustion of Flour protein (Nx5.7) 'as is' in 2017 and 2018 samples	Protein
	Gliadin as % total gluten protein	Direct Detect® Infrared Spectrometer	% gliadin
Water	% flour moisture	NIR	Flour moisture
Texture	Hardness	SKCS-Perten	Hardness
	Vitreousness	Visual scoring following sectioning (%)	Vitreousness
Fibre	Water-extractable arabinoxylan (WE-AX)	HPAEC of monosaccharides following mild acid hydrolysis, expressed as xylose	WE-(A)X
	Arabinose:xylose ratio in WE-AX	HPAEC of monosaccharides following mild acid hydrolysis	WE-A:X
	Relative viscosity	Capillary viscometry of aqueous extracts	RV
	Sucrose solvent retention capacity	ACCI Method 56-11.02	Sucrose SRC

	Total arabinose in AX	HPAEC of monosaccharides following mild acid hydrolysis expressed, as arabinose (adjusted for AGP)	TOT-A(X)
	Total arabinoxylan (AX)	HPAEC of monosaccharides following mild acid hydrolysis expressed as xylose	TOT-(A)X
	Arabinose:xylose ratio in TOT-AX	HPAEC of monosaccharides following mild acid hydrolysis.	TO-A:X
	Ratio of WE-AX:TOT-AX	HPAEC of WE and TOT monosaccharides following mild acid hydrolysis expressed as xylose	WE:TOT (A)X
	Total β-glucan (BG)	Enzymatic fingerprinting expressed in arbitrary units	TOT-BG
	β-glucan structure	Ratio of G3:G4 gluco-oligosaccharides released by enzyme digestion of β-glucan	G3:G4 BG
	Ratio of AX: β-glucan	Ratio of AX: β-glucan from enzymatic fingerprinting	AX:BG
	Soluble arabinogalactose peptide (AGP)	WE- AGP from monosaccharide analysis expressed as galactose	WE-G
	Total arabinogalactose peptide (AGP)	TOT-AGP from monosaccharide analysis expressed as galactose	TOT-G

4.1.1. Correlations and multivariate analysis of parameters measured in grain samples grown in four years

The Farrand equation (Farrand, 1969) is widely used to predict WA and is based on the determination of starch damage, protein content and moisture content. This equation was therefore used as the basis for exploring the effects of these and other grain components, using regression analysis. However, in order to carry out regression analysis it is important to ensure that the traits are not correlated. Correlations between the individual parameters were therefore initially determined and are shown as heat maps in Figures 3 and 4. Figure 3 shows strong correlations between starch damage, hardness and vitreousness. Because starch damage, hardness and vitreousness all behave similarly only one of these three traits needs be included as an explanatory variable in water absorption. To be consistent with the Farrand equation, starch damage was chosen. Similarly, correlations are also observed between fibre components, notable water-extractable (WE) xylose (used as a proxy for arabinoxylan) and the relative viscosity of aqueous extracts (Figure 4).

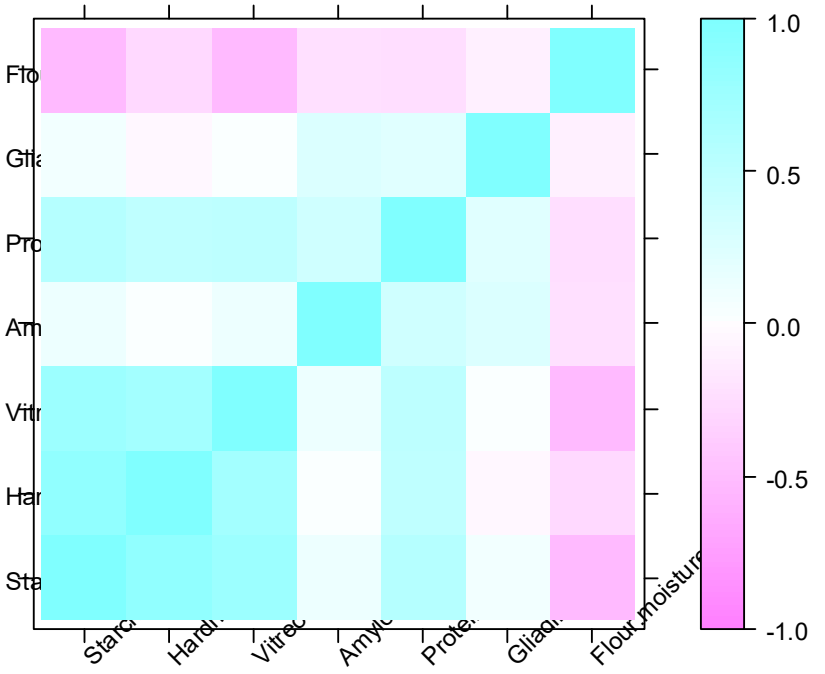

Figure 3. Heat map of correlations between grain parameters.

