CSIRO PUBLISHING

Functional Plant Biology, 2014, **41**, 1002–1008
http://dx.doi.org/10.1071/FP14060

Post-anthesis heat and a *Gpc-B1* introgression have similar but non-additive effects in bread wheat

Lancelot Maphosa[A,B,C], *Nicholas C. Collins*[A,B], *Julian Taylor*[B] *and Diane E. Mather*[A,B,D]

[A]Australian Centre for Plant Functional Genomics, Waite Research Institute, The University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia.
[B]School of Agriculture, Food and Wine, Waite Research Institute, The University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia.
[C]Present address: Department of Environment and Primary Industries, 110 Natimuk Road, Horsham, Vic. 3400, Australia.
[D]Corresponding author. Email: diane.mather@adelaide.edu.au

Abstract. High temperatures during grain filling can reduce the yield of wheat and affect its grain protein concentration. The *Gpc-B1* locus of wheat also affects grain protein concentration, but it is not known whether its effects interact with those of heat. The aim of this study was to investigate the effects of high temperature in lines with and without functional (high-protein) alleles at *Gpc-B1*. A highly replicated experiment was conducted in a glasshouse under control conditions (24/18°C, 14/10 h day/night), with half of the plants of each line or cultivar put into a heat chamber (37/27°C, 14/10 h day/night) at 15 days after anthesis for 3 days. Backcross derivatives with the *Gpc-B1* introgression segment differed from their recurrent parents more than those without that segment. In some respects, the effects of the *Gpc-B1* introgression were similar to those of the heat treatment: both could accelerate peduncle senescence, increase grain protein content and increase the percentage of unextractable polymeric protein. Unlike the heat treatment, *Gpc-B1* did not reduce grain weight, indicating that factors that hasten senescence do not necessarily limit grain size. The presence of the *Gpc-B1* segment did not exacerbate the effects of heat stress on any trait.

Additional keywords: protein, senescence, single nucleotide polymorphism, stress.

Received 22 February 2014, accepted 8 May 2014, published online 10 June 2014

Introduction

In both hexaploid bread wheat (*Triticum aestivum* L.) and tetraploid durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf.) Husn., a chromosome segment introgressed from wild emmer wheat (*Triticum turgidum* L. subsp. *dicoccoides* (Körn. Ex. Asch. and Graebn.)Thell.) is known to increase the concentration of protein, iron and zinc in the grain (Olmos *et al*. 2003; Uauy *et al*. 2006*a*, 2006*b*; Distelfeld *et al*. 2007). These effects have been attributed to a NAC transcriptional factor encoded by the *NAM-B1* gene at the *Gpc-B1* locus on chromosome arm 6BS (Uauy *et al*. 2006*b*). The functional *NAM-B1* allele from wild emmer acts by accelerating senescence, shortening the grain filling period and enhancing remobilisation of nitrogen from other plant organs to the developing grain (Waters *et al*. 2009; Brevis and Dubcovsky 2010). Grain weight, test weight and grain yield are also reduced (Brevis and Dubcovsky 2010; Brevis *et al*. 2010). The reduction in grain yield is not as severe as the reduction in grain weight, indicating that there are compensating effects in other yield components. The reduction in grain weight is greater in tetraploid than in hexaploid wheats, possibly because of a dosage effect: tetraploids have two other functional *NAM* genes (on chromosomes 6A and 2B), whereas hexaploids have four (on chromosomes 6A, 2B, 2D and 6D). Traits that typically show a positive association with high protein concentration, including flour water absorption, mixing time and loaf volume have also been reported to be affected by *Gpc-B1* (Brevis *et al*. 2010).

