A marker for stem water soluble carbohydrate remobilization to grain under drought

Jingjuan Zhang[1], Yunji Xu[2], Wei Chen[3], Bernard Dell[1], Rudy Vergauwen[4], Ben Biddulph[5], Nusrat Khan[1], Hao Luo[1], Rudi Appels[1], Wim Van den Ende[4] ✉

[1]School of Veterinary and Life Sciences, Murdoch University, South Street, Murdoch, WA, Australia, 6150; [2]School of Agriculture, Yangzhou University, 48 Wenhui East Road, Yangzhou, Jiangsu, China, 225009; [3]Institution of Soil and Water Conservation, Chinese Academy of Science and Ministry of Water Resources, 26 Xinong Road, Yangling, Shaanxi, 712100; [4]Lab of Molecular Plant Biology, Institute of Botany and Microbiology, KU Leuven, Kasteelpark Arenberg 31, B-3001 Leuven-Heverlee, Belgium, 2434; [5]Department of Agriculture and Food Western Australia, 3 Baron Hay Court, South Perth, WA, Australia 6151

Key Messages

- Genetic variation is involved in stem water soluble carbohydrate (WSC) (mainly fructan) remobilization to grain under drought.
- A key enzyme (1-FEH w3) degrades 2-1 linkages in fructan and contributes to stem WSC remobilization.
- A marker generated in the promoter region of 1-FEH w3 correlates with high stem fructan remobilization capacity and the 1-FEH w3 Westonia allele is associated with high grain weight under drought.

Aims

In recent decades, droughts have affected the wheat production worldwide. Severe drought can reduce wheat grain yield by more than 50% in Australia (Ji *et al.*, 2010). The cost of drought (2002/03) in terms of lost production was approximately $ 1.0 billion (AUD) in Western Australia (WA) (Setter & Waters, 2003). In the wheat fields of WA, water deficit intensifies from anthesis to about four weeks later and severe drought usually occurs from a month after anthesis to maturity (Conocono, 2002; Zhang, 2008). Thus, terminal drought is a major problem for wheat production in WA and it is also an increasing risk for wheat production in many parts of the world (Zhang *et al.*, 2009). A major limitation in this field is lack of efficient approaches of drought tolerant screening to accelerate the process of wheat drought tolerant breeding (Ji *et al.*, 2010).

The drought tolerant screening approach in crop is not only the ability of surviving under drought, but also the ability to produce harvest yield (Fleury *et al.*, 2010). Tolerant wheat varieties should be able to yield desirable grain under water-deficit environment. There are two types of carbon source for grain filling in wheat: current photosynthate in green tissues (predominantly the flag leaf) and storage carbohydrates in stems and leaf sheaths (Schnyder, 1993). Under terminal drought, storage carbohydrates become the major source for grain filling as photosynthesis ceases (Gallagher *et al.*, 1976; Bidinger *et al.*, 1977). Pre-anthesis reserves (stem WSC) contribute up to 57% of the grain yield of wheat (Gallagher *et al.*, 1976). The levels of stem WSC have been suggested as one of the selection criteria for drought tolerance in wheat (Volaire & Lelièvre, 1997; Wardlaw & Willenbrink, 2000; Foulkes *et al.*, 2007). However, stem WSC levels vary depending on the growth stages and conditions, and the remobilization of stem WSC clearly differs between genotypes (Evans & Wardlaw, 1996; Ruuska *et al.*, 2006; Ehdaie *et al.*, 2006b; Zhang *et al.*, 2009; Zhang *et al.*, 2015). Therefore, the efficiency of stem WSC remobilization to grain needs to be further defined (Užík & Žofajová, 2006; Rattey *et al.*, 2009).

The aim of this research is 1) to identify major enzymes and genes involved in WSC remobilization to grain; 2) to generate molecular markers for drought tolerant genotypic screening in wheat breeding.

Methods

Plant materials

Westonia, Kauz and their DH lines were used in this study. Westonia is developed in Western Australia and has a consistently high yield in medium and low rainfall regions of Western Australia. Kauz is developed by the International Maize and Wheat Improvement Center (CIMMYT, El Batan, Mexico) (Butler *et al.*, 2005) and considered to be drought tolerant (Rajaram *et al*., 2002; *FAO Plant Production and Protection Series* http://www.fao.org/DOCREP/006/Y4011E/Y4011E00.HTM). Both varieties have high WSC levels in stems (~40%) after anthesis (Zhang *et al.*, 2009). DH lines were first selected based on their similar flowering time and height with large genetic diversity.

Field experiments in Merredin

The field drought experiments were carried out in Merredin field station, Western Australia in 2011, 2012 and 2013. Plants were randomly sown by two or three replicates. Neutron pressure bombs (1.5 m depth below surface) were distributed evenly in each drought and well-watered treatments for soil moisture measurement and the data were recorded fortnightly.

Drought treatment was initiated at anthesis and no water was provided. Irrigated plants received 20 mm water on a weekly basis for 4 weeks after anthesis. In 2011, the plot area was 5 m^2. The drought treatment was set up in rainout shelters and the irrigated treatment was outside. In 2012 and 2013, the plot area was 1.8 m x 10 m. The drought treatment occurred under rainfall conditions.

The soil water content was reduced significantly by 30 and 60% at 10 cm depth at 14 days post anthesis (DPA) in the drought treatments as compared to the irrigated plants in 2011 and 2012, respectively (Fig. 1). In 2013, there was an exceptional 58.6 mm of rainfall from anthesis to maturity. Consequently, water content levels at 10 cm depth did not differ between the treatments before 20 DPA (Fig. 1). At the end of the season (40 DPA), soil water content under drought was 40, 20 and 10% as compared to the irrigated treatment at 10, 30 and 50 cm depth, respectively.