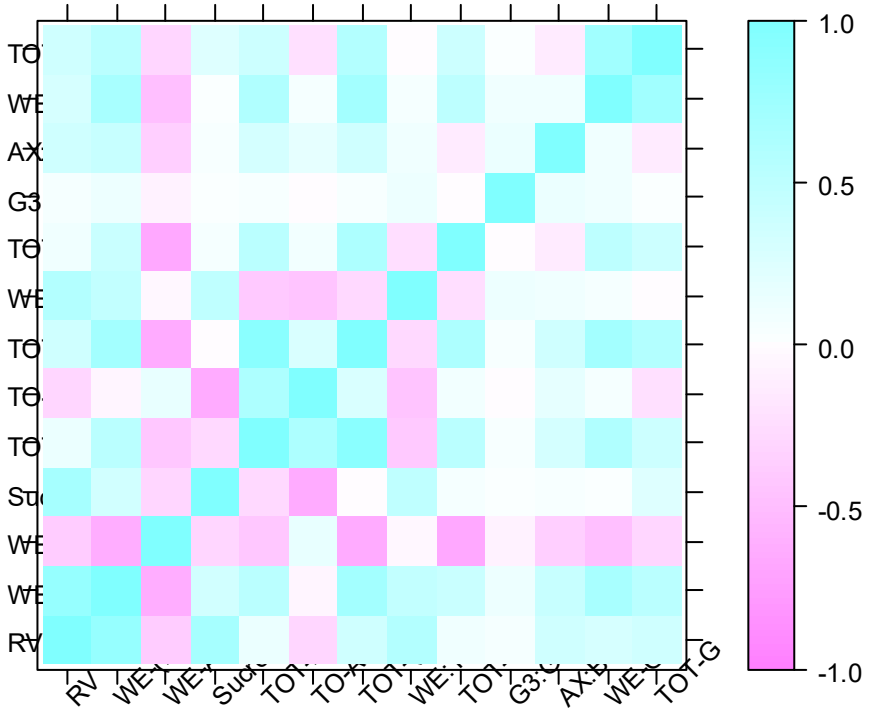

Figure 4. Heat map of correlations between parameters relating to fibre amount and composition.

Rather than selecting a subset of independent fibre measurements for further analysis, we decided to combine the fibre data by multivariate Principal Component Analysis (PCA). This identified 10 principal components which together accounted for 99% of the variation in the dataset. Figure 5A and B show the plots of PC1 against PC2, which together account for 60% of the total variance, coloured to show the cultivars and years. This shows a partial separation between years, but 2017 (which showed atypically low WA) was not clearly separated from the other years. The contributions of the traits to the PCs (loadings) are given in Table 4.

The 10 PCs are independent of each other and hence were appropriate to be included in the regression analysis.

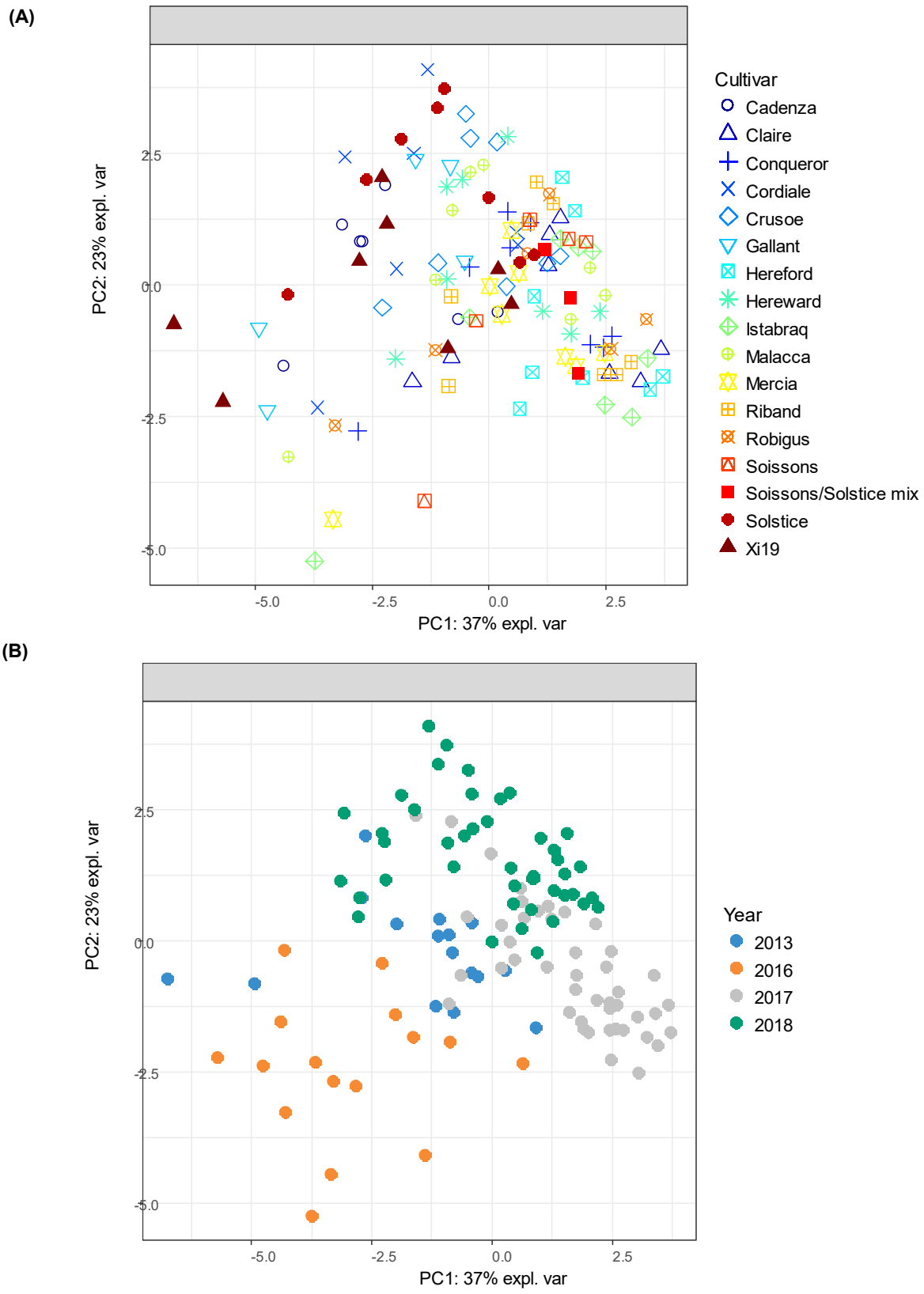

Figure 5. PCA (PC1 v PC2) of dietary fibre components, coloured by cultivar (A) and years (B).