The effects of *Gpc-B1* are similar in some respects to those that have been reported for heat stress during grain filling. Heat stress can shorten the duration of grain development (accelerate senescence) and affect grain yield and quality (Altenbach 2012). Starch synthesis is particularly sensitive to heat (Jenner 1994) and heat-induced loss of starch may largely account for the effects of heat on grain size and grain protein concentration. Heat stress has been reported to increase mixing time and loaf volume. This has been attributed to increased protein concentration (Li *et al*. 2013). However, Blumenthal *et al*. (1995) reported reduced mixing time due to heat. Differential responses in dough properties may be due to differences in the severity and timing of the heat stress. Increases in temperature up to 30°C can increase dough strength but further increases can decrease dough strength (Randall and Moss 1990). Heat-induced reductions in dough strength have been attributed to reductions in glutenin : gliadin ratios and the proportion of glutenin that is

present as very large polymers (Blumenthal *et al*. 1995). Heat has been reported to decrease the percentage unextractable polymeric protein (% UPP, which is used as an indicator of dough strength) when applied at later grain filling stages (Irmak *et al*. 2008) but to increase % UPP when applied in early stages of grain filling (Balla *et al*. 2011).

Here, to directly compare the effects of heat stress with those of *Gpc-B1* and to investigate how the effects of post-anthesis heat stress would affect the grain of wheat lines that carry the high-protein allele at *Gpc-B1*, pairs of backcross-derived wheat lines were grown with and without exposure to a high temperature treatment early in grain filling.

Materials and methods

Plant materials

The materials used here included the cultivars Gladius, Drysdale, Wyalkatchem, Burnside, Somerset and Glupro, a line designated RAC1262A (a selection from the breeding line RAC1262, which was later released as Gladius) and two pairs of backcross-derived lines (RS4-11-10 and RS4-11-16; WB4–1-6 and WB4-1-8). Gladius, a cultivar that is considered to be heat tolerant (Fleury *et al*. 2010) was derived from a complex cross involving derivatives of RAC875, Krichauff, Excalibur and Kukri. The pedigree of Drysdale is Hartog*3/Quarrion and that of Wyalkatchem is Machete///Gutha//Jacup*2/11th ISEPTON-135. Burnside (pedigree Glenlea*2///Pasqua*2/Glupro///Glenlea*6/Kitt) and Somerset (pedigree AC Minto*5/Glupro//Pasqua) both carry the high-protein allele at *Gpc-B1*. Glupro (pedigree Columbus/FA15-2/Len) is the source of the high-protein allele in both Burnside and Somerset.

RS4-11-10 and RS4-11-16 were derived from a marker-assisted backcrossing program using RAC1262A as the recurrent parent and Somerset as the donor parent. RS4-11-10 and RS4-11-16 were both derived from selfed progeny of a BC_4 progeny plant designated RS4-11. In the *Gpc-B1* region of chromosome 6B, RS4-11-10 is homozygous for donor-parent alleles and RS4-11-16 is homozygous for recurrent-parent alleles. Similarly, WB4-1-6 and WB4-1-8 were derived from selfed progeny of a BC_4 progeny plant (WB4-1) with Wyalkatchem as its recurrent parent and Burnside as its donor parent. In the *Gpc-B1* region, WB4-1-6 carries donor-parent alleles and WB4–1-8 carries recurrent-parent alleles. For brevity, RS4-11-10 and WB4-1-6 will be referred to here as *Gpc-B1*+ lines, and RS-11-16 and WB4-1-8 will be referred to here as *Gpc-B1*– lines.

DNA extraction and SNP genotyping

DNA was extracted from ~2.0 g of leaf tissue from one 4-week-old plant of each of Glupro, Somerset, Burnside, Gladius, Drysdale, RAC1262A, Wyalkatchem, RS4-11-10, RS4-11-16, WB4-1-6 and WB4-1-8, using a modified mini prep ball bearing extraction method (Pallotta *et al*. 2000). DNA concentration was quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Thermo Fisher Scientific, Wilmington, DE, USA). For each line, a 30 µL sample of about 100 ng μL^{-1} DNA was assayed on a 9K Infinium wheat SNP assay as described by Cavanagh *et al*. (2013).

Glasshouse experiment

Plants were grown in a naturally-lit glasshouse in Urrbrae, Australia and the evaporative cooling system was set on a 24/18°C max/min 24 h sine wave cycle. The experimental design consisted of 30 complete blocks. Each block consisted of 16 pots (arranged in two rows of eight) and contained four sub-blocks, each of which consisted of four pots (arranged two-by-two). Each of four pairs of lines (RS4-11-10 and RS4-11-16; WB4-1-6 and WB4-1-8; RAC1262A and Wyalkatchem; Gladius and Drysdale) was assigned at random to a sub-block within each block. For each sub-block, two pots were used for each of the two lines in the pair and the pots were assigned at random to positions within the sub-block. One plant of each line was allocated at random to be subjected to a heat treatment, while the other one was used as a control.

