Water deficits in wheat: fructan exohydrolase (1-FEH) mRNA expression and relationship to soluble carbohydrate concentrations in two varieties

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Received: *9 August 2008*
Accepted: *1 November 2008*

New Phytologist (2009) **181**: 843–850
doi: 10.1111/j.1469-8137.2008.02713.x

Key words: bread wheat, fructan 1-exohydrolase (1-FEH), green leaf retention, grain yield, terminal water deficit, water soluble carbohydrate (WSC).

Summary

• Terminal drought is a risk for wheat production in many parts of the world. Robust physiological traits for resilience would enhance the preselection of breeding lines in drought-prone areas.

• Three pot experiments were undertaken to characterize stem water-soluble carbohydrate (WSC), fructan exohydrolase expression, grain filling and leaf gas exchange in wheat (*Triticum aestivum*) varieties, Kauz and Westonia, which are considered to be drought-tolerant.

• Water deficit accelerated the remobilization of stem WSC in Westonia but not in Kauz. The profile of WSC accumulation and loss was negatively correlated with the mRNA concentration of 1-FEH, especially *1-FEH w3* (*1-FEH-6B*). Under water deficit, Westonia showed lower concentrations of WSC than Kauz but did not show a corresponding drop in grain yield.

• The results from pot experiments suggest that stem WSC concentration is not, on its own, a reliable criterion to identify potential grain yield in wheat exposed to water deficits during grain filling. The expression of *1-FEH w3* may provide a better indicator when linked to osmotic potential and green leaf retention, and this requires validation in field-grown plants.

Introduction

Grain filling in cereals depends on carbon from two sources, assimilates transferred directly to the grain and assimilates redistributed from reserve pools in vegetative tissues (Pheloung & Siddique, 1991; Kobata *et al.*, 1992; Schnyder, 1993). Reserve pools provide the substrate needed to maintain transport and supply of assimilate to grain during the dark period of the diurnal cycle and especially during mid–late grain-filling. In the latter period, the rate of dry matter accumulation in grain exceeds the rate of dry matter accumulation of the whole crop (Schnyder, 1993; Foulkes *et al.*, 2002). Soil drying after anthesis accelerates mobilization of stored carbohydrate reserves to the grain, which tends to induce a shorter but more intensive period of grain filling (Gallagher *et al.*, 1976; Bidinger *et al.*, 1977; Austin *et al.*, 1980; Yang *et al.*, 2000; Yang & Zhang, 2006).

The stems of rain-fed wheat have significantly higher average total carbohydrate (1.8-fold) and fructan (2.5-fold) concentrations compared with irrigated wheat (Kerepesi & Galiba, 2000; Conocono, 2002; Goggin & Setter, 2004). It has been estimated that pre-anthesis reserves contribute up to 74 and 57% of the grain yield of barley and wheat (Gallagher *et al.*, 1976), respectively, when crops suffered from post-anthesis water deficit (defined as terminal water deficit). Under terminal water deficit, the impact of shoot carbohydrate remobilization has a greater significance because post-anthesis assimilation is limited and grain growth depends, to a greater extent, on the translocation of carbohydrate reserves (Davidson & Birch, 1978; Nicolas *et al.*, 1985; Palta *et al.*, 1994; Blum *et al.*, 1997). Under terminal water deficit, there is generally an association between yield and high shoot carbohydrate concentrations at flowering (Nicolas & Turner, 1993; Blum *et al.*, 1997; Foulkes *et al.*, 2007; Xue *et al.*, 2008), but there is an inconsistent correlation between high stem water-soluble carbohydrate (WSC) and grain yield (Evans & Wardlaw, 1996; Ehdaie *et al.*, 2006; Ruuska *et al.*, 2006).

Fructan is the dominant form of long-term carbohydrate storage in the vegetative parts of temperate grasses and cereals (Blacklow *et al.*, 1984; Kühbauch & Thome, 1989; Turner *et al.*, 2008). At the stage of maximum WSC content, fructans represent 85% of the WSC in wheat stem internodes (Blacklow *et al.*, 1984; Turner *et al.*, 2008). It is significant that water deficit-resistant cocksfoot (*Dactylis glomerata*) manufactures more high DP fructans than related water deficit-sensitive plants under water-deficit conditions (Volaire & Lelièvre, 1997). The involvement of fructans in water deficit and cold tolerance has been suggested repeatedly (Wiemken *et al.*, 1995). The biosynthesis of fructans includes the enzymes fructosyltransferases and fructan exohydrolases (FEHs) (Goggin & Setter, 2004; Xue *et al.*, 2008). FEHs include 1-FEHs and 6-FEHs. Sucrose inhibits *1-FEH w1*, *w2* and *w3* involved in the degradation of fructan and therefore, when there is excess sucrose, the degradation of fructans does not occur (Van den Ende *et al.*, 2004). When the demand for grain filling is high and sucrose becomes limiting, fructans are degraded by 1-FEHs to release more sucrose and fructose. Therefore, *1-FEH w1*, *w2* and *w3* are very important for maintaining the flow of carbon required for grain filling. By contrast, 6-FEH is not inhibited by sucrose, suggesting that it might not be involved in reserve mobilization (Van Riet *et al.*, 2006). *1-FEH* is a focus for the current paper because it is postulated to be important not only during the period of fructan breakdown but also as a putative β-(2,1)-trimmer during the period of active fructan biosynthesis (Van den Ende *et al.*, 2003). *1-FEH* activities are elevated during the fructan breakdown phase in wheat stems (Van den Ende *et al.*, 2003; Yang *et al.*, 2004; Kawakami *et al.*, 2005; Van Riet *et al.*, 2008).