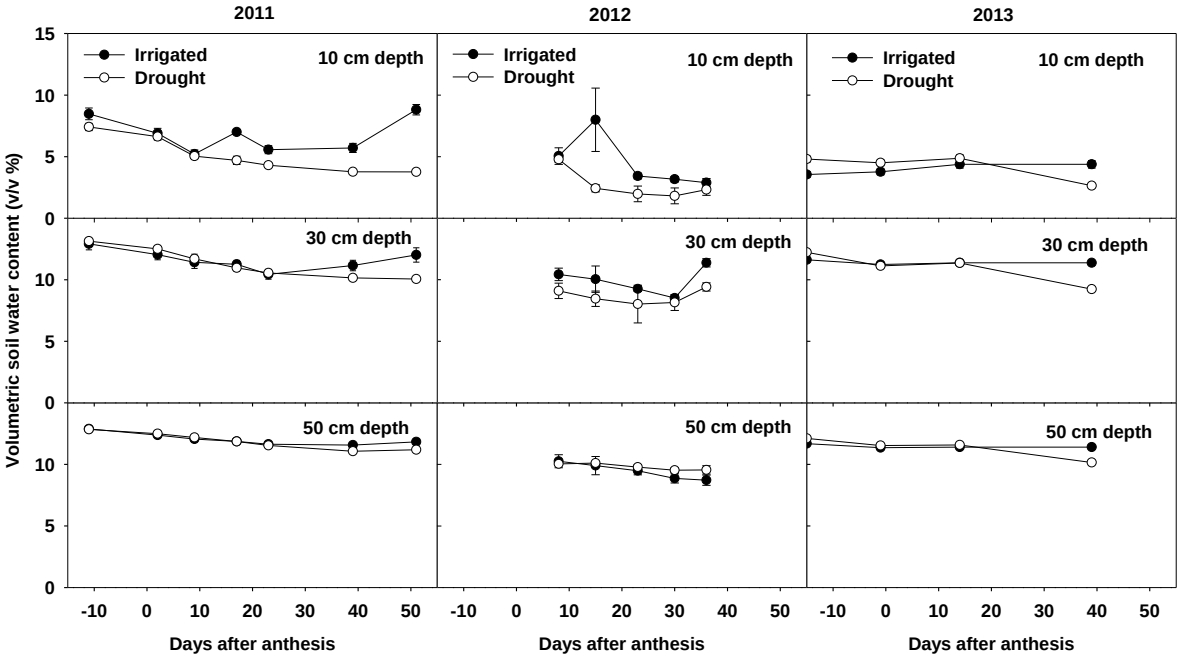

Fig. 1 Soil water content at 10, 30 and 50 cm depth, respectively, in drought experiments in 2011, 2012 and 2013 in Merredin field station. The days after anthesis is based on average of all lines planted. The vertical bars represent SE; values with the same letter are not different at *p* = 0.05.

Plant harvest

Four main stems of each plot were sampled weekly between 11:00 and 17:00 (Zhang *et al.*, 2008) from one week pre-anthesis to six weeks post-anthesis. The samples were immediately placed on dry ice and subsequently stored in a -20˚C freezer. Frozen plant samples were chopped into less than 5 mm pieces and divided into two parts. One was stored at -80°C for enzyme and RNA extraction, and the other was stored at -20°C, freeze-dried, and then oven-dried at 75°C for WSC analysis. The thousand grain weight (TGW), grain number per spike (KN) and grain weight per spike (GW) from main stems were recorded at harvesting time.

Sample measurements and analysis

Details in carbohydrate analysis, protein extraction and enzyme activity measurements, RNA extraction and real time PCR, database searches and sequence analysis, primer design, PCR amplification of genomic DNA, cloning and enzyme digestion, marker analysis, genetic map construction and QTL mapping were described previously (Zhang *et al.*, 2015). Post hoc Duncan's Multiple Range tests were used to identify significant groupings. Other statistical analyses were as described in Zhang et al., 2015.

Results

Genotype differences involved in the association between stem WSC and grain weight components under drought and irrigated conditions

Under drought, GW was reduced in most, but not all DH lines (Fig. 2). Since stem WSC are considered as a long storage carbon source for grain filling, they were recorded in a time course. Neither the maximal level of stem WSC, nor the total WSC at 17 to 20 DPA showed a significant correlation to GW, KN and TGW (data not shown), both in the main stem and in the harvest index cut. Some individual lines (e.g. lines 83, 277; Fig. 2) with average WSC levels achieved high GW under drought in 2011. Line 222, with the lowest stem WSC, achieved the highest GW in the irrigated condition, but not under drought. Line 207 showed the lowest GW and TGW but contained relatively high stem WSC under drought (Fig. 2). The levels of stem WSC remained similar in the Kauz parent line under drought and irrigated conditions. However, the GW of Kauz under drought was lower than that of irrigated plants. The stem WSC level in Westonia was higher in drought treated plants compared with the irrigated ones. There was no reduction in GW of Westonia under drought (Fig. 2).