Seeds were sown in pots containing a mixture of Waikerie sand and coco peat, 202 mg L^{-1} dolomite lime, 561 mg L^{-1} agricultural lime, 131 mg L^{-1} hydrated lime, 202 mg L^{-1} gypsum, 202 mg L^{-1} superphosphate, 505 mg L^{-1} iron phosphate, 33.7 mg L^{-1} iron chelate, 202 mg L^{-1} micronutrients (Scotts Micromax, MicroPlus Trace Element Fertilizer, Langley Fertilizers, Wangara WA, Australia), 505 mg L^{-1} calcium nitrate and 2022 mg L^{-1} slow-release fertiliser (Osmocote, Osmocote Exact Mini 16-3.4-9.1 (+1.2 Mg), Everris Australia, Bella Vista NSW, Australia). The pH of this mixture was between 6.0 and 6.5. Aquasol multi-nutrient fertiliser (nitrogen, phosphorus, potassium, sulfur, magnesium, manganese, copper, iron, zinc, boron and molybdenum) was dissolved in water (0.9 g L^{-1}) and applied to the soil at 2 week intervals after anthesis.

For each plant, the date on which anthers were first extruded from florets on the first spike (anthesis date) was recorded. That spike was tagged, and other tillers were removed to reduce competition for light and assimilates. Each plant allocated for heat treatment was transferred at 15 days after its anthesis date to a growth chamber (BDW120, Conviron, Winnipeg, MB, Canada), where pots were placed in tubs containing around 3 cm of water to minimise drought stress. The temperature of the growth chamber was held at 37°C for 8 h each day, with 3 h transition periods used either side to linearly ramp the temperature up and down from and to a night-time temperature of 27°C. Lighting (mixture of metal halide and tungsten incandescent) was at a maximum of 630 µM m^{-2} s^{-1} at spike height for 10 h each day, with a 2 h transition period used either side to step the intensity at 460 micromole m^{-2} s^{-1}. Average day/night RH in the chamber was measured at 60/80%. After 3 days in the growth chamber, the plants were returned to their original positions in the glasshouse and allowed to mature.

For each tagged tiller, the dates of anthesis and complete yellowing of the peduncle were recorded. Grain was harvested after all plants had ripened (complete yellowing of both peduncle and spike). The grains harvested from the spike of each tagged tiller were counted and weighed. Mean single grain weight was obtained by dividing grain weight by grain number.

Size-exclusion high-performance liquid chromatography (SE-HPLC)

Grain from each hand-threshed spike was crushed with a hammer. Flour that passed through a 280 µm sieve was retained. The

flour was allowed to rest for at least 7 days in a refrigerator before protein extraction. Protein was extracted from flour using an established method (Appelbee 2007). It involved weighing 25 mg of flour into a 1.5 mL tube and adding 1 mL of 0.05 M phosphate extraction buffer (pH 6.9). Phosphate extraction buffer was made by preparing two solutions, solution A (3.55 g Na_2HPO_4; 2.5 g sodium dodecyl sulfate (SDS); 500 mL water) and solution B (3 g NaH_2PO_4; 2.5 g SDS; 500 mL water) then gradually adding solution B to solution A until the pH reached 6.9. The protein extraction procedure was a two-step process. In the first step, the flour was mixed with 1 mL of phosphate extraction buffer, and centrifuged for 10 min at 15 682*g*. The supernatant (containing SDS-soluble protein) was aspirated from the pellet to a new tube. In the second step, 1 mL of phosphate extraction buffer was added to the pellet, the tube was sonicated at room temperature using a sonifier (Branson model B-12 cell disrupter, Danbury, CT, USA) set at 10 W (output four) for 30 s, then spun again and the supernatant (containing SDS-insoluble protein) was collected. Both extracts were filtered through 0.45 µm PVDF filters into 1-mL glass HPLC vials. Proteases were inactivated by incubating the vials at 80°C for 2 min.