Terminal drought is an increasing risk for wheat production in many parts of the world, especially those with a Mediterranean-type climate. Given the uncertainty of the usefulness of the stem WSC content, more robust physiological, biochemical or molecular traits are required to rapidly pre-screen wheat lines for resilience to terminal drought. Therefore, this study was undertaken to assess the correlation between the concentration of stem WSC and grain yield; to assess the expression of 1-FEH genes during remobilization of stem WSC; and to define leaf water relations during grain fill in two wheat varieties subjected to terminal water deficit. The varieties were chosen because they are high-yielding and have high concentrations of WSC.

Materials and Methods

Experiment design and cultivars

Three water-deficit experiments were carried out in pots over 2 yr using a split-pot design (well-watered/water deficit from anthesis) with three replications. Expt 1 (2005) was a preliminary experiment using four wheat (*Triticum aestivum* L.) cultivars, Bt-Schomburgh, Janz, Kauz and Westonia. Westonia has consistently high yields in the medium and low rainfall regions of Western Australia and had high concentrations of shoot carbohydrates during flowering (40 ± 5% of dry weight) (Conocono, 2002). Kauz has a high concentration of shoot carbohydrates (*c.* 40%) and is considered to be drought-tolerant (Rajaram *et al.*, 2002). Janz and Bt-Schomgurgh showed a lower yield (*c.* 2 t ha^{-1}) in Western Australia (Littlewood & Garlinge, 2002). In 2006, two water-deficit experiments were carried out to confirm and expand the 2005 results on three cultivars. Bt-Schomburgh was too heterogeneous and was excluded. Kauz and Westonia were of particular interest to define molecular targets that may influence concentrations of stem WSC in relation to water deficit in Expt 2. Expt 3 (2006) repeated Expt 2 for yield parameters.

General procedures

Plants were grown in glasshouse facilities at Murdoch University. Seeds were pre-germinated by soaking for 19 d at 4°C. Pre-germinated seeds were planted in 4 l free-draining pots filled with potting mix to give six plants per pot. The potting mix consisted of two parts composted pine bark, one part coconut fibre peat and one part river sand. Basal fertilizers (mg kg^{-1}: 1220 N, 368 P, 819 K plus a standard micronutrient mix) were incorporated into the potting mix. Pots were watered three times a day, using mats underneath the pots, until anthesis. In Expts 1 and 2, water was withheld from half the pots (water deficit) 1 d after anthesis while the other half were kept well watered (following the same regime as before anthesis) until maturity. In well-watered plants of Westonia and Kauz, water was withheld after 47 and 44 d post-anthesis (dpa), respectively, in Expt 1, and 39 and 37 dpa, respectively, in Expts 2 and 3. Weights of unwatered pots were recorded daily to determine the gravimetric water content (Fig. 1). In Expt 3, pots for the water-deficit part of the experiment were wrapped individually in plastic bags at anthesis to reduce evaporative water loss, and water was added to maintain the water content between 33 and 40% of the well-watered control.

Pots were rotated every other day to ensure that all plants received equal radiation exposure. Glasshouse temperature ranged from 15°C (night) to 35°C (day). The roof screen was set at 500 μmol m^{-2} s^{-1}, giving a range at midday from 400 to 700 μmol m^{-2} s^{-1}. The humidity was 60–70%.

Plant harvest

For WSC analysis and RNA extraction, plants of Expts 1 and 2 were harvested 2 wk before anthesis and then weekly after anthesis to grain maturity, between 11:00 and 17:00 h as previously described (Zhang *et al.*, 2008). Dry weights of the main stems, tillers and their respective ears were recorded. The leaf sheath was included with the stem.

