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Effect of heat and drought stress on the structure and composition of arabinoxylan and β-glucan in wheat grain

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ABSTRACT

The effects of heat (H), drought (D) and H + D (from 12th day after heading for 15 days) on the dietary fiber content and composition (arabinoxylan (AX) and β-glucan) of three winter wheat varieties (Plainsman V, Mv Magma and Fatima 2) were determined. Results showed that H and D stress decreased the TKW, the β-glucan contents of the seeds and the quantity of the DP3 + DP4 units, while the protein and AX contents increased. The highest amounts of AX and proteins were in the H + D stressed samples with heat stress also increasing the water extractability (WE) of the AX. However, while the content of AX content was generally increased by all stresses, drought stress had negative effect on the AX content of the drought tolerant Plainsman V. Fatima 2 behaved similarly to Plainsman V as regards to its drought tolerance, but was very sensitive to heat stress, while Mv Magma was the most resistant to heat stress.

1. Introduction

Wheat and other cereals are major sources of dietary fiber (DF) in the human diet, with bread alone accounting for about 20% of the total daily intake in the UK (Steer, Thane, Stephen, & Jebb, 2008). The major DF components in wheat grain are the cell wall polysaccharides, arabinoxylan (AX) and (1-3)(1-4)-β-D-glucan (β-glucan), which account for about 70% and 20%, respectively, of the total cell wall polysaccharides in the starchy endosperm (and hence white flour) (Mares & Stone, 1973). AX and β-glucan occur in soluble and insoluble forms, which may differ in their health benefits. Insoluble DF lowers transit time and increases fecal bulk, defecation frequency, and binding of carcinogens, while soluble DF reduces the risk of coronary heart disease and type II diabetes. DF components, in particular AX, also affect the processing properties of wheat, for example, in breadmaking (Courtin & Delcour, 2002), gluten-starch separation (Frederix, Van Hoeymissen, Courtin, & Delcour, 2004), the quality for livestock feed (Bedford & Schulze, 1998) and fermentation to produce alcohol for beverages and biofuel (Shewry, Freeman, Wilkinson, Pellny, & Mitchell, 2010; Shewry, Piironen et al., 2010).

Arabinoxylan (AX) has a backbone chain of β-D-xylopyranosyl (Xylp) residues linked through (1-4)-glycosidic linkages. Some of the Xylp residues are monosubstituted with α-L-arabinofuranosyl (Araf) residues at position 3, or disubstituted at positions 2 and 3 of the same Xylp residues (Hoffmann, Leeflang, de Barse, Kamerling, & Vliegenthart, 1991; Izydorczyk & Biliaderis, 1994; Perlin, 1951; Renard, Rouau, & Thibault, 1990). The AX in the secondary walls of the pericarp and seed coat tissues of the bran may also have 4-O-methyl α-D-glucuronic acid as an additional substituent at position 2 of Xylp units (Scooneveld-Bergmans, Beldman, & Voragen, 1999).

The (1-3,1-4)-β-D-glucans(β-glucan) are linear, unbranched polymers in which the β-D-glucopyranosyl residues are joined by both (1-3) and (1-4) glucosidic linkages. Single (1-3) linkages are separated by two or more (1-4) linkages and regions of two or three adjacent (1-4) linkages predominate. The distribution of oligosaccharides in β-glucan differs in different cereal species (Cui, Wood, Blackwell, & Nikiforuk, 2000; Lazaridou, Biliaderis, Micha-Screttas,

Abbreviations: AXOS, arabinoxylan oligosaccharides; D, drought; DP, degree of polymerization; DP3-4/DP5-11, DP3 + DP4/DP5 + DP6 + DP7 + DP8 + DP9 + DP10 + DP11; D, (xa2 + 3xx) + (xa3a2 + 3x) + (xa3xa2 + 3xx) sum of disubstituted AXOS; GOS, glucooligosaccharides; H, heat; H + D, heat and drought; HP-AEC, high performance-anion exchange chromatography; M, (xa3xx) + 2(xa3a3xx) + 2(xa3xa3xx) + (xa3a2 + 3x) + (xa3xa2 + 3xx) sum of monosubstituted AXOS; PCA, principal component analysis; TKW, thousand kernel weight; TOT-AX, total arabinoxylan; TOT, x + xx + xxx + xa3xx + xa3a3xx + xa3xa3xx + (xa2 + 3xx) + (xa3a2 + 3xx) + (xa3xa2 + 3xx) sum of all AXOS; US, x + xx + xxx sum of unsubstituted AXOS; WE-AX, water extractable arabinoxylan.

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& Steele, 2004), with the relative proportion of trisaccharide (DP3) decreasing from wheat (67–72%), to barley (52–69%) and oats (53–61%) and the relative amount of tetrasaccharide (DP4) following the opposite trend. Differences in the ratio of DP3:DP4 may also occur within the same cereal species, which may be attributed to genotypic and environmental factors (Jiang & Vasanthan, 2000; Miller, Wood, Pietrzak, & Fulcher, 1993; Storsley, Izydorczyk, You, Biliaderis, & Rossnagel, 2003; Wood, Weisz, Beer, Newman, & Newman, 2003).

The quantity and composition of DF in cereals are affected by both genetic and environmental factors, with genetic factors being the most important (Gebruers et al., 2010; Henry, 1989; Miller et al., 1993; Molina-Cano et al., 1997; Shewry, Freeman et al., 2010; Shewry, Piironen et al., 2010; Stuart, Loi, & Fincher, 1988). Gebruers et al. (2010) found that genotype determined ~50% or more of the variation observed in TOT-AX and WE-AX in wheat flour, WE-AX in bran and β-glucan in wholemeal of wheat, whereas the impact of the GxE interaction was relatively low. This was confirmed by further analyses of the same dataset (Shewry, Freeman et al., 2010; Shewry, Piironen et al., 2010) which showed that AX and β-glucan content are highly heritable and hence an appropriate target for plant breeding.