Table 4. Contributions (loadings) of fibre components to PCs 1 to 10.

Percentage variance of each PC	37.3	23.5	12.1	7.7	7.3	4.6	3.7	1.5	0.9	0.8
	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**	**PC10**
RV	-0.277	0.367	0.193	-0.066	0.196	-0.004	0.395	0.223	-0.010	0.063
WE-(A)X	-0.403	0.169	0.170	0.049	0.153	-0.220	-0.012	0.240	0.001	0.101
WE-A:X	0.347	-0.046	0.021	0.322	0.469	0.090	0.317	0.000	-0.286	0.571
Sucrose SRC	-0.115	0.463	-0.107	-0.193	-0.134	0.241	0.461	-0.577	0.076	-0.022
TOT-A(X)	-0.334	-0.351	0.128	0.010	0.105	0.016	0.225	0.045	0.272	0.014
TOT-A:X	-0.020	-0.457	0.322	0.070	0.179	-0.301	0.354	-0.368	-0.163	-0.426
TOT-(A)X	-0.413	-0.193	0.023	-0.066	0.023	0.185	0.098	0.237	0.258	0.174
WE:TOT (A)X	-0.033	0.469	0.179	0.191	0.169	-0.548	-0.177	0.000	-0.047	-0.171
TOT-BG	-0.294	-0.151	-0.348	-0.187	-0.374	-0.404	0.145	0.002	-0.558	0.291
G3:G4 BG	-0.048	0.044	0.214	0.739	-0.603	0.096	0.162	0.055	0.013	0.014
AX:BG	-0.162	0.014	0.650	-0.210	-0.072	0.355	-0.332	-0.154	-0.457	0.131
WE-G	-0.368	-0.058	-0.166	0.304	0.197	-0.075	-0.392	-0.571	0.191	0.311
TO-G	-0.304	0.044	-0.390	0.296	0.285	0.394	-0.056	0.114	-0.431	-0.468

4.1.2. Modelling the contributions of parameters to WA.

In order to determine which components are of most importance in determining WA we developed a statistical model based upon the analytical data. Regression analysis was carried out as above (having accounted for Year as a random effect), with the baseline model (based on the Farrand equation) being:

WA = Starch damage + Protein + Flour Moisture.

This model was then modified by introducing either individual fibre traits or the principle components (PCs) discussed above.

The baseline model gave an approximate percentage variance accounting for 74.9% of the variation, therefore only traits which improved upon this %age are listed in Table 5, with plots showing the addition of selected traits in Figure 6. The greatest improvement was achieved by adding all fibre PCs, which improved the variance accounted for to 90.36%. However, both TOT-BG and TOT-A(X) also gave improvements, to 84.2% and 79.9%, respectively.

Table 5: Regression analysis of WA, adding fibre components to the Baseline equation

Model	Term	Mean square	F value	P value	Approx. % variance
Baseline	Starch damage	448.23	241.827	2.20E-16	74.91445
	Protein	20.76	11.198	0.001133	
	Flour Moisture	36.54	19.712	2.19E-05	
fibre 1	Starch damage	336.25	181.522	2.20E-16	77.79059
	Protein	17.52	9.4562	0.002707	
	Flour Moisture	37.95	20.489	1.56E-05	
	WE-A:X	3.16	1.7052	0.194406	
fibre 2	Starch damage	351.57	191.6235	2.20E-16	76.0267
	Protein	25.34	13.8141	0.000323	
	Flour Moisture	38.43	20.9471	1.28E-05	
	Sucrose.SRC	3.94	2.1472	0.145827	
fibre 3	Starch damage	419.54	225.5657	2.20E-16	79.93681
	Protein	19.56	10.5168	0.001606	
	Flour Moisture	32.43	17.4346	6.08E-05	
	TOT-A(X)	3.41	1.8317	0.180315	
fibre 4	Starch damage	438.86	234.7569	2.20E-16	77.06628
	Protein	21.08	11.2738	0.001094	
	Flour Moisture	32.76	17.5234	5.84E-05	
	TOT-A:X	1.08	0.5793	0.448344	
fibre 5	Starch damage	378.19	203.3615	2.20E-16	78.61681
	Protein	18.64	10.0251	0.002051	
	Flour Moisture	33.68	18.1095	4.49E-05	
	TOT-(A)X	2.73	1.4692	0.228565	
fibre 6	Starch damage	452.14	246.8569	2.20E-16	77.07794
	Protein	23.58	12.8731	0.000508	
	Flour Moisture	32.69	17.8492	5.05E-05	
	WE:TOT (A)X	4.92	2.6845	0.104294	
fibre 7	Starch damage	340.84	213.15	2.20E-16	84.18824
	Protein	16.5	10.32	0.001811	
	Flour Moisture	27.26	17.049	7.25E-05	
	TOT-BG	31.59	19.753	2.19E-05	
fibre all PC	Starch damage	230.491	142.3593	2.20E-16	90.33559
	Protein	26.441	16.3311	0.000107	
	Flour Moisture	20.947	12.9378	0.000515	
	fibre_PC_1	1.182	0.7298	0.431233	
	fibre_PC_2	16.903	10.4396	0.079864	
	fibre_PC_3	1.921	1.1862	0.512546	
	fibre_PC_4	8.593	5.307	0.065957	
	fibre_PC_5	8.889	5.4904	0.102056	
	fibre_PC_6	14.064	8.6865	0.019946	
	fibre_PC_7	0.764	0.4716	0.494934	
	fibre_PC_8	3.233	1.9965	0.161404	
	fibre_PC_9	1.644	1.0155	0.319889	
	fibre_PC_10	1.647	1.0172	0.318971	
fibre PC 1+2+6	Starch damage	350.98	209.9616	2.20E-16	84.85038
	Protein	23.72	14.1867	0.000282	
	Flour Moisture	30.07	17.989	4.82E-05	
	fibre_PC_1	5.66	3.3845	0.07066	
	fibre_PC_2	19.38	11.5922	0.00106	
	fibre_PC_6	14.26	8.528	0.00431	