At maturity, main stem and tiller ears were weighed separately and the spikelet and floret number determined. The ears were threshed manually, grains per main stem and tillers were

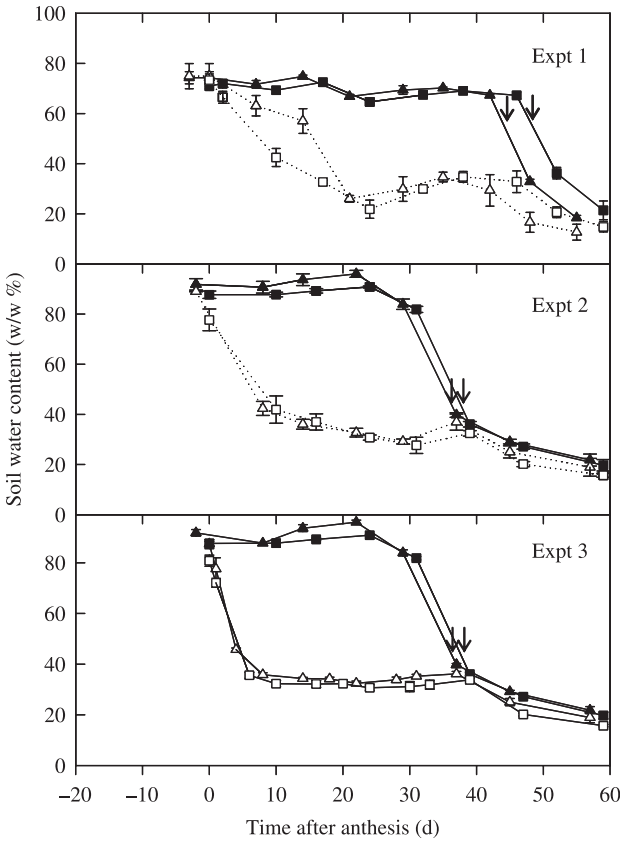

Fig. 1 Soil water profiles for the water-deficit Expts 1, 2 and 3. Soil drying commenced at anthesis (day 1) for the spring wheat (*Triticum aestivum*) varieties, Kauz (triangles) and Westonia (squares). Closed symbols, well-watered; open symbols, water deficit; vertical bars represent ± SE of the mean of three replicates; arrows indicate the time of water withdrawal from well-watered plants.

counted and then oven-dried (75°C) for 3 d, and the dry weight was determined.

Carbohydrate analysis

Sample preparation was as described in Zhang *et al.* (2008). Carbohydrates were extracted from the stem (sheath included) using boiling deionized water and quantified by colorimetry using the anthrone reagent (Fales, 1951; Yemm & Willis, 1954). Fructose was used as standard sugar.

Leaf water relations

Leaf water potential (Ψ_{leaf}) was measured weekly after anthesis on two leaves for each pot using a pressure chamber (Model 3000, Soil Moisture Corp., Santa Barbara, CA, USA) in Expt 2. Well-illuminated tiller flag leaves were chosen randomly for this measurement.

To determine leaf osmotic potential (Ψ_π), in Expt 2, main stem flag leaves were removed, placed in sealed plastic bags on dry ice, then stored at −80°C. Four main stem flag leaves from each pot were cut into pieces and homogenized when frozen. Ψ_π was measured on expressed sap with a Fiske 1/10 Osmometer (Fiske Associates Norwood, MA, USA).

Green leaf retention

Flag leaves from the main stem in Expt 2 were cut into small pieces (*c.* 1 mm wide) and the pigments were extracted by shaking in 20 ml of 85% acetone at 20°C overnight. The absorbance of the extract was measured with a B&L Spectronic 20 spectrophotometer at 663 and 644 nm and the concentrations of chlorophyll *a* and *b* were calculated (Arnon, 1949).

Photosynthesis

Measurements of gas exchange were made on flag leaves in Expt 2 using a CIRAS-2 infrared gas analyser (IRGA) (PP Systems, Hitchin, UK) connected to a PLC-6 Parkinson leaf cuvette. Photosynthesis rate (A), transpiration rate (E) and stomatal conductance (g_s) were calculated according to von Caemmerer & Farquhar (1981). All measurements were taken under constant light intensity set to 1500 μmol mol^{-1} photosynthetic photon flux density (PPFD) with the PLC-6 internal halogen light attachment. The cuvette environment was maintained at the following parameters: CO_2 concentration was set at 360 ppm, cuvette H_2O supplied at 8.2 millibars and cuvette temperature maintained at 29°C, which was representative of glasshouse temperature during the measure-ment period.