Despite the high heritability there are also significant effects of the environment on DF. Early studies reported that drought and heat stress during flowering increased the concentration of AX (Hong, Rubenthaler, & Allen, 1989), while high temperature and drought after flowering had the opposite effect (Laurentin & Douglas, 2003). However, the degree of stress also appears to be important as Coles et al. (1997) showed that the concentration of AX in wheat was increased by mild drought after flowering but decreased under severe drought. High temperature from flowering time also increased the AX concentration in the grain, particularly WE-AX (Zhang, Liu, Chang, Anthony, & Anyia, 2010). When temperature and water stress occurred at the same time, as is usual under natural conditions in Europe, the grain tended to have higher levels of DF components when grown in hot and dry (Hungary) than in cool wet (UK) conditions (Gebruers et al., 2010). Toole et al. (2007) showed that the degree of substitution of the AX with arabinose (termed branching) in the cell walls of the starchy endosperm decreased during development, and that this occurred more rapidly when the plants were grown at a higher temperature with restricted water availability. Hence, environmental factors may affect both the amount and structure of AX. However, the results from the studies are not consistent which may be due to differences in the genotypes and growing sites tested (Li, Morris, & Bettge, 2009).

Whereas β-glucan is a relatively minor component in wheat, it accounts for about 70% of the cell wall polysaccharides in the barley starchy endosperm (Fincher, 1975). The effects of the environment on the β-glucan content of cereals have therefore mainly been studied in barley. However, the results are again inconsistent. Coles, Jamieson, and Haslemore (1991) found a relationship between β-glucan synthesis and transpiration, with low β-glucan content occurring in environments, such as drought stress, where stomata are closed to limit transpiration (Coles et al., 1991). This is supported by the results of Narasimhalu et al. (1995), who reported higher β-glucan contents under wet conditions. Later Swanston, Ellis, PerezVendrell, Voltas, & MolinaCano (1997) reported higher β-glucan contents in samples grown in a hot and dry country (Spain) than in the cool and wet climate of Scotland. Güler (2003) also reported decreased β-glucan content when the barley plants were grown with irrigation. However, while it has been shown that the contents of total β-glucan (Morgan & Riggs, 1981; Swanston et al., 1997) and WE β-glucan (Nilssen, Sahlstrřm, Knutsen, Holtekjřlen, & Kjersti Uhlen, 2008) are greater under hot and dry conditions, a short and very severe period of high temperature stress may reduce the β-glucan content (Savin, Stone, Nicolas, & Wardlaw, 1997). MacNicol, Jacobsen, Keys, and Stuart (1993) found that the timing of the heat or drought stress is important and that drought stress occurring late in the grain-filling period had no effect on β-glucan content.

The conflicting results from these studies demonstrate the need to carry out more controlled studies of the effects of environmental factors on cereal DF, and in particular to quantify the effects of individual factors on different fiber components. We have therefore carried out detailed analyses of three wheat varieties grown under controlled conditions of heat, drought and heat + drought, in order to determine their impact on the amounts and compositions of the major DF components (AX and β-glucan) in whole grain.

2. Materials and methods

2.1. Plant material and growing conditions

Three winter wheat cultivars with different genetic backgrounds were selected: Plainsman V (USA), Fatima 2 (Hungary) and Mv Magma (Hungary). The hard red wheat cv. Plainsman V has a high protein content and good drought tolerance. The mid-early maturing cv. Fatima 2 is known for its excellent yield potential and good milling and baking quality. Mv Magma is also a mid-early maturing variety, with good resistance to diseases and winter frost. It has average milling quality and is suitable for breadmaking.

Plants were grown in a Conviron PGV-36 climate chamber in the Martonvásár phytotron. Pots with a volume of 3500 cm^3, containing a known quantity of a 3:2:1 ratio of soil, Vegasca, and sand, were each planted with four germinated wheat seeds. After six weeks of vernalization at 4 °C, the plants were grown at controlled temperature using the T2NY2 climate program (Tischner, Rajkaine Vegh, & Kőszegi, 1997) until the start of the stress treatment. The experiment consisted of four treatments: control (C), heat stress (H), drought stress (D), and heat + drought stress (H + D). For each cultivar, there were a total of 16 pots, four pots per treatment, with four plants per pot. The treatment began on the 12th day after heading (at Zadoks-75 developmental stage, Tottman & Makepeace, 1979) and was continued for 15 days. The temperature was programmed at 24/20 °C (day/night, with 12.5 h at 24 °C) in the control chamber (Tischner et al., 1997) and at 35/20 °C (with 8 h at 35 °C) for the heat stress treatment. The soil moisture was adjusted to 60–70% of natural water content (NWC, taken as 100%) for the control treatment and 40–45% for the drought stress treatment. Water was added on a weight basis. The light intensity was adjusted to 350 μmol/m^2/s during the stress treatments.

2.2. Methods

Ten grams of seed from each sample was milled using a Perten Laboratory Mill 3100 (with 0.5 mm sieve) to produce wholemeal. Wholemeal samples were immediately cooled and stored at −20 °C.

2.2.1. Thousand kernel weight

Test weight and thousand kernel weight were determined by standard MSZ 6367/4-86 (1986) method. Duplicate analyses were carried out on each sample.

2.2.2. Protein

Crude protein contents were determined by the Kjeldahl method consistent with ICC method 105/2 using the Kjeltec 1035 Analyzer instrument. Duplicate analyses were carried out on each sample.

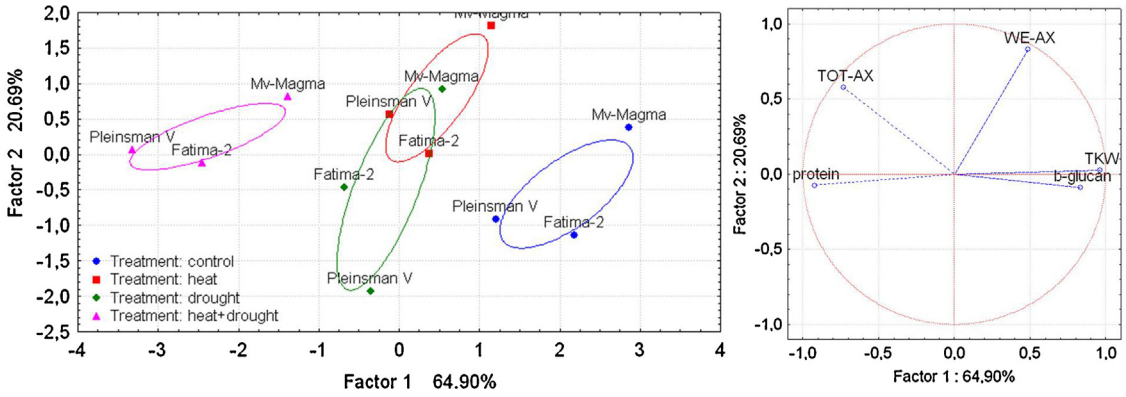

Fig. 1. Principal component analysis for the 3 varieties (Plainsman V, Mv-Magma, Fatima 2) based on compositional properties: TKW, protein, β-glucan, TOT-AX, WE-AX. Results grouped by different stress treatments and colored differently. (TKW, thousand kernel weight; TOT-AX, total arabinoxylan; WE-AX, water extractable arabinoxylan).