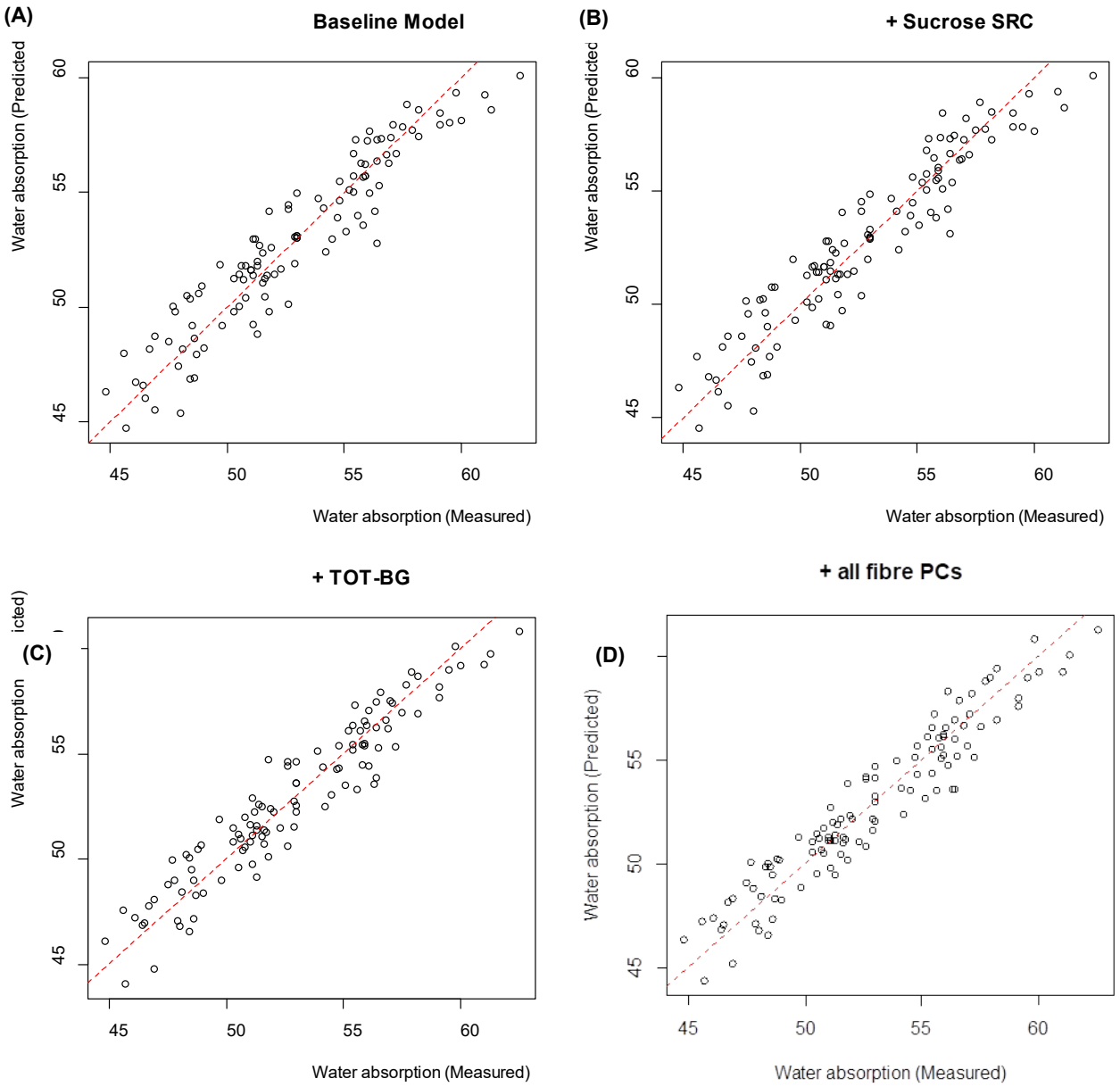

Figure 6. Comparison of determined WA with values predicted using the Baseline model, and after adding Sucrose SRC (B), TOT-BG (C) and all fibre PCs (D) to the baseline model. Adding Sucrose SRC did not improve the model predictions whilst both TOT-BG and the fibre PCs improved model predictions.

To further investigate the effect of Year, an interaction term was included in the fixed effects model. This interaction term was marginally significant when added to the baseline model, but was unimportant when added to the fibre PC model. Thus, the differences observed in WA between years are additive once the relationships with starch, protein, flour moisture and fibre are accounted for.

4.1.3. Conclusions

The baseline model based on the Farrand equation accounted for almost 75% of the variation in WA between the samples and this was increased to just over 90% by adding variation in fibre components. However, this increase was only achieved by including principal components based on a range of fibre parameters. When considered individually, the single fibre component which resulted in a substantial increase in the predictive power of the model was total β-glucan, which increased the prediction to 84% when added to the baseline equation.