RNA extraction and real-time PCR

Total RNA was extracted from the main stem (sheath included) as described in Zhang *et al.* (2008). Quantitative reverse transcription-PCR (qRT-PCR) was carried out using the Corbert Rotor-Gene RG-3000 (Corbett Research, Queensland, Australia). qRT-PCR reactions were performed in triplicate with the Power SYBR® Green PCR Master Mix and contained 1 μl of a 1 : 500 or 1 : 50 dilution of template cDNA. The PCR conditions used were one cycle at 95°C for 10 min, 40 cycles at 92°C for 15 s and 58°C for 60 s. Cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcript concentration in the different samples were used to normalize the amount of FEH transcripts. Specific primer pairs were designed based on single nucleotide polymorphisms (SNPs) in exon 3 of the three genes (Zhang *et al.*, 2008). Gene expression was quantified using a standard relative standard curve method (as recommended by Corbett Research).

Statistical analysis

STATGRAPHICS Centurian XV (Statistical Graphics Corporation, Princeton, NJ, USA) was used for ANOVA. Post-hoc comparisons were conducted using Duncan's multiple range test at $P = 0.05$.

Table 1 Plant grain weight and ratio comparisons in well-watered (–) and water-deficit (+) treatments of four wheat (*Triticum aestivum*) varieties

		Janz		Kauz		Westonia		Bt-Schomburgh	
		–	+	–	+	–	+	–	+
Grain weight[c] (g per plant)	Expt 1	4.1 a[a] (0.2)	3.43 bc (0.2) 0.84[b] a (0.03)	4.08 a (0.4)	3.36 bc (0.2) 0.83 a (0.05)	3.9 ab (0.4)	3.3 c (0.3) 0.84 a (0.02)	3.88 ab (0.06)	2.86 c (0.29) 0.74 a (0.06)
	Expt 2	3.64 b	2.5 c (0.1) 0.70 a (0.04)	4.93 a (0.1)	2.42 c (0.1) 0.49 b (0.02)	4.3 ab (0.5)	2.7 c (0.1) 0.64 a (0.03)	–	–
	Expt 3	3.4 b (0.3)	1.3 c (0.1) 0.44 a (0.02)	4.4 a (0.2)	1.2 c (0.1) 0.27 b (0.01)	3.4 b (0.3)	1.4 c (0.1) 0.42 a (0.04)	–	–

( ), ± SE.
[a]Mean ± SE, values with the same letter are not different at $P = 0.05$.
[b]Ratio of water deficit (+) versus well-watered (–) plants.
[c]Total grain weight.
Bt-Schomburgh was not included in Expts 2 and 3 because it was heterogeneous.

Table 2 Plant physiological responses to well-watered (–) and water deficit (+) treatments in two wheat (*Triticum aestivum*) varieties

	Kauz		Westonia	
	–	+	–	+
Time after anthesis (d)	27	27	28	28
Chlorophyll content (mg g^{-1} DW)	4.69 ± 0.92 a[a]	1.5 ± 0.02 b	4.5 ± 0.53 a	1.5 ± 0.14 b
Tiller leaf water potential (MPa)	–0.55 ± 0.07 a	–3.4 ± 0.3 c	–0.4 ± 0.06 a	–3.0 ± 0.12 bc
Main stem flag leaf osmotic potential (MPa)	–0.87 ± 0.01 a	–1.64 ± 0.07 bc	–1.2 ± 0.03 ab	–2.41 ± 0.12 d
Main stem osmotic potential (MPa)	–1.18 ± 0.04 a	–1.79 ± 0.07 b	–1.21 ± 0.04 a	–2.23 ± 0.32 b
Photosynthesis rate (*A*) (CO_2 mol m^{-2} s^{-1})	15.27 ± 0.77 ab	–0.38 ± 0.28 c	14.05 ± 0.72 b	0.4 ± 0.4 c
Transpiration rate (*E*) (mmol m^{-2})	4.64 ± 0.28 a	0.44 ± 0.06 c	3.68 ± 0.11 b	0.44 ± 0.09 c
Instantaneous water-use efficiency (*A*/*E*)	3.30 ± 0.15 a	–1.08 ± 0.89 c	3.82 ± 0.16 a	1.13 ± 1.15 b
Main stem water-soluble carbohydrate (% DW)	12.6 ± 2.35 a	9.04 ± 3.08 abc	11.37 ± 0.72ab	5.86 ± 0.94 bc

[a]Mean ± SE, values with the same letter are not different at $P = 0.05$.

Results

Physiological responses to water deficit

Grain yield depression Water deficit depressed the grain weight of Kauz and Westonia in all three experiments (Table 1). In Expts 2 and 3, the grain weight ratio (water deficit: well-watered) of Kauz (0.49; 0.27) was significantly lower ($P < 0.05$) than Westonia (0.64; 0.42) (Table 1). In Expt 1, there was no difference in the grain weight ratio between varieties, presumably because of the moderate water deficit (Fig. 1). In well-watered plants in Expt 2, the grain weight of Kauz (4.9 g per plant) was similar to that of Westonia (4.3 g per plant) and higher than that of Janz (3.64 g per plant). In Expt 3 well-watered plants, the grain weight of Kauz (4.4 g per plant) was higher ($P < 0.05$) than those of Westonia (3.4 g per plant) and Janz (3.4 g per plant) (Table 1). There was no significant difference between Westonia and Janz in both well-watered and water-deficit treatments (Table 1). Expt 1 showed that Bt-Schomburgh was heterogenous. For further study, Westonia and Kauz were chosen for detailed analysis.