2.2.3. Quantitative determination of total and water extractable pentosans

Total and water extractable pentosans were determined using a colorimetric method as described by Douglas (1981) and Finnie, Bettge, and Morris (2006). 12.5 ml MQ water was added to 62.5 mg flour and shaken (suspension1, TOT-AX). 0.5 ml of the suspension (1) was diluted to the double volume with water and than 5 ml freshly prepared extraction solution was added (93.2 (v/v) % acetic acid, 1.69 (v/v) % HCL, 0.85 (w/v) % phloroglucinol and 0.017 (w/v) % glucose). Tubes were placed into a boiling water bath for 25 min and after cooling the absorbance of the samples were measured at 552 and 510 nm. Suspension 1 was shaken for 30 min to determine water extractable pentosans (suspension 2, WE-AX). Suspension 2 was centrifuged and 0.5 ml of the supernatant was diluted two-fold with water and 5 ml of extraction solution was added. The sample was then boiled for 25 min and after cooling, the absorbance was measured at 552 and 510 nm. Comparison of the absorbance values with those of D-(+) xylose standards allowed the pentose concentration to be determined. Duplicate analyses were carried out on each sample. Pentosans will be referred to as 'arabinoxylan' in further sections of the paper.

2.2.4. Enzyme fingerprinting of arabinoxylan

The protocol was adapted from Ordaz-Ortiz, Devaux, and Saulnier (2005). 1 ml of 80% (v/v) ethanol was added to 100 mg of flour and heated in a 95 °C water bath for 5–10 min to inactivate the enzymes. After centrifugation, the residue was washed with 80% (v/v) ethanol and then with 95% (v/v) ethanol and dried using a Speedvac centrifugal evaporator. The dried powder was resuspended in 1 ml of water containing 16 U of endoxylanase and 2 U of lichenase and incubated at 40 °C for 16 h with continuous rotation. After centrifugation, 0.6 ml of the supernatant was heated for 10 min in a 95 °C hot water bath to inactivate the enzymes. The samples were then centrifuged and filtered using 0.45 μm Millex-HV syringe driven filters.1:20 diluted samples were injected onto an HPAEC system (high-performance anion-exchange chromatography) using a Carbopac PA1 analytical column (4 × 250 mm) (Ordaz-Ortiz et al., 2005). Duplicate analyses were carried out on each sample. Measures of the amounts of unsubstituted, mono- and di-substituted xylose residues in the AXOS are derived from the AXOS peak areas (Toole et al., 2010), calculated as:

$$M = (xa3xx) + 2(xa3a3xx) + 2(xa3xa3xx) + (xa3a2 + 3x) + (xa3xa2 + 3xx)$$

and

$$D = (xa2 + 3xx) + (xa3a2 + 3x) + (xa3xa2 + 3xx),$$

$$US = x + xx + xxx \text{ and}$$

$$TOT = x + xx + xxx + xa3xx + xa3a3xx + xa3xa3xx + (xa2 + 3xx) + (xa3a2 + 3xx) + (xa3xa2 + 3xx)$$

is the total AXOS.

2.2.5. Quantitative determination of β-glucan

The total amount of mixed-linkage β-glucan in wholemeal samples was determined using a Megazyme kit (Megazyme, Bray, Ireland) (ICC Standard No. 166; AACC Method 32-23). Duplicate analyses were carried out on each sample.

2.2.6. Enzyme fingerprinting of β-glucan

100 mg samples were pre-treated with ethanol as for pentosan analysis, suspended in water (1 ml) and digested overnight at 40 °C with continuous stirring, using a lichenase solution (100 μl, 16 U) (Ordaz-Ortiz et al., 2005; Saulnier et al., 2009). After inactivating the enzyme by heating at 95 °C for 5 min, the samples were centrifuged and filtered through 0.45 μm Millex-HV syringe driven filter. 1:20 diluted samples were injected on an HPAEC system (high-performance anion-exchange chromatography) using a Carbopac PA1 analytical column (4 mm × 250 mm) (Ordaz-Ortiz et al., 2005). Duplicate analyses were carried out on each sample.

2.2.7. Statistical analyses

Statistical analysis was carried out using Microsoft Excel and Statistica 6.0 software.

3. Results and discussion

3.1. Grain composition

The DF content and composition of whole grain of three winter wheat varieties (Plainsman V, Mv Magma and Fatima 2) grown under heat, drought and combination of the two stress factors were determined. A colorimetric assay for pentose sugars was used to determine WE-AX and TOT-AX. Principal component analysis (PCA) of the data for thousand kernel weight (TKW), protein and DF fractions show that heat and drought stress had clear effects on the composition and TKW of the grain (Fig. 1). In particular, heat and drought stress resulted in lower TKW and β-glucan contents relative to the control, while the protein and AX contents were increased. The highest contents of TOT-AX and proteins were in the H + D stressed samples, while heat stress alone resulted in higher proportions of WE-AX than drought or H + D stress together (Fig. 1 and Table 1.). These results are consistent with those reported

Table 1
Mean and standard deviation of thousand kernel weight (TKW), protein, β-glucan, TOT-AX and WE-AX content measured in the stressed samples. sd, standard deviation; d.m., dry matter.