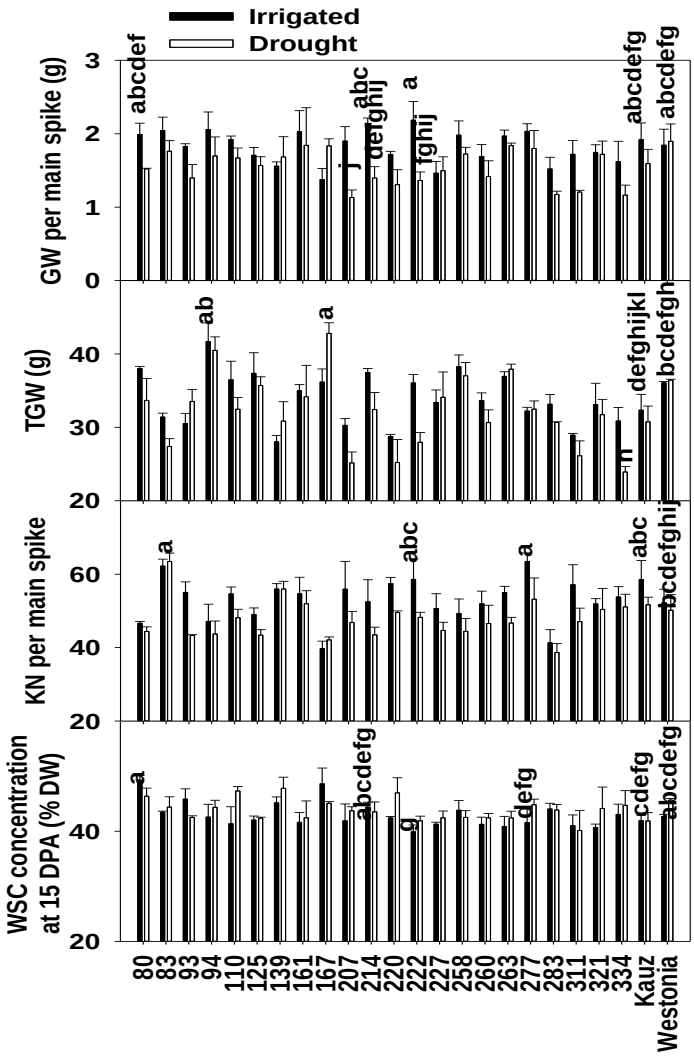

Fig. 2 The association between stem water soluble carbohydrates (WSC) and core phenotypes, including grain weight (GW) per main spike, thousand grain weight (TGW) and kernel number (KN) per main spike in Westonia, Kauz and their 22 double haploid (DH) lines under irrigated (close bars) and drought conditions (open bars) in 2011. The vertical bars represent SE. Values with the same letter are statistically not different at *P* = 0.05. DPA: Days Post Anthesis; DW: dry weight.

The decrease of bifurcose and increase of fructose under drought

The overall total stem WSC levels of Westonia and Kauz followed rather similar patterns post anthesis (Fig. 3a). To consider the changes of stem WSC in greater detail, the levels of specific stem WSC components were quantified. The main WSC components include glucose, fructose, sucrose, 1-kestose, 6-kestose, bifurcose and mixed fructans. Fructan levels increased up to 15 DPA and decreased thereafter (Fig. 3b). Fructan levels decreased faster under drought in comparison with irrigated conditions and between 15 and 25 DPA they decreased slower in Kauz than in Westonia (Fig. 3b). Between 20-30 DPA, the levels of bifurcose, a major and most persistent fructan in wheat stems, were significantly lower in both drought (0.44 ± 0.05%) and irrigated (0.82±0.03%) plants in Westonia compared with Kauz (drought: 0.66 ± 0.02% and irrigated: 1.05 ± 0.05%) (Fig. 4a, upper panel). The levels of fructose were higher in Westonia (12.9 ± 0.15%) than in Kauz (10.1 ± 0.90%) in drought treated plants over the same time period (20-30 DPA) (Fig. 4a, lower panel).

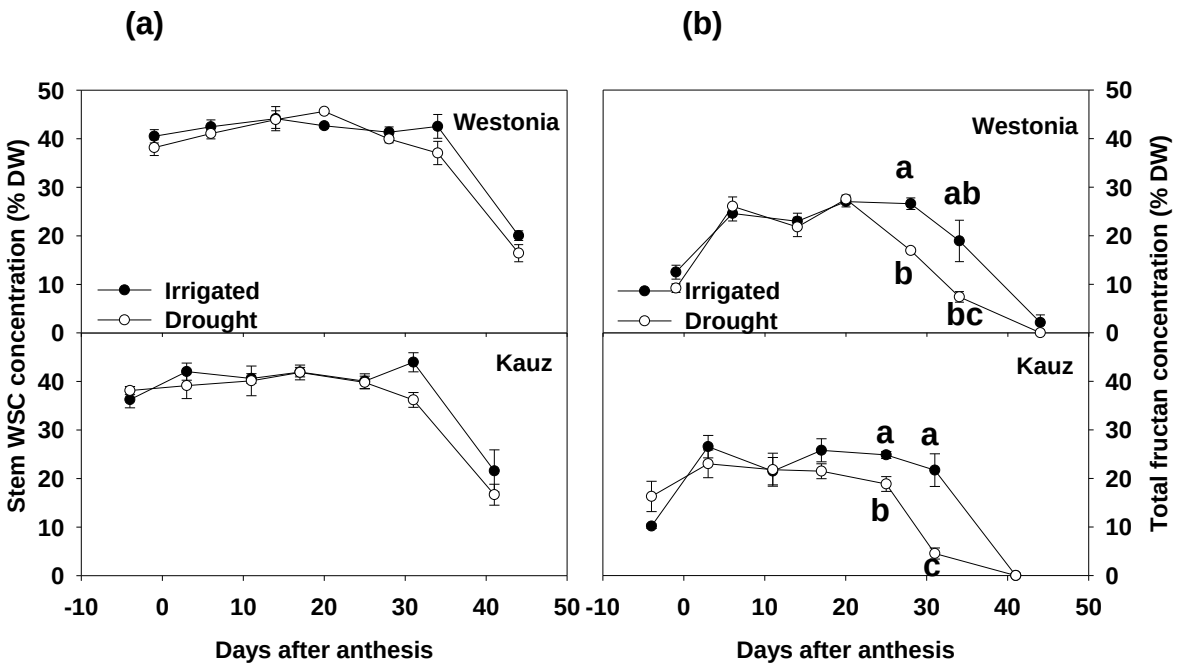

Fig. 3 Stem water soluble carbohydrates WSC (a) and fructan (b) levels in Westonia and Kauz under drought (open circles) and irrigated conditions (closed circles) at Merredin field station in 2011. The vertical bars represent SE. Values with the same letter are statistically not different at *P* = 0.05.