The protein extracts were run on a Waters (Milford, MA, USA) HPLC system using a Protein-Pak 300TM column (C18, 300 Å pore size, 3.5 µm particle size, 150 × 4.6 mm) along with a Waters 717 plus auto sampler, a Waters 600 system controller and a Waters 486 detector. The samples were run in 50% (v/v) acetonitrile containing 0.1% trifluoroacetic acid (TFA), for 50 min (soluble fraction) or 40 min (insoluble fraction) (Batey *et al*. 1991) at a flow rate of 0.5 mL min^{-1}. The software Millennium32 (ver. 3.2, Waters, Milford, MA, USA) was used for data acquisition and processing. Protein was detected by UV absorbance at 214 nm (Stone and Nicolas 1996) and the areas under specific peaks were used to estimate amounts of soluble and insoluble protein. The first major peak eluting at ~14.5 min in the soluble fraction was defined as total soluble polymeric protein and the first major peak in the insoluble fraction eluting at ~15.5 min was defined as total insoluble polymeric protein. Two aliquots of each sample were each run separately through the HPLC and the values averaged. Total polymeric protein and percentage of SDS-unextractable polymeric protein (% UPP) were calculated as follows:

$$\text{total polymeric protein} = \text{area of soluble polymeric protein peak} + \text{area of insoluble polymeric protein peak}, \quad (1)$$

$$\%\ \text{UPP} = (\text{area of insoluble polymeric protein peak} / \text{total polymeric protein}) \times 100. \quad (2)$$

Total protein measurement

For each line, total grain protein concentration was estimated using grain harvested from each of three blocks of the experiment. Total nitrogen content was measured using the Dumas total combustion method (Buckee 1994) using an Elementar Rapid N III Nitrogen Analyser, ver. J (Elementar, Hanau, Germany). Total protein concentration expressed on an 'as is' basis was obtained by multiplying nitrogen concentration by 5.7 (conversion factor for wheat).

Statistical analysis

For each of the measured traits, a spatial analysis was conducted using a linear mixed model (Gilmour *et al*. 1997) that took into account the design factors of the experiment (blocks and sub-blocks) and the layout of pots within the glasshouse. The model also contained a fixed treatment-by-line interaction term which provided adjusted means for the measured traits of each factorial combination of the treatment levels. All models were analysed using ASReml-R (Butler *et al*. 2009) in the R statistical computing environment (R Development Core Team 2012). With each trait model, post-analysis multiple comparisons were performed to test the significance of the effects of the heat treatment on each of the lines and to directly compare the lines within pairs (Gladius and Drysdale, RAC1262A and Wyalkatchem, RS4-11-10 and RS4-11-16, and WB4-1-6 and WB4-1-8) under heat treatment and control conditions. Correction for the family-wise error rate (FWER) for the number of comparisons performed was done using a Bonferroni-corrected significance level calculated as 0.05 divided by number of comparisons (eight for time to anthesis and 16 for the other traits).

Results

As expected, data for SNP markers that had previously been mapped on chromosome 6B (Cavanagh *et al*. 2013), confirmed the presence of donor-parent (Somerset or Burnside) introgressions in both *Gpc-B1*+ lines (RS-11-10 and WB4-1-6) (Fig. 1). In addition to containing the *Gpc-B1*-containing segment derived from wild emmer wheat, these introgressions could include flanking regions derived from the original donor Glupro and donor wheat cultivars Somerset and Burnside. Considering that there would have been at least five opportunities for recombination in the development of the BC_4-F_1-derived lines, the introgression segments on chromosome 6B are surprisingly long (up to 220 cM in RS4-11-10 and up to 151 cM in WB4-1-6), possibly indicating suppression of recombination around the *Gpc-B1* locus. Elsewhere in the genome, only a few polymorphisms were detected between the backcross derivatives and their recurrent parents (Fig. S1, available as Supplementary Material to this paper), with RS4-11-10, RS4-11-16, WB4-1-6 and WB4-1-8 differing from their recurrent parents for only two, seven, four and five markers respectively. Thus, although differences between the *Gpc-B1*+ lines and their *Gpc-B1*− counterparts cannot be attributed exclusively to the presence or absence of the functional *NAM-B1* allele at the *Gpc-B1* locus, it is reasonable to attribute them to the presence or absence of the *Gpc-B1*-containing introgression. With continued backcrossing there would be further opportunities for recombination, and it might be possible to reduce the introgression segment size, especially if marker-based selection is used to favour recombinant progeny. However, given that the donor parents used here are modern cultivars, there is no reason to expect negative effects from these long introgression segments.