Variety	Treatment	TKW g/1000 kernel	TKW sd	Protein % (d.m.)	Protein sd	β-Glucan mg/g (d.m.)	β-Glucan sd	TOT-AX mg/g (d.m.)	TOT-AX sd	WE-AX mg/g (d.m.)	WE-AX sd
Mv-Magma	Control	28.84	1.00	16.10	0.21	7.57	0.60	37.96	2.85	8.31	0.64
Mv-Magma	Heat	24.76	2.65	19.60	0.42	6.32	0.44	48.12	0.87	8.99	0.32
Mv-Magma	Drought	24.06	1.25	17.20	0.14	5.19	0.21	48.23	3.89	7.78	0.37
Mv-Magma	Heat + drought	17.60	2.35	24.30	0.28	3.94	0.02	47.93	1.72	7.73	0.40
Fatima-2	Control	28.64	2.96	18.10	0.14	7.23	0.16	35.60	1.24	6.78	0.55
Fatima-2	Heat	17.94	1.56	21.90	0.07	6.05	0.19	39.00	1.07	7.81	0.11
Fatima-2	Drought	19.63	0.75	21.80	0.21	5.02	0.61	45.80	2.30	6.50	0.26
Fatima-2	Heat + drought	12.34	1.60	26.80	0.21	4.76	0.48	52.58	1.01	6.35	0.19
Pleinsman V	Control	23.12	1.74	20.70	0.35	6.75	0.24	36.28	3.50	7.01	0.45
Pleinsman V	Heat	18.29	0.45	24.50	0.35	5.69	1.06	42.02	0.70	8.17	0.27
Pleinsman V	Drought	19.42	1.87	22.90	0.35	5.03	0.21	34.87	0.61	5.87	0.30
Pleinsman V	Heat + drought	8.67	0.64	32.00	0.14	5.10	0.51	54.70	0.67	6.52	0.83

previously, which showed that heat (Coles et al., 1997; Zhang et al., 2010), drought (Coles et al., 1997) and H + D stress (Gebruers et al., 2010) increased the amount of TOT-AX, while heat stress also increased the amount of WE-AX (Zhang et al., 2010). Coles et al. (1991) similarly reported that drought reduced the amount of β-glucan, while Savin et al. (1997) also reported negative effect of short and severe heat stress on β-glucan content.

In our study the TKW was decreased by 14.1–37.4% under heat stress, by 16.0–31.5% under drought stress and by 39.0–62.5% under H + D stress, depending on the variety (Table 1.) At the same time the β-glucan content was decreased under heat, drought and H + D stress by 15.8–16.6%, 25.5–31.4% and 24.5–47.9%, respectively. The contents of protein and TOT-AX content were increased by 18.4–21.7% and 9.5–26.8%, respectively, under heat stress and by 48.1–54.6% and 26.3–50.8% under H + D stress. Under drought stress alone the protein content was increased by 6.8–20.4% but the effect on TOT-AX varied between varieties, being reduced by 3.9% in the drought tolerant variety Plainsman V but increased by up to 28.6% in the other varieties. The content of WE-AX was also increased under heat stress (by 8.1–16.5%), but decreased under drought (by 4.2–16.3%) and H + D (by 6.4–7.0%) stress.

As the compositions of the seeds were highly correlated with the TKW (Table 2), the results were also calculated on a single kernel basis and compared with the control samples (Fig. 2.). This showed decreases in the amounts of all measured components when compared to the controls. Heat stress had the smallest effect on the compositions of all the three varieties, with Mv Magma having the most stable contents of protein and TOT-AX under heat stress. The effects of drought on TKW and on the contents of protein and β-glucan did not differ statistically from the effects of heat, but the differences in the contents of TOT-AX in heat and drought stressed grain of Fatima 2 and Plainsman V did differ significantly, as did the contents of WE-AX in heat and drought stressed grains of Mv Magma and Fatima 2. H + D stress together had the greatest effects, resulting in drastic decreases in all of the parameters that were measured (Fig. 2).

The effects of stress on the composition of wheat grain are likely to depend on the timing of the stress, as individual components differ in their temporal accumulation in the seed. Wilson et al. (2006) studied the deposition of individual cell wall polysaccharides in developing barley endosperm, showing that callose and cellulose are deposited first (between 3 and 4 days after pollination (DAP) followed by β-glucan (5 DAP), hetero-(1/4)-β-mannan (6), AGP (7 DAP) and AX (8 DAP), although there is a possibility that AX is deposited earlier. Philippe, Barron et al. (2006), Philippe, Saulnier, and Guillon (2006) showed that β-glucan is deposited in the walls of the starchy endosperm cells of wheat during the cellularization phase and later in the walls of the aleurone layer. FT-IR microspectroscopy of the cell walls in the developing starchy endosperm of wheat showed that AX was deposited only after cellularization and that, during differentiation, AX features dominated (Philippe, Barron et al., 2006; Philippe, Saulnier et al., 2006). Feruloylated AX is first deposited in the walls of the aleurone and the starchy endosperm of wheat grain at 13 DAP and continues to accumulate until the aleurone cells are differentiated at 19 DAP (Philippe, Barron et al., 2006; Philippe, Saulnier et al., 2006). Similar patterns of deposition of β-glucan and AX were reported by Toole et al. (2010). Our stress treatments started on the 12th day after heading (Zedoks-75) and continued for 15 days. This is equivalent to the period from 9 to 24 days after anthesis, when the accumulation of AX, β-glucan and protein occur. The stress treatment was started at different times after anthesis for each cultivar, but these times corresponded to the same phenophase.

Table 2
Correlation of the different compositional properties based on the results of the three varieties ($r_{5\%} = 0.5324$, $r_{1\%} = 0.6614$, $r_{0.1\%} = 0.7800$).