1-FEH w3 is likely the main enzyme associated with β-(2-1) fructan degradation

In monocots, fructan levels are determined by the balance between fructan biosynthesis and degradation (Van den Ende *et al.*, 2003). The enzyme activities involved in fructan synthesis and degradation were determined on the same samples that were used for WSC analysis. Under drought conditions, the total 1-FEH activity was strongly induced and reached an earlier maximum in Westonia (28 DPA) as compared with Kauz (31 DPA) (Fig. 4b, upper panel). Bifurcose and 1-FEH enzyme activities correlated well over the 0-35 DPA period (Fig. 5). Overall, there is a tendency that drought treatment reduces the enzyme activities involved in fructan synthesis, for example, 1-SST, 6-SST, 1-FFT and 6-SFT activities (data not shown). 1-FEH activities are higher compared to the other enzymatic activities, including soluble acid INV activity (data not shown), although it should be noted that 6-FEH activities were tested at 5 mM substrate and 1-FEH activities at 50 mM. Adjusting both FEHs to the same millimolar levels suggests that both activities could be equally important.

Three isoforms of 1-FEH (1-FEH w1, w2 and w3) can be found in wheat (Van Riet *et al.*, 2008). To understand better which isoform contributes the most to the total 1-FEH enzyme activity, the gene expression levels of three *1-FEH* genes were determined. The data show that the *1-FEH w3* expression was much higher than the expression levels of *1-FEH w1* and *w2*, suggesting that the 1-FEH w3 enzyme is the most prominent form contributing to the total 1-FEH activity involved in WSC remobilization under drought (Fig. 4b, lower panel). Drought strongly induced the *1-FEH w3* gene expression in Westonia and Kauz. Moreover, the level of *1-FEH w3* gene expression peaked much earlier in Westonia compared with Kauz under drought. Furthermore, the *1-FEH w3* gene expression level was significantly higher in Westonia than in Kauz under drought around 15 DPA (Fig. 4b, lower panel), preceeding the 1-FEH activity optimum by about one week (Fig. 4b up panel). This was also associated with faster bifurcose reduction and fructose increases in comparison with Kauz (Fig. 4a).

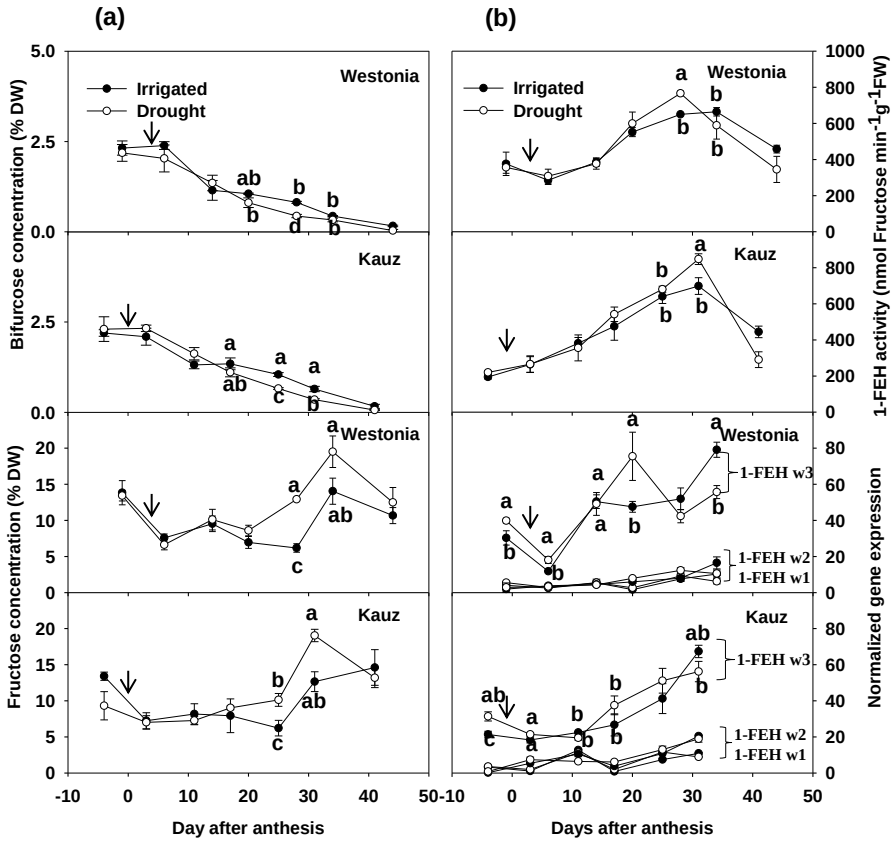

Figure 4 (a) The profile of wheat stem (sheath included) bifurcose concentration (upper panel) and fructose concentration (lower panel) in Westonia and Kauz under irrigated and drought conditions. (b) Total 1-FEH enzyme activity (upper panel) and normalized gene expression patterns of *1-FEH w1*, *w2* and *w3* (lower panel). The vertical bars represent SE. Values with the same letter are statistically not different at *P* = 0.05. Arrows indicate the moment when the drought treatment was initiated.