Photosynthesis Photosynthesis rates (*A*) in the two wheat varieties were initially similar and then declined, as the soil water content declined from 80 to 34% (Fig. 1). The leaf transpiration rate of both varieties also decreased sharply from 5 – 8 to 0.5 mmol m^{-2} and then remained above 0 (data not shown). Comparison of Kauz and Westonia at 26.8 and 28 d after anthesis (Table 2), when they had comparable chlorophyll contents of 1.5 mg g^{-1}, showed that *A* for Westonia was 0.4 μmol m^{-2} s^{-1} while that for Kauz had fallen to –0.38 μmol m^{-2} s^{-1}.

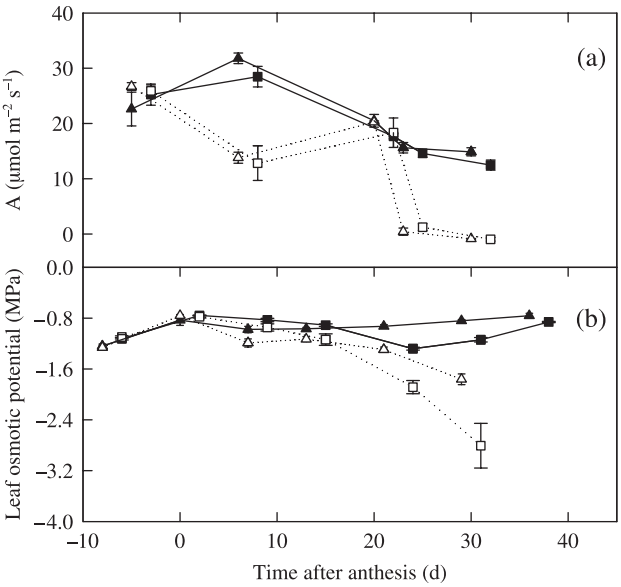

Fig. 2 Dehydration phenotypes of the spring wheat (*Triticum aestivum*) varieties Kauz and Westonia under the same water deficit in Expt 2 plotted against time after anthesis. (a) Flag leaf photosynthesis potential (*A*); (b) changes in the main stem flag leaf osmotic potential. Triangles, Kauz; squares, Westonia; closed symbols, well-watered; open symbols, water deficit; vertical bars represent ± SE of the mean of three replicates.

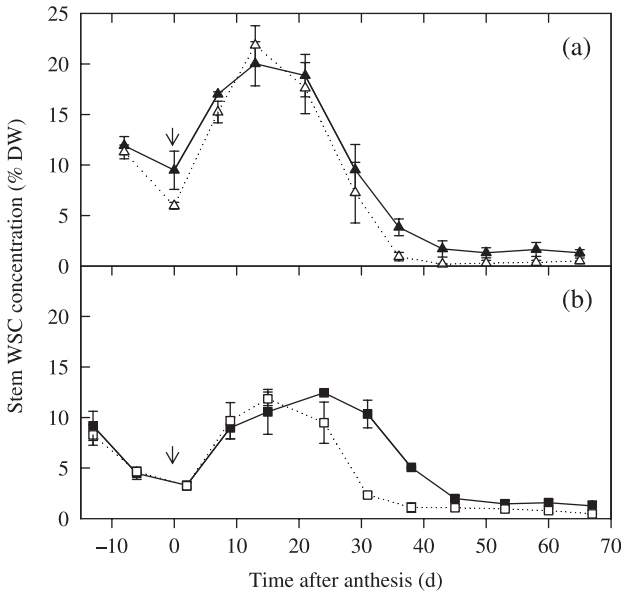

Fig. 3 Change in stem water soluble carbohydrate (WSC) concentration with time after anthesis in Expt 2 in spring wheat (*Triticum aestivum*) varieties, (a) Kauz and (b) Westonia; closed symbols, well-watered; open symbols, water deficit; arrow, flowering; vertical bars represent ± SE of the mean of three replicates.

Green leaf retention Even though the soil water contents were similar (Fig. 1), Kauz visually senesced faster than Westonia; that is to say, no green leaves remained at 36 d post-anthesis, whereas the tiller leaves of Westonia remained green at 39 d post-anthesis. The flag leaf chlorophyll content (FLC) in water-deficit plants declined 1 d earlier in Kauz than in Westonia (26.8 versus 28 d post-anthesis), falling to 1.5 mg g^{-1} (Table 2). At the same time, in well-watered plants, the leaf chlorophyll contents of Kauz and Westonia were similar at 4.7 and 4.5 mg g^{-1}, respectively (Table 2).