	TKW (g)	Protein (%)	β-Glucan (mg/g)	TOT-AX (mg/g)	WE-AX (mg/g)
TKW (g)	1.00				
Protein (%)	−0.96	1.00			
β-Glucan (mg/g)	0.72	−0.62	1.00		
TOT-AX (mg/g)	−0.64	0.58	−0.61	1.00	
WE-AX (mg/g)	0.43	−0.44	0.34	0.03	1.00
DP3	0.53	−0.52	0.66	−0.12	0.15
DP4	0.61	−0.53	0.80	−0.57	−0.03
DP3/DP4	0.03	−0.12	−0.01	0.50	0.22
DP3-4/DP5-11	0.65	−0.60	0.80	−0.36	0.18
X	−0.98	0.92	−0.66	0.70	−0.32
XX	−0.68	0.60	−0.58	0.49	−0.23
Unsubstituted (US)[a]	−0.77	0.68	−0.63	0.55	−0.26
Total (TOT)[a]	0.08	−0.12	0.05	−0.05	0.15
Monosubstituted AX (M)[a]	0.25	−0.30	0.14	−0.20	0.26
Disubstituted AX (D)[a]	−0.11	0.08	−0.07	0.16	0.01
M/D	0.62	−0.65	0.32	−0.65	0.32
US/M	−0.53	0.51	−0.39	0.42	−0.28
US/D	−0.24	0.20	−0.27	0.12	−0.17
US/M + D	−0.45	0.43	−0.36	0.34	−0.25
US/TOT	−0.45	0.42	−0.38	0.34	−0.23
M/TOT	0.72	−0.76	0.36	−0.62	0.49
D/TOT	−0.48	0.50	−0.24	0.54	−0.21

[a] Measures of the amounts of unsubstituted, mono- and di-substituted xylose residues in the AXOS are derived from the AXOS peak areas, calculated as: M = (xa3xx) + 2(xa3a3xx) + 2(xa3xa3xx) + (xa3a2 + 3x) + (xa3xa2 + 3xx) and D = (xa2 + 3xx) + (xa3a2 + 3x) + (xa3xa2 + 3xx), US = x + xx + xxx and TOT = x + xx + xxx + xa3xx + xa3a3xx + xa3xa3xx + (xa2 + 3xx) + (xa3a2 + 3xx) + (xa3xa2 + 3xx) is the total AXOS.

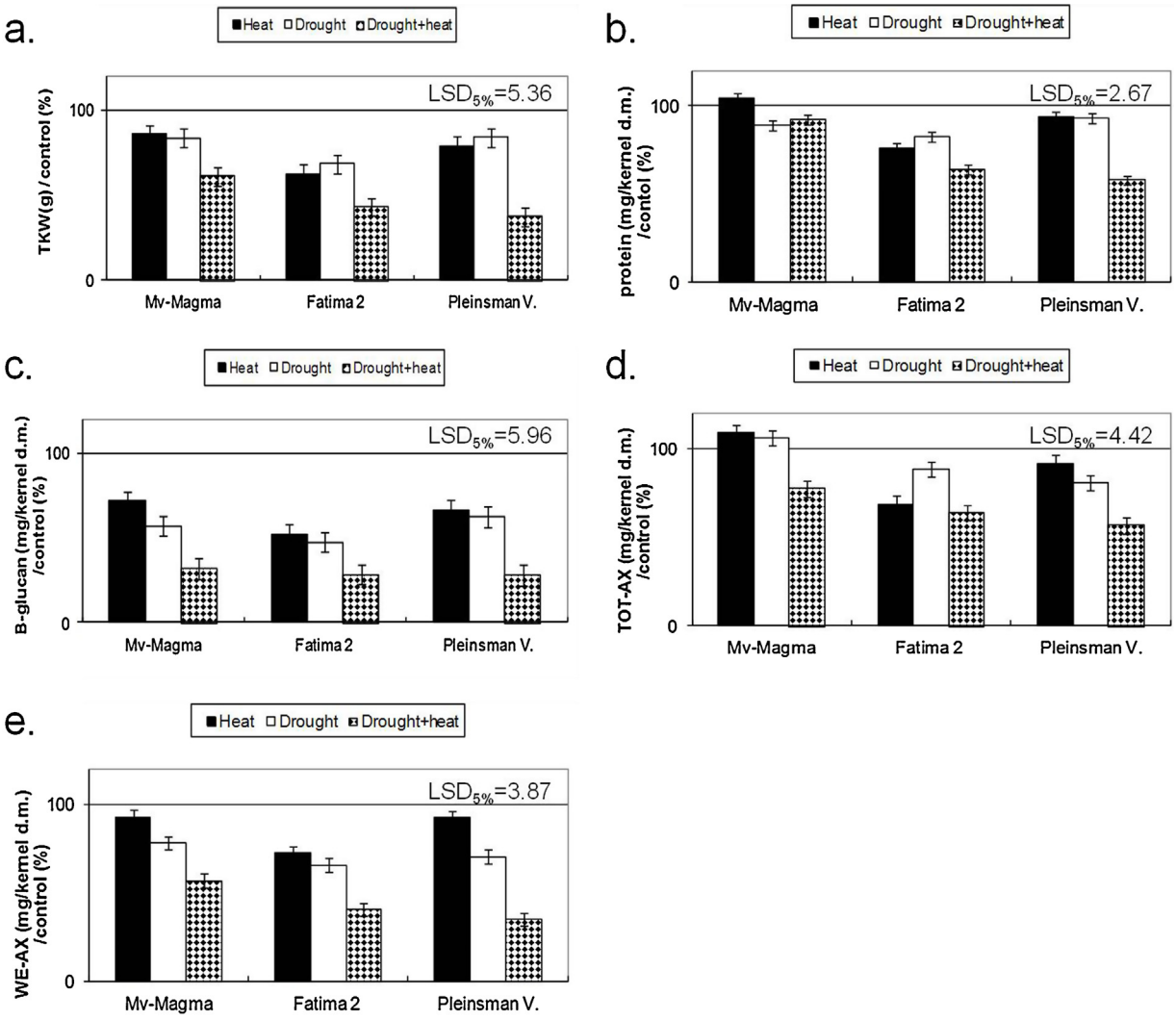

Fig. 2. Compositional properties calculated as they are found in a single kernel and related to the untreated control. (a) TKW, (b) protein, (c) β-glucan, (d) TOT-AX and (e) WE-AX content per kernel per control (%). (TKW, thousand kernel weight; TOT-AX, total arabinoxylan; WE-AX, water extractable arabinoxylan).