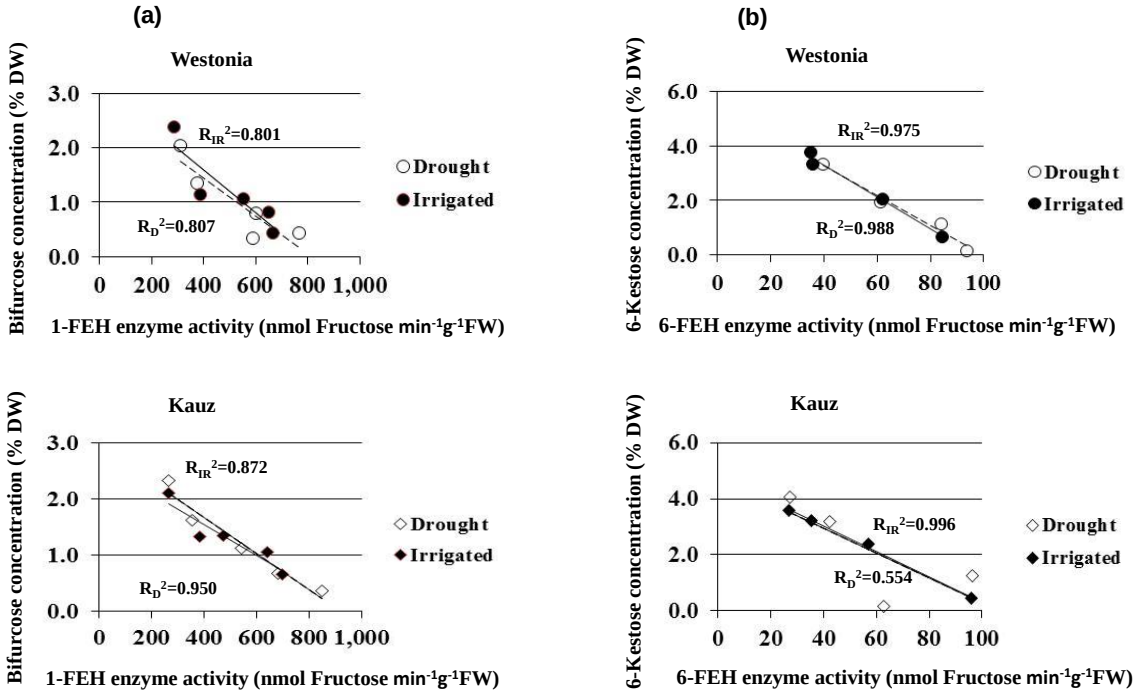

Fig. 5 The correlation of stem (sheath included) bifurcose concentration and 1-FEH enzyme activities between 0 to 35 DPA (a) and 6-kestose concentrations and 6-FEH activities between 15 to 45 DPA (b) in Westonia and Kauz, respectively, under irrigated and drought conditions.

A CAP marker for 1-FEH w3

Due to the putative importance of the *1-FEH w3* gene expression, the *1-FEH w3* gene sequences of Westonia and Kauz were more thoroughly investigated. No SNP could be detected within the ORF gene and downstream regions. Fortunately, one SNP was detected in the promoter region of *1-FEH w3* between Westonia and Kauz (Fig. 6a). A unique BsoB1 restriction enzyme cuts the SNP site in Westonia but not in Kauz. A 14 bp band difference was detected between Westonia and Kauz on a 2.5% agarose gel (Fig. 6d,e). This Cleaved Amplified Polymorphic (CAP) marker was then used in the DH population of Westonia and Kauz and the *1-FEH w3* gene was mapped to the short arm of 6B, about 1 cM away from the SSR marker wmc494 (Zhang *et al.*, 2015).