4.2. Comparison of doubled haploid (DH) and parental from the cross Yumai 34 x Valoris lines, differing in AX content.

The comparison of cultivars grown in different years showed that the fibre content contributed to differences in WA. To explore this effect in more detail we compared a series of lines which had been selected to exhibit variation in AX content in a randomly segregating genetic background. Single samples grown in large plots were milled and subjected to analyses as in Table 3.

The full analyses of the samples are given in Appendix 2 and the data for fibre components summarised in Figure 7. Data for selected fibre components are shown graphically in Figure 7A, B and C.

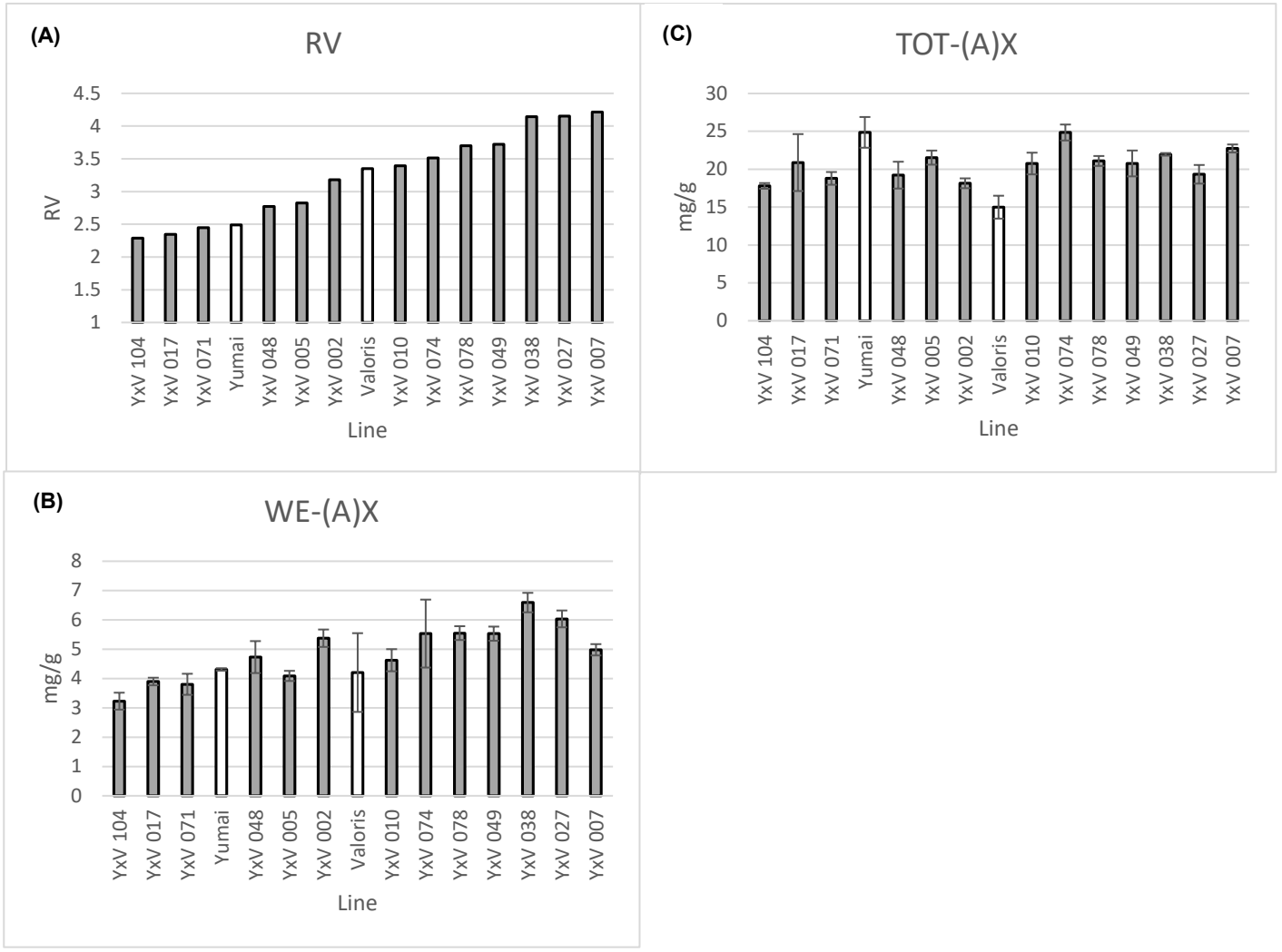

Figure 7. Water-extractable AX determined as relative viscosity (RV) (A) and by monosaccharide analysis (B) and total AX (determined by monosaccharide analysis) (C) in the Yumai 34 x Valoris lines. Samples are displayed in the same order in all panels.

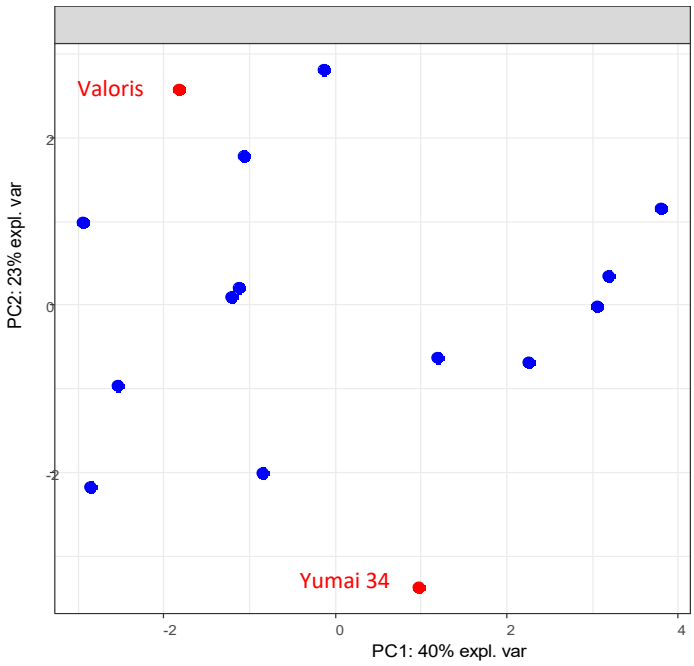

Figure 8. The first two principal axes from PCA of fibre components in the lines differing in fibre content (D). Parents indicated in red.