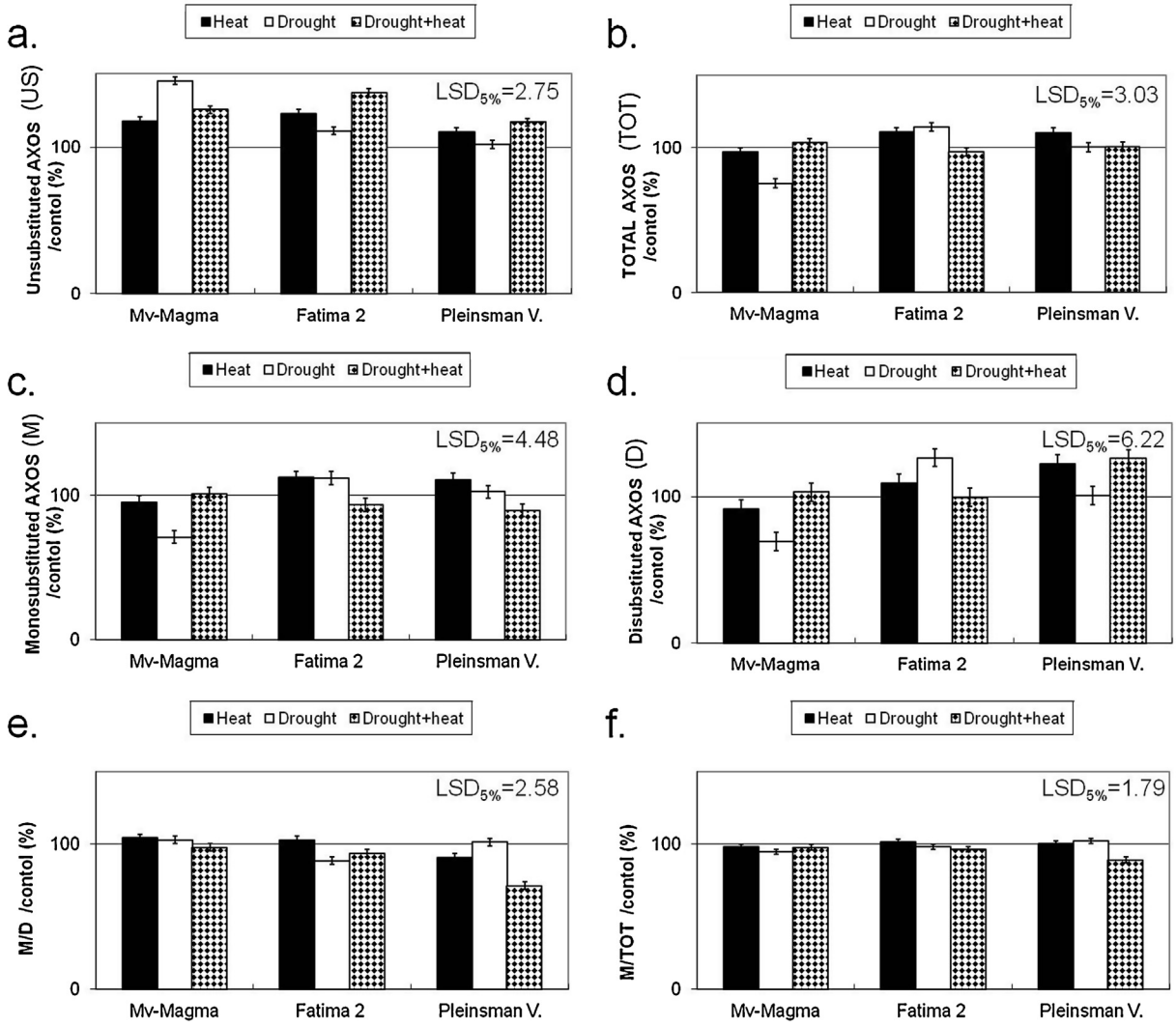

Fig. 3. Quantity of arabinoxylan units after enzymatic fingerprinting in stressed samples related to the untreated controls. (a) Unsubstituted AXOS (US), (b) total AXOS (TOT), (c) monosubstituted AXOS (M), (d) disubstituted AXOS (D), (e) M/D ratio and (f) M/TOT ratio. AXOS, arabinoxylan oligosaccharide; measures of the amounts of unsubstituted, mono- and di-substituted xylose residues in the AXOS are derived from the AXOS peak areas, calculated as M = (xa3xx) + 2(xa3a3xx) + 2(xa3xa3xx) + (xa3a2 + 3x) + (xa3xa2 + 3xx), D = (xa2 + 3xx) + (xa3a2 + 3x) + (xa3xa2 + 3xx), US = x + xx + xxx AXOS and TOT = x + xx + xxx + xa3xx + xa3a3xx + xa3xa3xx + (xa2 + 3xx) + (xa3a2 + 3xx) + (xa3xa2 + 3xx) is the total AXOS.

3.2. Amount and composition of arabinoxylan

AX is the most important dietary fiber in wheat cell walls, accounting for 1.35–2.75% dm in flour and 13.2–22.1% dm in bran (Gebruers et al., 2008). AX molecules vary in structure, including the proportions and distributions of unsubstituted, monosubstituted and disubstituted xylose residues. Information on these differences is provided by digestion with a specific endoxylanase, releasing AX oligosaccharides (AXOS) which can be separated and quantified by HP-AEC. The structures of the separated AXOS can also be assigned as described by Ordaz-Ortiz et al. (2005) and the patterns used as 'fingerprints' for the samples. The proportions of AXOS released by digestion of the different samples are shown in Figs. 3 and 4 while Fig. 5 displays the data relating to AX (the contents of TOT-AX and WE-AX and the patterns of AXOS) as a PCA plot.

The pattern of AXOS was affected by the stresses, with increases in the proportions of xylose (x) and xylobiose (xx) or of di-substituted AXOS (xa2 + 3xx) and mixed-substituted AXOS (xa3a2 + 3xx, xa3xa2 + 3xx) at the expense of monosubstituted AXOS (xa3xx, xa3a3xx, xa3xa3xx). These results are clearly reflected in the qualitative changes in the unsubstituted AXOS (US) and in the ratio monosubstituted AXOS (M) to di-(D) and to total (TOT) AXOS in Fig. 3. Variance analysis also supported this finding, as the mean values of the three varieties for unsubstituted AXOS and for M/D were significantly different between the treatments. The proportions of unsubstituted AXOS was significantly higher in all treatments than in the control samples, while the M/D values were significantly lower than the control only when H + D stress were expressed together. The M/TOT ratio behaved similarly to the M/D ratio with less difference between the drought and H + D stressed sample means.