Main stem and flag leaf osmotic potential (LOP) Using an FLC of 1.5 mg g^{-1} dry weight as a point of comparison (by extrapolation) under water deficit, the flag leaf Ψ_π (−2.4 MPa) of Westonia was significantly lower ($P < 0.05$) than that of Kauz (−1.64 MPa) (Fig. 2b). The main stem Ψ_π values of Westonia and Kauz were −2.23 and −1.79 MPa, respectively, but they were not significantly different (Table 2). In well-watered plants, the flag leaf Ψ_π was between −0.8 and −1.3 MPa (Fig. 2b; Table 2) and did not differ between the two varieties.

Stem WSC concentration in relation to grain dry weight

Under water-deficit conditions (Expt 2), the timing of the drop in stem WSC concentrations was advanced by 9 d in Westonia but was unchanged in Kauz, relative to well-watered plants. The stem WSC of Kauz and Westonia reached a peak at 13 and 15 dpa at values of 21.9 and 11.9% (Figs 3, 4), respectively. The total WSC contents of Kauz and Westonia were 140 and 50 mg per stem, respectively. The stem WSC concentration and total WSC content of Kauz were higher ($P < 0.05$) than those of Westonia. However, the total grain weight per plant and the main stem grain weight of these two varieties were similar.

In well-watered plants in Expt 2, the stem WSC concentration and the total stem WSC of Kauz (20%, 145 mg per stem) were higher ($P < 0.05$) than those of Westonia (12.5%, 55 mg per stem) (Figs 3, 4) and peaked at 13 and 24 dpa, respectively. The main grain weight of Kauz (1.4 g per ear) was higher ($P < 0.05$) than in Westonia (0.9 g per ear). However, the grain weight per plant of Kauz (4.9 g per plant) was similar to that of Westonia (4.3 g per plant).

In Expt 1, the time to reach a peak concentration of WSC in Westonia was 14 d earlier in water-deficit plants relative to well-watered plants, while the time to peak WSC for Kauz did not differ with treatment (Supporting Information, Fig. S1). The maximum stem WSC concentration of Kauz (25.7%) was higher than in Westonia (18.7%) (Fig. 4). The total stem WSC of Kauz (398 mg per stem) was also significantly higher than that of Westonia (223 mg per stem). The main stem grain weight of Kauz (1.8 g per ear) was greater ($P < 0.05$) than Westonia (1.1 g per ear) (Fig. 4). However, the grain weights per plant were similar (Fig. 4).

Correlation of WSC and 1-FEH gene expression

Three forms of FEH are evaluated in this manuscript: *1-FEH w1* (*1-FEH-6A*), *1-FEH w2* (*1-FEH-6D*) and *1-FEH w3*

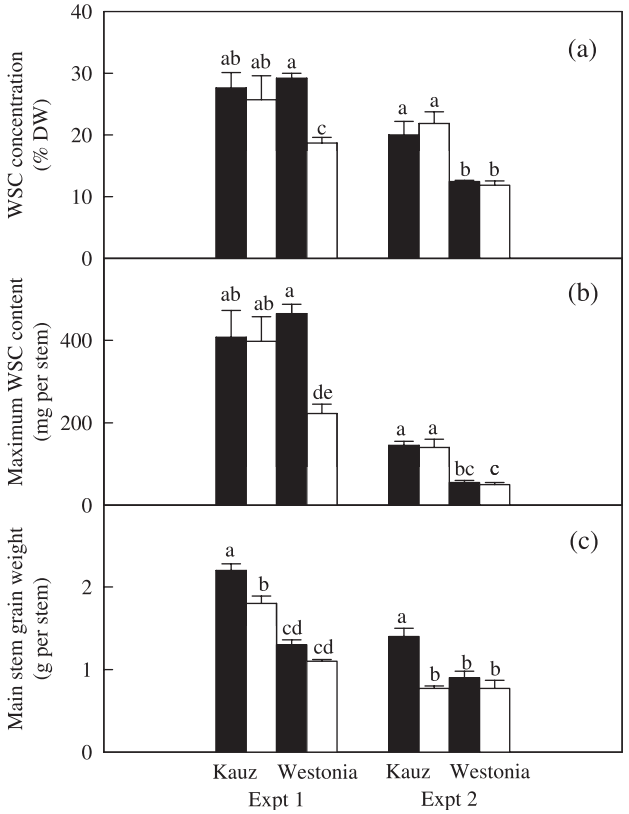

Fig. 4 The main stem water-soluble carbohydrate (WSC) and total WSC content at the maximum values (see Fig. 3 and Fig. S1) shown in relation to the main stem grain weight in well-watered (closed bars) and water-deficit treatments (opens bars) in spring wheat (*Triticum aestivum*) varieties Kauz and Westonia. Bars with the same letter are not different at $P < 0.05$ (see Tables 1, 2). The vertical bars represent ± SE of the mean of three replicates. (a) Comparisons of maximum WSC concentration; (b) comparisons of maximum WSC content; (c) comparisons of main stem grain weight.