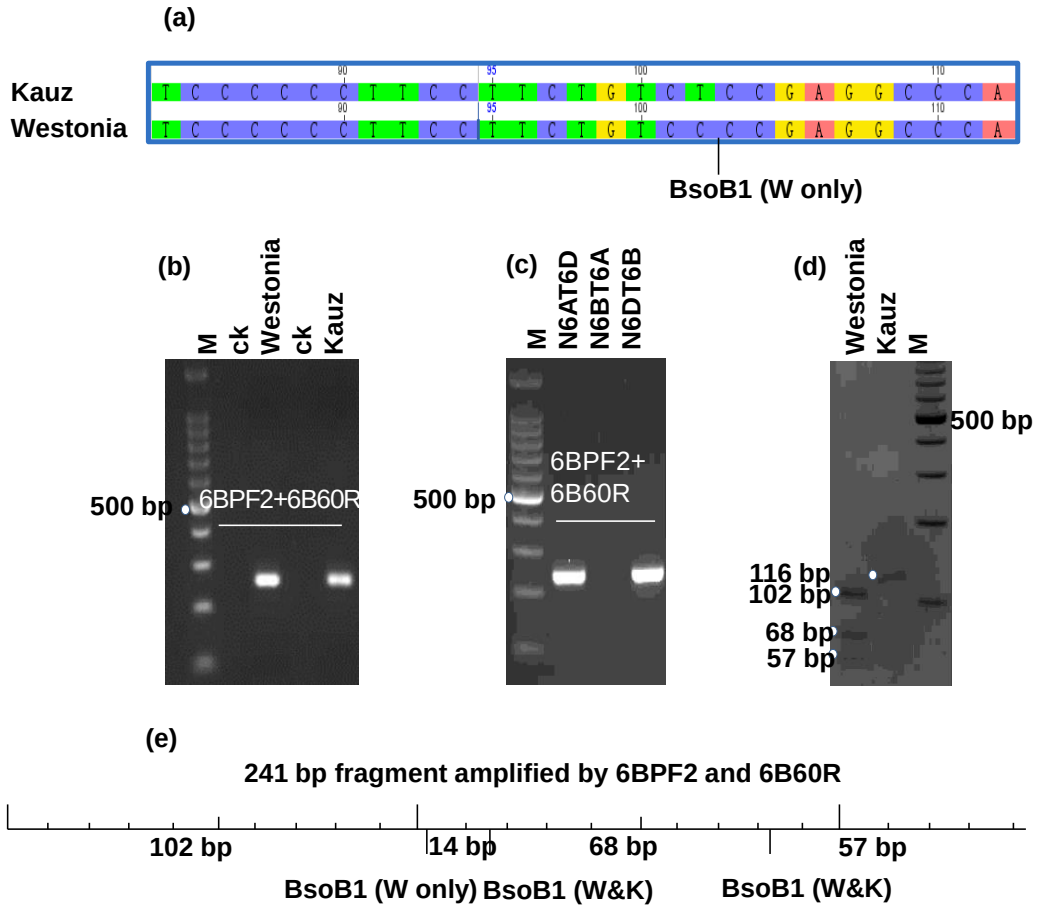

Fig. 6 Generation of a cleaved amplified polymorphic (CAP) marker. (a) One (single nucleotide polymorphism) SNP was located in the promoter region of *1-FEH w3*. (b) The SNP promoter region was amplified from *1-FEH w3* in Westonia and Kauz, and (c) from nulli (N)-tetra (T) lines N6AT6D, N6BT6A and N6DT6B. (d and e) A CAP marker was generated based on the 14 bp fragment length difference between Westonia and Kauz after overnight digestion on the 6BPF2/6B60R amplification product with the restriction enzyme BsoB1 (CYCGRG). ck is the negative control. M represents standard marker.

Westonia alleles of the 1-FEH w3 gene in DH lines are associated with high 1-FEH w3 gene expression under drought

To further confirm whether the CAP marker is associated to the *1-FEH w3* gene expression level, four Westonia type DH lines (DH 321, DH 83, DH 139 and DH 167) and three Kauz type DH lines (DH 263, DH 311 and DH 125) were randomly selected and analysed for their *1-FEH w3* gene expression. DH lines of Westonia and Kauz with similar anthesis time were compared to each other. Under drought, three out of four DH lines with the Westonia allele showed significantly high *1-FEH w3* gene expression levels between 10 to 30 DPA compared to lines containing the Kauz alleles (Fig. 7). Only DH 167, with an exceptionally low KN per spike (Fig. 1), showed no difference in *1-FEH* w3 gene expression under drought and irrigated conditions (data not shown).

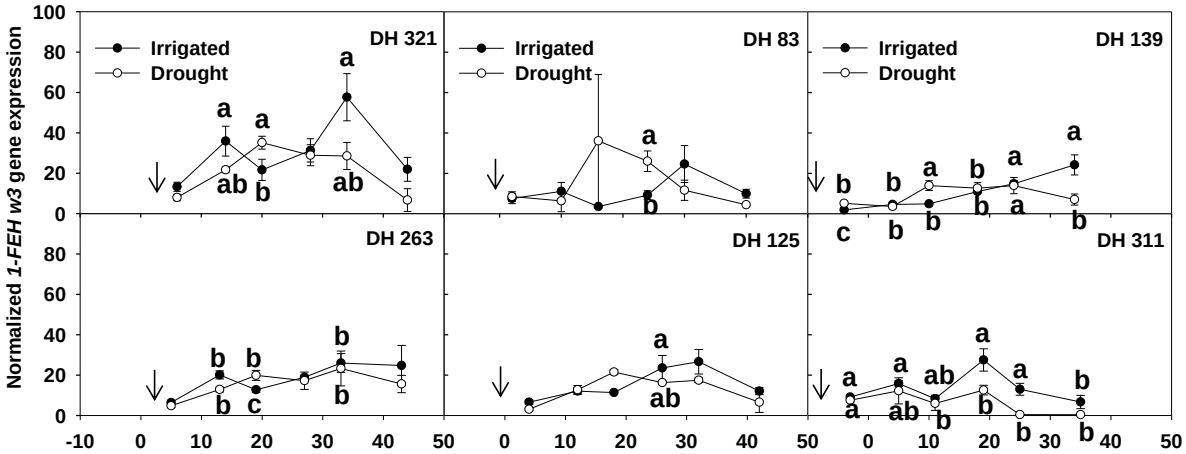

Fig. 7 The 1-FEH w3 gene expression patterns in DH lines of Westonia and Kauz using the same tissue sources of stem WSC analysis. Top panel: Westonia alleles including DH 321, DH 83 and DH 139; lower panel: Kauz alleles including DH 263, DH 125 and DH 311. The vertical bars represent SE; values with the same letter are not different at *p* = 0.05. Arrows indicate the time of the drought treatment.

The CAP marker of 1-FEH w3 correlates with a superior TGW under drought

In the drought experiments in Merredin, 22, 21 and 17 DH lines were used in 2011, 2012 and 2013, respectively. In addition to the parental Westonia and Kauz lines, 29, 23 and 18 plots for each *1-FEH w3* genotype per treatment were analysed in 2011, 2012 and 2013, respectively. The GW and TGW in lines with Westonia type *1-FEH w3* alleles were higher than those obtained from lines with Kauz type *1-FEH w3* alleles under both drought and irrigated conditions across three years (Fig. 8). The GW of lines with Westonia type allele was also significantly higher compared with those carrying Kauz type alleles under drought treatment in 2011. The reduction of TGW in Kauz type under drought was significant when considered across three years, but it did not reach the level of significance for Westonia type in 2011 and 2012 (Fig. 8). There was no difference in KN per spike between the different *1-FEH w3* genotypes, although the KN per spike was greatly reduced under drought in 2011 and 2012.