4.2.1. Multivariate analysis of fibre components

PCA of the fibre components identified 7 PCs which together accounted for 99% of the total variance (Table 6). The first two PCs, which together accounted for 63% of the total variance, are shown in Figure. 8, which shows good separation of the samples, with the parental lines clearly separated from the progeny. The loadings for all 7 PCs are given in Table 6.

Table 6. Contributions (loadings) of fibre components to PCs 1 to 7

Percentage variance of each PC	39.85	23.25	17.84	8.327	4.689	2.949	2.103
	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**
RV	-0.27701	0.195765	-0.4036	-8.05E-02	0.221264	-0.30017	-0.29182
WE-(A)X	-0.27051	0.170304	-0.4461	8.75E-02	0.052067	0.298248	0.293284
WE-A:X	-0.33217	0.154699	0.21825	-2.59E-01	0.459094	-0.05578	0.307611
Sucrose SRC	-0.34255	-0.22778	0.162774	3.48E-01	-0.10564	-0.17016	-0.11571
TOT-A(X)	-0.14917	-0.50715	-0.16238	-7.73E-05	0.101421	0.309097	0.107519
TOT-A:X	-0.17066	-0.19424	0.489752	-3.20E-01	0.151321	0.282399	0.212037
TOT-(A)X	-0.09184	-0.45523	-0.34936	1.27E-01	0.114523	0.210895	0.050373
WE:TO (A)X	-0.21507	0.456517	-0.19843	-3.32E-02	-0.02579	0.166766	0.284881
TOT-BG	0.364751	-0.0357	-0.08171	4.55E-01	0.135319	0.038297	0.373851
G3:G4 BG	0.339636	0.219304	-0.02194	-1.35E-01	0.166953	0.642372	-0.43588
AX:BG	-0.39712	-0.13254	-0.08008	-1.54E-01	-0.21813	0.149513	-0.38871
WE-G	0.202048	-0.14344	-0.26251	-5.82E-01	-0.51539	-0.1105	0.301818
TO-G	0.263478	-0.24243	-0.23202	-3.01E-01	0.565726	-0.30435	-0.093

4.2.2. Regression analysis of fibre and WA.

Regression analysis was carried out as above, using the same baseline model based on the Farrand Equation:

WA = Starch damage + Protein+ Flour Moisture.

This was then modified by introducing either individual fibre traits or the PCs discussed above. The baseline model gave an approximate percentage variance accounting for 86.01% of the variation, so only traits which improved this are listed in Table 7, with plots of the effects of the addition of selected traits being displayed in Figure 9.

The best improvement was achieved by adding PCs 1 and 3, which improved the variance accounted for to 96.77%. However, both the addition of all fibre PCs, RV and WE-(A)X also gave improvements, to 95.18, 94.21% and 96.73%, respectively. The addition of Sucrose SRC did not give any improvement.