Similar changes in AX structure, from a highly substituted form to a less substituted form, have been reported to occur in developing seeds by Philippe, Barron et al. (2006), Philippe, Saulnier et al. (2006) and Toole et al. (2007). They found that during development the rate of restructuring was faster at higher temperature with restricted water availability from 14 days after anthesis with differences in the rate of restructuring occurring between two cultivars (Toole et al., 2007). This refers to the importance of timing of the abiotic stresses released on plants during their development, which surely influencing the synthesis and structuring of the AXOS.

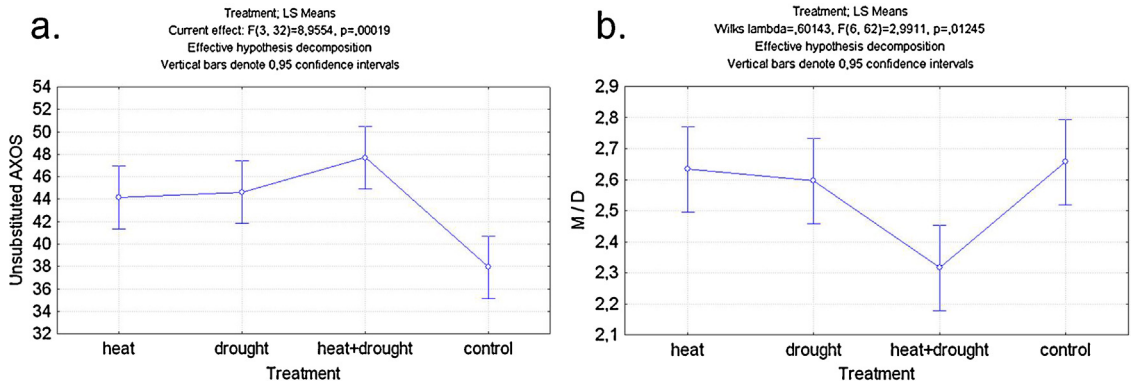

Fig. 4. One-way ANOVA analysis of arabinoxylan units after enzymatic fingerprinting. (a) Unsubstituted AXOS (US), (b) M/D ratio. (AXOS, arabinoxylan oligosaccharide; M, monosubstituted AXOS; D, disubstituted AXOS (D); US, unsubstituted AXOS). Measures of the amounts of unsubstituted, mono- and di-substituted xylose residues in the AXOS are derived from the AXOS peak areas, calculated as M = (xa3xx) + 2(xa3a3xx) + 2(xa3xa3xx) + (xa3a2 + 3x) + (xa3xa2 + 3xx) and D = (xa2 + 3xx) + (xa3a2 + 3x) + (xa3xa2 + 3xx), US = x + xx + xxx AXOS.

3.3. Amount and composition of β-glucan

β-Glucan is present in lower amounts in wheat than AX, 0.5–0.95% dm in wholemeal (Gebruers et al., 2008). As discussed above, the amount of β-glucan was lower in grain grown under heat and drought stress, which is also supported by PCA analysis (Fig. 6.). Heat and drought had similar effects on Mv Magma and Fatima 2, with the combined effects being significantly greater than the individual effects. By contrast, heat stress had a greater effect on the β-glucan content of Plainsman V than drought, which is consistent with its higher drought tolerance.

Digestion of β-glucan with lichenase releases glucooligosaccharides (GOS) with degrees of polymerization (DP) of up to about 10, with the DP3 and DP4 GOS being the major forms. The ratio of the DP3 and DP4 units varied between the samples from 1.92 to 2.30, but there were no significant differences between the three treatments or between the treatments and the control samples (Fig. 7a.). However, heat, drought and H + D, all resulted in significant deceases in the ratio of short to long units [(DP3 + DP4)/(DP5 + DP6 + DP7 + DP8 + DP9 + DP10 + DP11)], with no significant differences between the three treatments (controls 3.39–5.34, treated samples 1.76–2.83) (Figs. 6 and 7b).

3.4. Variety differences

Balla et al. (2011) have previously reported the effects of heat and drought during grain filling on the composition of protein and starch and the breadmaking quality of five winter wheat varieties (Plainsman V, Fatima 2, Mv Mambo, Mv Mariska, Bezostaja 1), three of which were included in this study. They found that drought and H + D had much greater effects on the yield and quality characters, including protein content, than heat stress alone and this was also true for the other parameters analyzed in this study. However, the differences between the effects of heat and drought were smaller in the present study.

Plainsman V is known to be drought tolerant and this was apparent in our study. Thus, while the content of TOT-AX generally increased under all stresses, drought stress had negative effects on the total content (decreasing by 3.9%) and on the water-extractability (by 16.3%) of AX in Plainsman V. By contrast, heat stress not only increased the TOT-AX content (by 15.8%) of Plainsman V but also increased the water extractability (by 16.5%). Heat and drought stress together increased the proportions of X and disubstituted (xa2 + 3xx) AXOS in Plainsman V. The effect of drought and heat were similar on the β-glucan content and structure of Plainsman V.