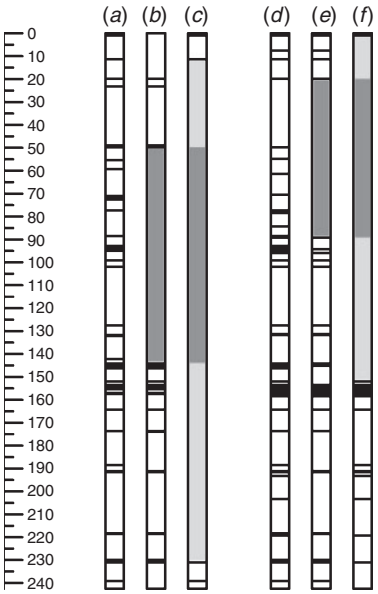

Fig. 1. Genetic maps of wheat chromosome 6B showing consensus-map positions at which polymorphisms were detected: (*a*) among Glupro, Somerset and RAC1262A; (*b*) between Somerset and Glupro (with shading indicating the possible extent of the Glupro-derived introgression in Somerset); (*c*) between RS4-11-10 and Somerset (with shading indicating the possible extent of the Somerset-derived introgression in RS4-11-10 and the dark-shaded part likely to have been derived from Glupro); (*d*) among Glupro, Burnside and Wyalkatchem; (*e*) between Burnside and Glupro (with shading indicating the possible extent of the Glupro-derived introgression in Burnside); and (*f*) between WB4-1-6 and Burnside (with shading indicating the possible extent of the Somerset-derived introgression in WB4-1-6 and the dark-shaded part likely to have been derived from Glupro). The scale on the left indicates genetic distances in cM.

The two recurrent parents differed substantially in flowering time, with Wyalkatchem reaching anthesis 13 days after RAC1262A (Table 1). Compared with RAC1262A, Wyalkatchem consistently (i.e. under both control conditions and after the heat treatment) had a shorter grain filling duration (anthesis to complete peduncle senescence), more grains per spike, larger grain and lower grain protein concentration. Each of the other lines reached anthesis at a very similar time as the line with which it had been paired (Gladius with Drysdale; RS4-11-10 with RS4-11-16; WB4-1-6 with WB4-1-8) (Table 1). For these pairs of lines, there were numerous cases in which the two lines differed for a particular trait under one set of conditions but not the other (Table 2). There were no cases in which the direction of a significant difference differed between the two sets of conditions.

In the Wyalkatchem background, the *Gpc-B1* segment significantly increased grain protein content (20.0% for WB4-1-6 compared with 16.8% for WB4-1-8). In the higher-protein RAC1262A background, the difference (22.2% for RS4-11-10 compared with 20.6% in RS4-11-16) was not quite significant with the stringent (Bonferroni-corrected) Type I error control employed here. This failure to detect significant differences in protein content for one pair of lines is probably due to the relatively low number of replicates used for this trait. With more replicates, the statistical test would likely have had sufficient power to detect an increase in grain protein content due to *Gpc-B1* in both backgrounds.

Table 1. Number of days from sowing to anthesis in four pairs of wheat lines and *P*-values for comparisons of lines within pairs

Statistically significant differences at a Bonferroni-corrected significance level of 0.006 (0.05/8) are indicated: *

Wheat line or comparison	Days from sowing to anthesis	*P*-value
Drysdale	71	–
Gladius	68	–
Drysdale vs Gladius	–	0.123
RAC1262A	63	–
Wyalkatchem	76	–
RAC1262A vs Wyalkatchem	–	0.002*
RS4-11-10 (*Gpc-B1*)	66	–
RS4-11-16 (non-*Gpc-B1*)	66	–
RS4-11-10 vs RS4-11-16	–	0.856
WB4-1-6 (*Gpc-B1*)	75	–
WB4-1-8 (non-*Gpc-B1*)	76	–
WB4-1-6 vs WB4-1-8	–	0.277

As expected, the heat treatment, which was applied after grain had set, had no effect on the number of grains per spike. In each of the eight lines evaluated, the period between anthesis and complete peduncle senescence was significantly shortened by exposure to heat (Table 2). The heat treatment reduced grain weight in Drysdale, Wyalkatchem, RS4-11-10, WB4-1-6 and WB4-1-8, increased grain protein concentration in WB4-1-8 and increased % UPP in WB4-1-6.