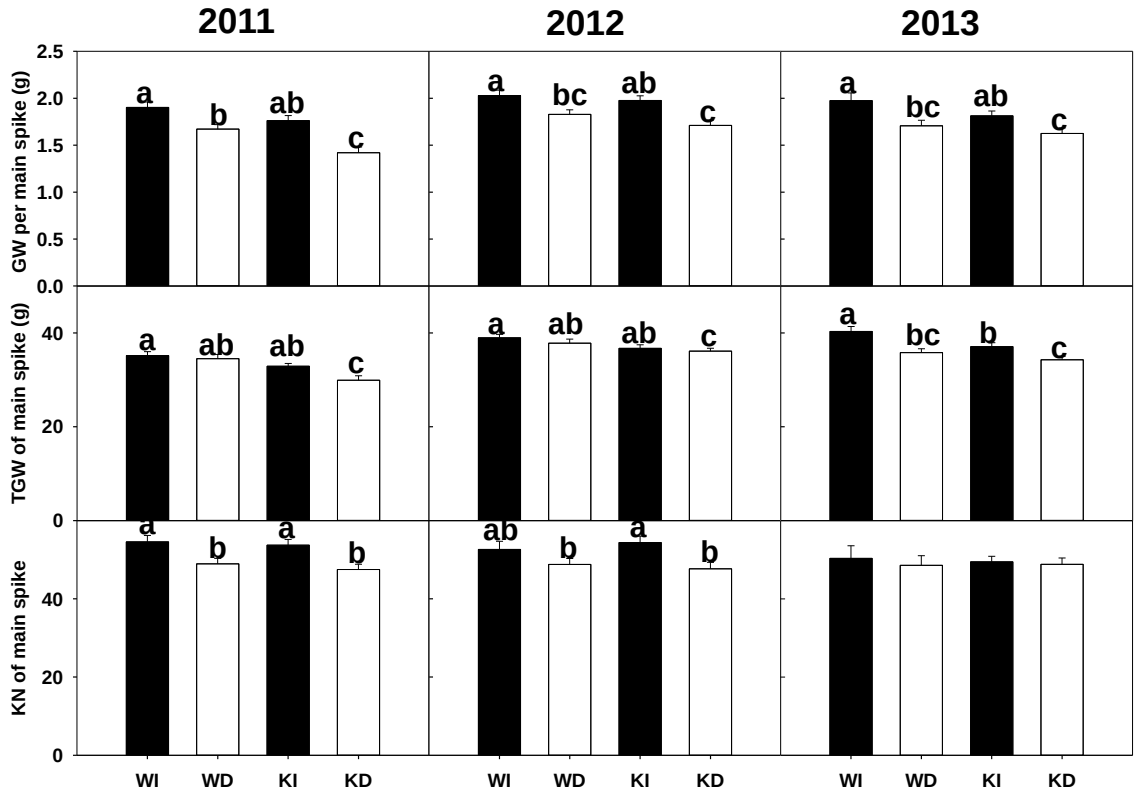

Figure 8 The core phenotypes of grain weight (GW) per spike, thousand grain weight (TGW) and kernel number (KN) per spike associated with the cleaved amplified polymorphic (CAP) marker of *1-FEH w3* in selected double haploid (DH) lines and the parental lines of Westonia and Kauz under irrigated (closed bars) and drought (open bars) conditions in 2011, 2012 and 2013. WI: *1-FEH w3* Westonia type under irrigated condition; WD: *1-FEH w3* Westonia type under drought condition; KI: *1-FEH w3* Kauz type under irrigated condition; KD: *1-FEH w3* Kauz type under drought condition. The vertical bars represent SE. Values with the same letter are not different at *P* = 0.05.

The role of 6-FEH during stem fructan remobilization

Besides bifurcose, 6-kestose also represents a prominent fructan in wheat stems. The 6-kestose concentration declined sharply in both lines after 20 DPA. This degradation occurred faster under drought. The profile of 6-FEH activity could be closely correlated with the degradation patterns of 6-kestose between 15 to 45 DPA (Fig. 5), suggesting that 6-kestose could be one of the preferred substrates of wheat 6-FEH enzymes, warranting deeper investigations into 6-FEHs in wheat stems during carbon remobilization under terminal drought.

Conclusion

The levels of fructan-dominated WSC do not always correlate well with grain yield. High level of stem WSC combined with FEH-mediated remobilization efficiency may contribute to high TGW, especially under drought. The rate of the fructan degradation showed genotypic differences in fructan remobilization efficiency. The gene expression data indicated that *1-FEH w3* was likely the main gene involved in the total 1-FEH enzyme activity. A CAP marker generated from the SNP in the promoter region distinguished two genotypes (Westonia and Kauz) with different levels of *1-FEH w3* gene expression. The Westonia genotype was linked to high gene *1-FEH w3* expression and high TGW indicating that the high gene expression of *1-FEH w3* contributed to the high levels of the stem WSC remobilization. The CAP marker of *1-FEH w3* may be useful for the selection for high stem WSC remobilization and high TGW in wheat breeding under terminal drought.

High levels of 6-FEH enzyme activity and high correlation (R^2=0.99) with the degradation of 2-6 linked fructans led us to also investigate the role of 6-FEH in WSC remobilization under terminal drought as *β*-(2-6) linkage fructans are the predominated form in stems (Pollock & Cairns, 1991). The results suggest that the combined activities of 1-FEH and 6-FEH contribute to the high utilization of stem WSC in grain filling under drought stress.

Because of the climate change, drought stress has impacted wheat grain yield severely. This threatens the viability of farming marginal environments, such as family stress and disintegration with concomitant negative impacts on rural society and land management. New approaches are needed to provide wheat breeders with tools to quickly select drought tolerant lines. Understanding and manipulating the mobilization of fructans in the stem and the consequence for grain filling in wheat will give more stable grain yield, provide more resilience to unpredictable drought environment and greater flexibility to wheat producers.