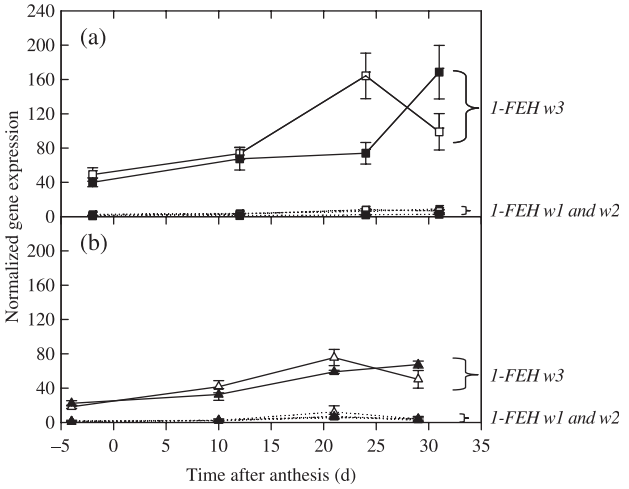

Fig. 5 1-FEH gene expression in the stem (sheath included) following anthesis in spring wheat (*Triticum aestivum*) varieties Kauz and Westonia. Gene transcription accumulation was assayed using qRT-PCR on tissue from either water-deficit (open symbols) or well-watered (closed symbols) plants. *1-FEH w1* (*1-FEH 6A*) (dash line), *1-FEH w2* (*1-FEH-6D*) (dashed line) and *1-FEH w3* (*1-FEH-6B*) (solid line) gene expression patterns measured in (a) Westonia and (b) Kauz. Vertical bars represent ± SE of the mean of three replicates.

(*1-FEH-6B*). These showed differences in expression of mRNA both within and between varieties in different treatments. For Westonia, water deficit activated the accumulation of *1-FEH w3* earlier, compared with well-watered plants, whereas it remained unchanged in Kauz. The qRT-PCR results (Fig. 5) showed that *1-FEH w3* (*1-FEH-6B*) had significantly higher concentrations of messenger RNA compared with the concentrations of *1-FEH w1* (*1-FEH-6A*), and *1-FEH w2* (*1-FEH-6D*). The expression of *1-FEH w3* (*1-FEH-6B*) in Westonia was *c.* 40 units 2 d before anthesis and continued increasing steadily in both water-deficit and well-watered plants. Twelve days after anthesis, it increased in water-deficit plants, reaching a maximum of *c.* 170 units at 24 dpa, and then decreased. By contrast, in well-watered plants, the concentrations of *1-FEH w3* (*1-FEH-6B*) continued to increase after the 24 dpa reference point. The expression of *1-FEH w3* (*1-FEH-6B*) in Kauz was *c.* 20 units at 4 d before anthesis and steadily increased up to *c.* 40 units at 10 dpa, and then increased at a faster rate until the maximum of *c.* 80 units in both water-deficit and well-watered plants. The accumulation of *1-FEH w1* (*1-FEH-6A*) and *1-FEH w2* (*1-FEH-6D*) were relatively low in both water-deficit and well-watered Kauz and Westonia, the highest reaching *c.* 10 units.

The accumulation of *1-FEH w1* (*1-FEH-6A*), *1-FEH w2* (*1-FEH-6D*) and especially, *1-FEH w3* (*1-FEH-6B*) was negatively correlated with the pattern of WSC variation at the same stage (Figs 3, 5). The WSC concentration of Westonia decreased 15 dpa in water-deficit plants while the accumulation of *1-FEH w3* (*1-FEH-6B*) increased. In well-watered plants, the stem WSC concentration in Westonia dropped at *c.* 24 dpa, while the accumulation of *1-FEH w3* (*1-FEH-6B*) started increasing. On the other hand, the WSC concentration of Kauz in water-deficit plants was similar to that of well-watered plants and the expression of *1-FEH w3* (*1-FEH-6B*) in Kauz was not affected by water deficit.

As noted before, the stem WSC concentration of Westonia (water-deficit, 11.9%; well-watered, 12.5%) was significantly lower than that of Kauz (water-deficit, 21.9%; well-watered, 20%) (Fig. 4). The lower WSC concentration in Westonia reflects the higher expression of *1-FEH w3* in this variety.