Discussion

Although the cultivars Gladius and Drysdale did not differ in time to anthesis, they differed from each other in grain number, senescence, single grain weight and grain protein content. In Drysdale, heat treatment accelerated senescence more than in Gladius (by 11 days compared with 7 days) and significantly reduced grain weight. In Gladius, which is considered to be heat tolerant (Fleury *et al*. 2010), exposure to heat accelerated senescence by 7 days but did not significantly reduce grain weight. Similarly, RAC1262A (Gladius or a close sister line of Gladius) did not show significant reduction in grain weight under heat stress. In Drysdale, the acceleration of senescence was greater (11 days) and grain weight was significantly reduced, possibly due to early senescence shortening the grain filling duration and interfering with the supply of substrates to the developing grain. Heat stress also shortens the duration of grain filling and in sensitive cultivars it decreases the rate of grain growth (Tashiro and Wardlaw 1989; Stone and Nicolas 1995*a*, 1995*b*; Viswanathan and Khanna-Chopra 2001; Zahedi and Jenner 2003; Altenbach 2012).

Given that the whole plant was exposed to the heat treatment, the heat-induced reduction in grain weight may have involved interference with important physiological processes in vegetative tissues and/or in the developing grain. These could include nutrient uptake, carbon translocation and starch synthesis. Soluble starch synthase, which catalyses starch biosynthesis, is particularly sensitive to high temperatures, leading to reduction of starch deposition in the developing grain under heat (Jenner

Table 2. Adjusted mean values for number of grains per spike, number of days from anthesis to senescence (complete peduncle yellowing), single grain weight, percentage flour protein and percentage unextractable polymeric protein (% UPP) in four pairs of wheat lines in control and heat treatments and *P*-values for comparisons of lines within pairs and for the effects of post-anthesis exposure to heat for 3 days

Statistically significant *P*-values indicated: *, below the Bonferroni-corrected significance levels of 0.0031 (0.05/16), indicating statistical significance of the corresponding comparisons

	Grains per spike		Days from anthesis to senescence		Single grain weight (mg)		% Protein		% UPP	
	Control	Heat	Control	Heat	Control	Heat	Control	Heat	Control	Heat
Wheat line					Adjusted mean					
Drysdale	51	50	60	49	49	38	17.3	18.0	34.9	36.1
Gladius	32	32	61	55	38	35	20.0	20.8	46.8	48.3
RAC1262A	28	28	66	59	34	33	20.0	20.3	46.9	48.0
Wyalkatchem	37	36	62	54	47	43	17.7	17.7	39.7	41.5
RS4–11–10 (*Gpc-B1*)	29	28	60	52	36	33	22.2	21.5	48.4	49.1
RS4–11–16 (non-*Gpc-B1*)	31	29	62	57	38	34	20.6	22.2	46.9	48.0
WB4–1-6 (*Gpc-B1*)	41	39	56	51	46	40	20.0	19.5	39.9	42.9
WB4–1-8 (non-*Gpc-B1*)	38	38	63	55	48	40	16.8	19.9	39.0	39.9
Comparison					*P*-value					
Drysdale vs Gladius	0.000*	0.000*	0.158	0.000*	0.000*	0.036	0.000*	0.000*	0.002*	0.001*
RAC1262A vs Wyalkatchem	0.000*	0.000*	0.003*	0.000*	0.000*	0.000*	0.000*	0.000*	0.005	0.002*
RS4-11-10 vs RS4-11-16	0.215	0.464	0.022	0.000*	0.264	0.091	0.006	0.204	0.006	0.410
WB4-1-6 vs WB4-1-8	0.132	0.337	0.000*	0.001*	0.080	0.702	0.000*	0.295	0.000*	0.583
Heat vs control										
Drysdale	0.612		0.000*		0.000*		0.147		0.347	
Gladius	0.906		0.000*		0.071		0.070		0.410	
RAC1262A	0.734		0.000*		0.369		0.194		0.724	
Wyalkatchem	0.710		0.000*		0.001*		0.033		0.969	
RS4-11-10 (*Gpc-B1*)	0.263		0.000*		0.003*		0.167		0.060	
RS4-11-16 (non-*Gpc-B1*)	0.541		0.000*		0.015		0.408		0.410	
WB4-1-6 (*Gpc-B1*)	0.723		0.000*		0.000*		0.316		0.000*	
WB4-1-8 (non-*Gpc-B1*)	0.369		0.000*		0.000*		0.000*		0.530	