Keywords: Grain filling, Single Nucleotide Polymorphism, Stem water soluble carbohydrate remobilization, Terminal drought

Acknowledgements

This work was supported by Murdoch University, Grains Research & Development Corporation 'grant number UMU00039', the Sir Walter Distinguished Collaborator Scheme at Murdoch University. The work involved collaborations with the Yangzhou University and Chinese Academy of Science and Ministry of Water Resources in China, and with KU Leuven, Belgium. The authors are very grateful to InterGrain for providing Westonia and Kauz DH population and Irene Waters, Tim Setter and Merredin staff (the Department of Agricultural and Food, Western Australia) for their support and help.

GRDC Project number: UMU00039

Paper reviewed by: The most contents were peer reviewed and published in New Phytologist.

References

Bidinger F, Musgrave RB, Fischer RA. 1977. Contribution of stored pre-anthesis assimilate to grain yield in wheat and barley. *Nature* **270**: 431-433.

Butler JD, Byrne PF, Mohammadi V, Chapman PL, Haley SD. 2005. Agronomic performance of *Rht* alleles in a spring wheat population across a range of moisture levels. *Crop Science* **45**: 939-947.

Conocono EA. 2002. *Improving yield of wheat experiencing post-anthesis water deficits through the use of shoot carbohydrate reserves.* PhD PhD thesis, University of Western Australia Perth.

Ehdaie B, Alloush G, Madore M, Waines J. 2006b. Genotypic variation for stem reserves and mobilization in wheat: II. Postanthesis changes in internode water-soluble carbohydrates. *Crop Science* **46**: 2093-2103.

Evans LT, Wardlaw F. 1996. *Photoassimilate Distribution in Plants and Crops. Source-Sink Relationships.* New York: Marcel Dekker.

Fleury D, Jefferies S, Kuchel H, Langridge P. 2010. Genetic and genomic tools to improve drought tolerance in wheat. *Journal of Experimental Botany* **61**(12): 3211-3222.

Foulkes MJ, Sylvester-Bradley R, Weightman R, Snape JW. 2007. Identifying physiological traits associated with improved drought resistance in winter wheat. *Field Crops Research* **103**(1): 11-24.

Gallagher JN, Biscoe PV, Hunter B. 1976. Effects of drought on grain growth. *Nature* **264**: 541-542.

Ji X, Shiran B, Wan J, Lewis DC, Jenkins CLD, Condon AG, Richards RA, Dolferus R. 2010. Importance of pre-anthesis anther sink strength for maintenance of grain number during reproductive stage water stress in wheat. *Plant, Cell & Environment* **33**(6): 926-942.

Pollock CJ, Cairns AJ. 1991. Fructan metabolism in grasses and cereals. *Annual Review of Plant Physiology and Plant Molecular Biology* **42**(1): 77-101.

Rattey A, Shorter R, Chapman S, Dreccer F, van Herwaarden A. 2009. Variation for and relationships among biomass and grain yield component traits conferring improved yield and grain weight in an elite wheat population grown in variable yield environments. *Crop and Pasture Science* **60**(8): 717-729.

Ruuska SA, Rebetzke GJ, van Herwaarden AF, Richards RA, Fettell NA, Tabe L, Jenkins CLD. 2006. Genotypic variation in water-soluble carbohydrate accumulation in wheat. *Functional Plant Biology* **33**(9): 799-809.

Schnyder H. 1993. The role of carbohydrate storage and redistribution in the source-sink relations of wheat and barley during grain filling- a review. *New phytologist* **123**: 233-245.

Setter T, Waters I. 2003. Review of prospects for germplasm improvement for waterlogging tolerance in wheat, barley and oats. *Plant and Soil* **253**: 1-34.

Užík M, Žofajová A. 2006. Translocation and accumulation of dry matter in winter wheat genotypes. *Cereal Research Communications* **34**(2): 1013-1020.

Van den Ende W, Clerens S, Vergauwen R, Van Riet L, Van Laere A, Yoshida M, Kawakami A. 2003. Fructan 1-exohydrolases. *β*-(2,1)-trimmers during graminan biosynthesis in stems of wheat? purification, characterization, mass mapping, and cloning of two fructan 1-exohydrolase isoforms. *Plant Physiology* **131**(2): 621-631.

Van Riet L, Altenbach D, Vergauwen R, Clerens S, Kawakami A, Yoshida M, Van den Ende W, Wiemken A, Van Laere A. 2008. Purification, cloning and functional differences of a third fructan 1-exohydrolase (1-FEHw3) from wheat (*Triticum aestivum* L.). *Physiologia Plantarum* **133**(2): 242-253.

Volaire F, Lelièvre F. 1997. Production, persistence, and water-soluble carbohydrate accumulation in 21 contrasting populations of *Dactylis glomerata* L. subjected to severe drought in the south of France. *Australian Journal of Agricultural Research* **48**(7): 933-944.

Wardlaw IF, Willenbrink J. 2000. Mobilization of fructan reserves and changes in enzyme activities in wheat stems correlate with water stress during Kernel filling. *New phytologist* **148**(3): 413-422.

Zhang J. 2008. *Water deficit in bread wheat: characterisation using genetic and physiological tools.* PhD PhD thesis, Murdoch University Perth.

Zhang J, Dell B, Conocono E, Waters I, Setter T, Appels R. 2009. Water deficits in wheat: fructosyl exohydrolase (1-FEH) mRNA expression and relationship to soluble carbohydrate concentrations in two varieties. *New phytologist* **181**: 843-850.

Zhang J, Xu Y, Chen W, Dell B, Vergauwen R, Biddulph B, Khan N, Luo H, Appels R, Van den Ende W. 2015. A wheat 1-FEH w3 variant underlies enzyme activity for stem WSC remobilization to grain under drought. *New phytologist* **205**(1): 293-305.