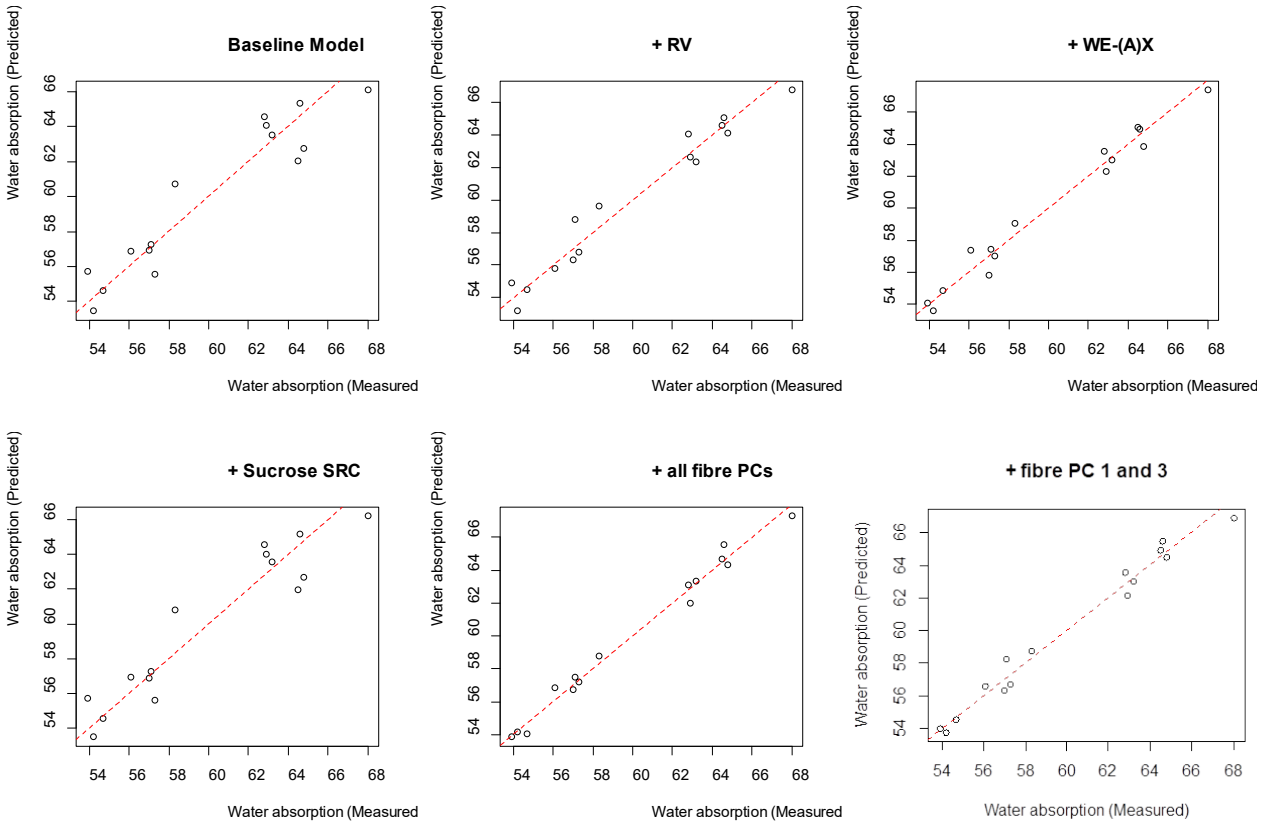

Figure 9. Regression analysis of WA, adding fibre components to the baseline equation.

Table 7. Regression analysis of WA, adding fibre components to the Baseline equation

Model	Term	F value	P value	Adjusted R^2 (reported as % variance accounted for)
Baseline	Starch damage	73.5603	3.35E-06	86.01
	Protein	15.5104	0.002319	
	Flour Moisture	0.0023	0.962908	
fibre 1	Starch damage	177.7883	1.08E-07	94.21
	Protein	37.487	0.000112	
	Flour Moisture	0.0055	0.942499	
	RV	16.586	0.002241	
fibre 2	Starch damage	314.8097	6.90E-09	96.73
	Protein	66.3783	1.00E-05	
	Flour Moisture	0.0097	0.923544	
	WE-(A)X	37.0758	0.000117	
fibre 3	Starch damage	94.3777	2.07E-06	89.10
	Protein	19.8998	0.001214	
	Flour Moisture	0.0029	0.958086	
	TOT-A:X	4.113	0.070033	
fibre 4	Starch damage	83.0704	3.69E-06	87.61
	Protein	17.5156	0.001872	
	Flour Moisture	0.0026	0.960674	
	TOT-(A)X	2.4221	0.150689	
fibre 5	Starch damage	109.9273	1.03E-06	90.64
	Protein	23.1784	0.000708	
	Flour Moisture	0.0034	0.954769	
	WE:TOT(A)X	6.4382	0.029496	
fibre 6	Starch damage	118.6462	7.22E-07	91.33
	Protein	25.0168	0.000536	
	Flour Moisture	0.0037	0.953011	
	TOT-BG	7.742	0.019369	
fibre all PC	Starch damage	213.4555	0.000128	95.18
	Protein	45.0076	0.00257	
	Flour Moisture	0.0066	0.939302	
	fibre_PC_1	9.3709	0.037615	
	fibre_PC_2	0.5014	0.517985	
	fibre_PC_3	16.1098	0.015948	
	fibre_PC_4	0.1551	0.713826	
	fibre_PC_5	1.2991	0.317999	
	fibre_PC_6	0.4796	0.526725	
	fibre_PC_7	0.0038	0.953751	
fibre PC1 + PC3	Starch damage	318.2076	2.484e-08	96.77
	Protein	67.0948	1.832e-05	
	Flour Moisture	0.0098	0.9233477	
	fibre_PC_1	13.9696	0.0046427	
	fibre_PC_3	24.6142	0.0007792	

4.2.3. Conclusions

The baseline model based on the Farrand equation accounted for 86% of the variance in WA in the samples, which was increased to 96.77% by the addition of two PCs for variation in fibre traits. However, improvements were also achieved by adding variation in single traits related to fibre content and properties, and particularly soluble fibre: to 96.7% by adding WE-AX (determined as xylose) and 94.2% by adding RV. RV is the relative viscosity of aqueous extracts and is largely determined by water-soluble fibre, notably WE-AX. WE-β-glucan would also be expected to contribute to RV as soluble forms of β-glucan from barley and oats are known to form highly viscous solutions (Lazaridou and Biliaderis, 2007). However, β-glucan is a relatively minor component in wheat flour and its solubility is low (with about 10% of the total being soluble (Nemeth et al., 2010)). Nevertheless, the addition of the ratio of total AX:BG resulted in some improvement of the prediction, to 91.3%, and β-glucan is also a component of PC1. This indicates that soluble β-glucan may also contribute, though it appears less important in this population than in the cultivars described previously.

4.3. Effect of nitrogen fertilisation on WA and AX content

Five modern wheat cultivars were grown in three replicate plots at Rothamsted in 2016, with 100 and 200 kg N/ ha. Triplicate plots were combined prior to milling. Fibre analyses were performed on flour samples showing little effect of nitrogen fertilisation on relative viscosity (RV), which is a measure of WE-AX, or TOT-AX (Figure 10). A two-sample t-test utilising the single rep cultivars as replicates of the nitrogen treatment (n=5), showed there was insufficient evidence to suggest a difference in WA due to nitrogen treatment (p=0.105). (t-test analysis is shown in annex **)