Discussion

Genotype variation in response to water deficit

The wheat varieties Kauz and Westonia provided a useful comparison here because, during the course of this study, they appeared to respond differently to water deficit. Under

water-deficit conditions, Kauz senesced earlier than Westonia. The remobilization of stem WSC in Westonia was accelerated by water deficit while it was similar in both water-deficit and well-watered treatments in Kauz. Accordingly, under water deficit, the main stem grain weight of Westonia was similar to that under the well-watered treatment while the grain weight of Kauz was significantly reduced by water deficit.

It was evident from the experiments in this study that the concentrations of WSC did not correlate well with grain weight under water deficit. In the more severe water-deficit conditions of Expt 2, for example, the stem WSC concentration and the total WSC concentration of Kauz were significantly higher relative to Westonia under water deficit. However, the related main-stem grain weight and the whole-plant grain weight of Kauz were not different from Westonia. Similar results were observed in well-watered plants in this study and match observations by other workers (Evans & Wardlaw, 1996; Ehdaie *et al.*, 2006; Ruuska *et al.*, 2006; Turner *et al.*, 2008). The data in the present study indicate that differences in remobilization rates of stem WSC are more important than the absolute concentrations of WSC.

The main storage form of WSC in the stem and leaf sheath is fructan (Blacklow *et al.*, 1984; Kühbauch & Thome, 1989; Gebbing *et al.*, 1998). During the mobilization of fructan from stems and subsequent gain in grain weight, *1-FEH* activity increases considerably (Wardlaw & Willenbrink, 2000; Van den Ende *et al.*, 2003). Water deficit substantially enhanced the activities of FEH and this correlated with the reduction in WSC in the stem, consistent with increased remobilization from the stem to the grain (Yang *et al.*, 2004). Also consistent with these observations is the demonstration that the normalized gene expression of *1-FEH w2* (Van Riet *et al.*, 2006) and *1-FEH w3* (Van Riet *et al.*, 2008) showed maximal accumulation in the stem relative to other organs during grain filling in wheat.

The mRNA accumulation patterns of *1-FEH w1*, *w2* and especially *w3* in this study in Kauz and Westonia were negatively related to the pattern of high value of stem WSC (mainly fructan) accumulation assayed in the same period. For example, in Westonia, the stem WSC concentration in water-deficit plants dropped earlier compared with the well-watered plants. Accordingly, the accumulation of *1-FEH w3* increased earlier relative to the well-watered plants. This mRNA accumulation result correlates with available studies on FEH enzyme activities (Wardlaw & Willenbrink, 2000; Van den Ende *et al.*, 2003; Yang *et al.*, 2004) and confirms that FEH is positively correlated with the total fructan remobilization from wheat stems (Wardlaw & Willenbrink, 2000; Yang *et al.*, 2004; Yang & Zhang, 2006).

The mRNA accumulation of *1-FEH w3* in Kauz was slightly higher in water-deficit relative to well-watered plants. By contrast, in Westonia, the concentration of *1-FEH w3* mRNA in water-deficit plants was double that in well-watered plants. This indicated that the *1-FEH w3* was activated by water deficit in Westonia but not in Kauz. It is important to note that the concentrations of *1-FEH w3* mRNA were 10–20 times those of *1-FEH w1* and *w2*, which indicated that *1-FEH w3* is potentially the key gene driving fructan mobilization (Zhang *et al.*, 2008). The expression of *1-FEH w3* in Westonia was double that in Kauz, and this might explain the relatively low amount of stem WSC in Westonia, compared with Kauz, in both water-deficit and well-watered conditions. Consistent with this interpretation, osmotic potentials indicated that Westonia had a higher solute content in the main-stem flag leaf than did Kauz under water deficit. Even though *1-FEH w1* and *w2* were slightly higher in water-deficit plants compared with well-watered plants for both Kauz and Westonia, the highest values attained were below 10 units.

In conclusion, this study has demonstrated that the mRNA concentration of *1-FEH w3* was consistently associated with the concentration of stem WSC and the efficiency of the apparent remobilization of the stem WSC in two different varieties of wheat. The expression of 1-*FEH w3*, which has significant function in the degradation of fructan, was accelerated by terminal water deficit in Westonia but not in Kauz. This result indicates that the amount of stem WSC mobilized during grain filling is not only dependent on the concentration of the stem WSC but also on the efficiency of the remobilization process. Apart from green leaf retention and high stem WSC concentration for pre-screening water-deficit resistance in wheat, the dominant expression of *1-FEH w3* might be another indicator of the high efficiency of the remobilization of WSC.

Acknowledgements

We thank Murdoch University and the Molecular Plant Breeding CRC for providing financial support for this project.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Stem water-soluble carbohydrate (WSC) concentration and the related grain dry weight per ear plot with time after anthesis in Expt 1.

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