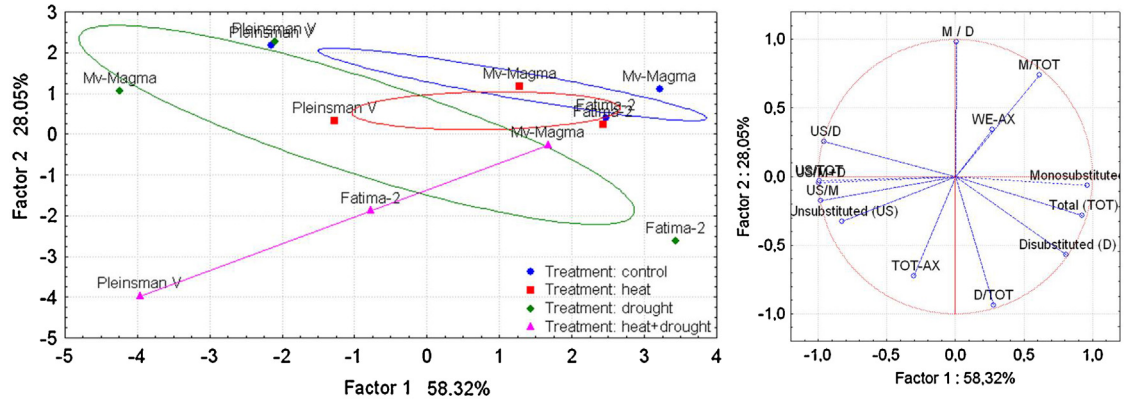

Fig. 5. Principal component analysis for the 3 varieties (Plainsman V, Mv-Magma, Fatima 2) based on enzymatic fingerprinting of arabinoxylans. Results grouped by different stress treatments and colored differently. (Unsubstituted AXOS (US), total AXOS (TOT), monosubstituted AXOS (M), disubstituted AXOS (D), M/D-, M/TOT-, D/TOT-, US/D-, US/M-, US/M + D-, US/TOT-ratios, AXOS, arabinoxylan oligosaccharide; TOT-AX, total arabinoxylan; WE-AX, water extractable arabinoxylan). Measures of the amounts of unsubstituted, mono- and di-substituted xylose residues in the AXOS are derived from the AXOS peak areas, calculated as M = (xa3xx) + 2(xa3a3xx) + 2(xa3xa3xx) + (xa3a2 + 3x) + (xa3xa2 + 3xx), D = (xa2 + 3xx) + (xa3a2 + 3x) + (xa3xa2 + 3xx), US = x + xx + xxx AXOS and TOT = x + xx + xxx + xa3xx + xa3a3xx + xa3xa3xx + (xa2 + 3xx) + (xa3a2 + 3xx) + (xa3xa2 + 3xx) is the total AXOS.

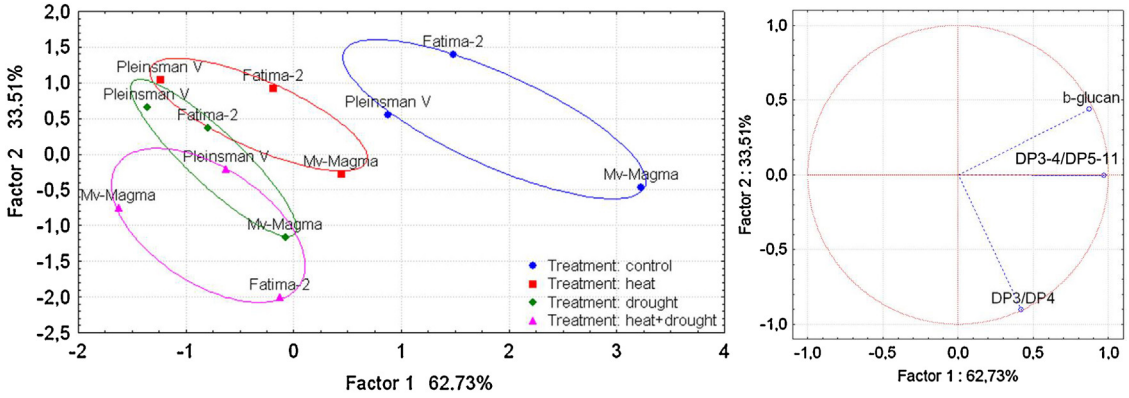

Fig. 6. Principal component analysis for the 3 varieties (Plainsman V, Mv-Magma, Fatima 2) based on enzymatic fingerprinting of β-glucan. Results grouped by different stress treatments and colored differently. (DP, degree of polymerization; DP3-4/DP5-11 is DP3 + DP4/DP5 + DP6 + DP7 + DP8 + DP9 + DP10 + DP11).

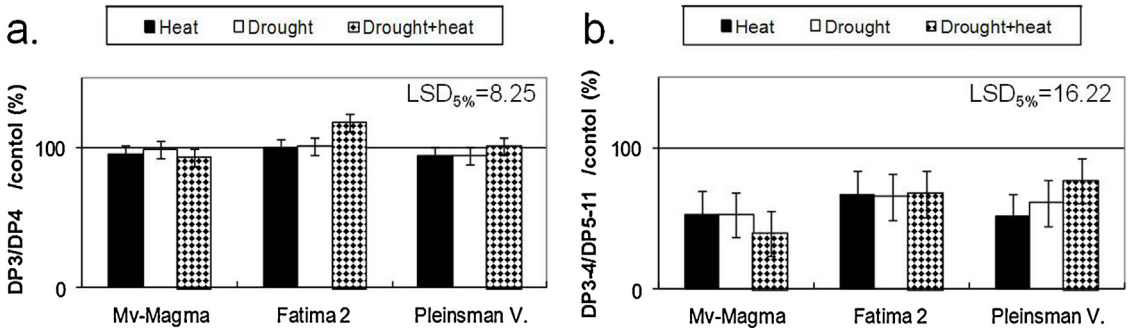

Fig. 7. Quantity of β-glucan units after enzymatic fingerprinting in stressed samples related to the untreated controls. (a) DP3/DP4, (b) DP3-4/DP5-11. (DP, degree of polymerization, DP3-4/DP5-11 is DP3 + DP4/DP5 + DP6 + DP7 + DP8 + DP9 + DP10 + DP11).

Mv Magma was the most stable to heat stress in terms of the contents of protein and TOT-AX, but drought resulted in greater effects, with decreased protein and an increase in XX released by digestion of AX. Drought also had greater effects on the proportions of mono-, di- and mixed-substituted AXOS in Mv Magma than in the other cultivars. Drought also had more significant effect on the β-glucan content of Mv Magma than heat.

Fatima 2 behaved similarly to Plainsman V with regard to its drought tolerance, but was more sensitive to heat stress which had effects on TKW and the contents of protein and TOT-AX (Fig. 2). The proportions of di- and mixed-substituted AXOS also increased due to drought, while the proportions of X and XX were only increased when H + D stress occurred at the same time. The effect of drought and heat were similar on the β-glucan content and structure of Fatima 2.

4. Conclusions

The effect of the heat and drought stress treatments applied for 15 days after the 12th day after heading were studied on the arabinoxylan and β-glucan content and structure of wheat grain.

Significant differences were found between the genotypes as regards to their tolerance to abiotic stresses, but heat and drought stress decreased the TKW, the β-glucan contents of the seeds and the quantity of the DP3 + DP4 units, while the protein and AX contents increased in all varieties. At the same time the proportions of unsubstituted AXOS and the ratio of the longer β-glucan units increased under stressed conditions. These overall statements possibly will allow an effective selection to be made during wheat breeding for higher dietary fiber content and better resistance.

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