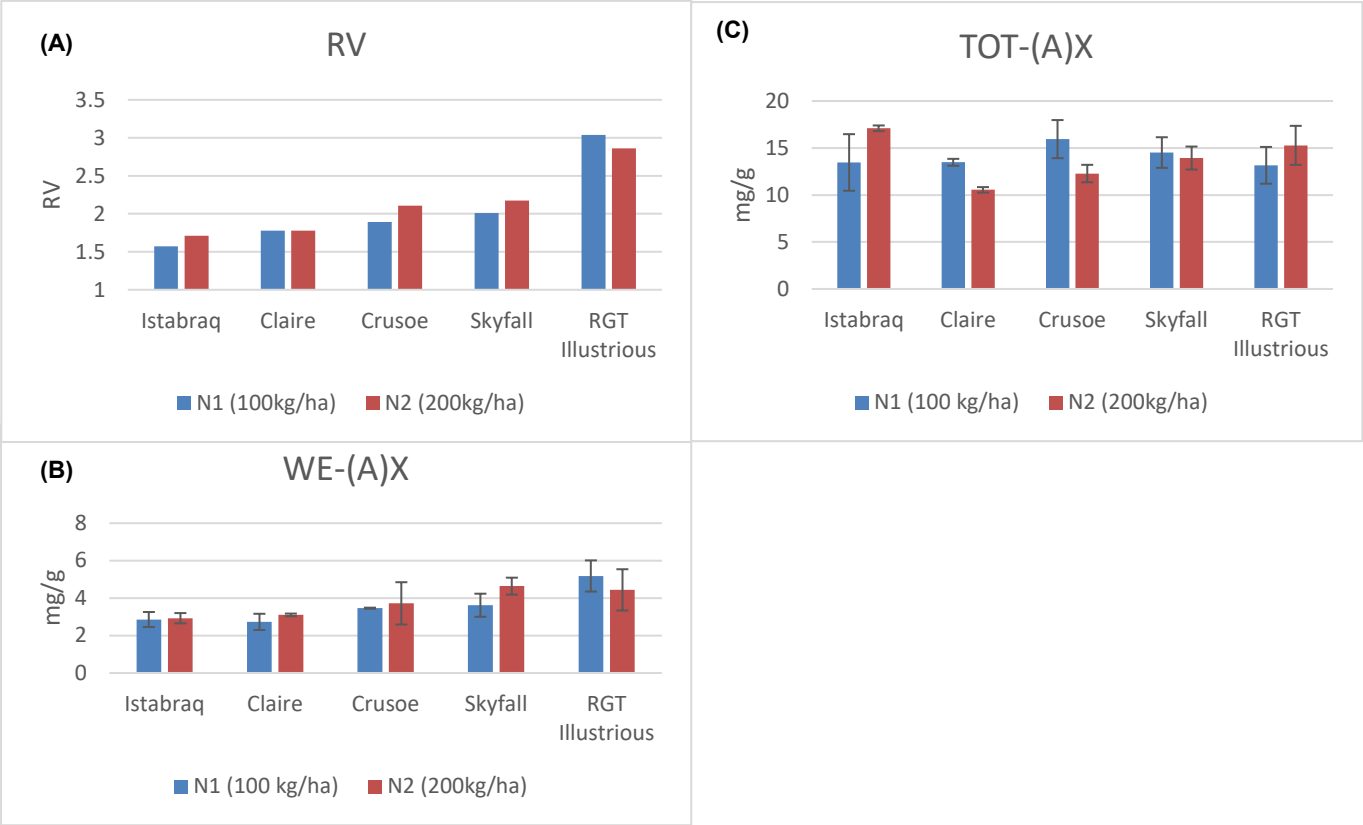

Figure 10. Effect of nitrogen fertilisation on water-extractable (A, B) and total (C) fibre components in elite cultivars grown in 2016.

5. Discussion

The experiments described above were carried out to answer three questions:

1. What components are responsible for the differences in WA between “typical” and “atypical” years?
2. Do fibre components have a specific role in determining differences in WA?
3. Is WA affected by nitrogen fertiliser?

In order to answer them, we selected three sets of samples.

For the first question, we compared sets of cultivars from the WGIN archives and field trials that had been grown in typical (2016 & 2018) and atypical years (2013 & 2017). Determination of WA showed little difference between the 2013, 2016 and 2018 samples, but WA was generally lower for the 2017 samples.

A range of traits were measured and tested for their ability to improve the prediction when added to the Farrand equation as a baseline. No improvements were observed by including data for starch composition (% amylose) or protein composition (% gliadins), but the addition of PCs derived from analyses of fibre components did markedly improve the prediction, from about 75% to over 90%. A smaller improvement was achieved by adding data for β-glucan, a minor fibre component. Hence,

although it can be concluded that fibre components were the major contributor to the differences in WA between the samples, it was not possible to identify a single component which could form the basis for a simple analytical test in a non-specialist laboratory.

Comparison of a smaller set of the WGIN cultivars grown at 100 and 200 kg N/Ha in 2016 showed no significant differences in WA related to nitrogen fertilisation, answering question 3.

In order to answer the second question, we used a series of near-isogenic lines selected to vary in their content of arabinoxylan fibre in flour. These lines are genetically related, with backgrounds derived from the cultivar used for the cross: Yumai 34 (Chinese) and Valoris (French). The related genetic background of the lines may have accounted for the fact that the baseline model accounted for a higher proportion of the total variance in WA than in the first study, 86% compared to 75%. Improvements were again achieved by adding PCs related to fibre content, to over 96%, but also by adding two single traits related to soluble fibre: soluble arabinoxylan (to 96.7%) and relative viscosity of aqueous extracts (to 94.2%).

The results, therefore, demonstrate that fibre components contribute to a substantial extent to the variation in WA between grain samples between different years. Furthermore, the comparison of lines differing in arabinoxylan amount and composition indicate a specific role for soluble fibre. This provides the basis for further studies in which simpler analytical procedures (for soluble AX and RV) could be used to determine the validity of these observations on larger sample sets, which could lead to predictive tests suitable for use by millers.

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