1994). Since a large proportion of the wheat grain is starch, suppression of starch synthesis would be expected to reduce grain weight. Reduction of starch synthesis might be expected to be accompanied by increased grain protein concentration due to a concentration effect. Here, significant heat-induced reduction in grain weight was accompanied by significant increase in grain protein content only in the *Gpc-B1*− line WB4-1-8. We noted that although the heat treatment resulted in a significant increase in grain protein content of that line, and the *Gpc-B1* introgression segment significantly increased grain protein content in the same background (WB4-1-6 vs WB4-1-8), there was no further increase due to the combination of *Gpc-B1* and heat. Grain protein concentration may have already reached an upper limit.

In contrast to the effects of heat, the *Gpc-B1* introgression segment accelerated senescence without significantly decreasing grain weight, indicating that factors that hasten senescence do not necessarily limit grain size. Although acceleration of senescence by *Gpc-B1* was expected (Uauy *et al*. 2006*b*; Brevis and Dubcovsky 2010), we noted that the extent of this effect differed between genetic backgrounds (Wyalkatchem vs RAC1262A) and between the control and heat treatments.

Estimation of % UPP made it possible to investigate the effects of *Gpc-B1* and/or heat on a functional property of grain protein. Measurement of % UPP, which requires only a small sample of flour, provides an indication of the extent of glutenin polymerisation and is a predictor of dough strength. These characteristics are affected by allelic variation at the *Glu-1* loci, which encode high-molecular-weight glutenins (HMW-GS). Gladius, RAC1262A, RS4-11-10 and RS411-16 all carry the *Glu-A1a*, *Glu-B1b* and *Glu-D1d* alleles, which encode the HMW-GS *Ax1*, *Bx7*, *By8*, *Dx5* and *Dy10*. Wyalkatchem, WB4-1-6 and WB4-1-8 all carry the *Glu-A1a*, *Glu-B1b* and *Glu-D1a* alleles, which encode the HMW-GS *Ax1*, *Bx7*, *By8*, *Dx2* and *Dy12*. Drysdale carries *Glu-A1a*, *Glu-B1i* and *Glu-D1d* alleles, which encode the HWM-GS *Ax1*, *Bx17*, *By18*, *Dx5* and *Dy10*. According to effect estimates presented by Eagles *et al*. (2002), the allelic combination carried by Gladius should lead to stronger dough (measured as the maximum dough resistance to extension, R_{max}) than the combinations carried by Wyalkatchem and Drysdale. Consistent with these expectations, the lines carrying the Gladius allelic combination had consistently higher % UPP than Drysdale or those carrying the Wyalkatchem combinations, under control conditions and after post-anthesis heat treatment. Such differences in dough strength are partly due to the extent of glutenin polymerisation.

Accumulation of SDS-insoluble polymers has been shown to begin at ~31 days after anthesis and to be accompanied by reductions in monomers and SDS-soluble polymers (Gupta *et al*. 1996; Carceller and Aussenac 1999). Consistent with

this, exposure to heat late in grain filling (in field experiments or applied at 25 days after anthesis) has been reported to reduce % UPP and dough mixing time and strength (Irmak *et al*. 2008; Cavanagh *et al*. 2010). In contrast, earlier heat treatments (applied at 10 or 12 days after anthesis) have been reported to increase % UPP (Balla *et al*. 2011) and increase SDS sedimentation height : volume ratio (another indicator of glutenin polymerisation and dough strength) (Beecher *et al*. 2012). In the experiment conducted here, plants were exposed to heat early in grain filling (15 days after anthesis) and for only 3 days. In the *Gpc-B1*− lines, this heat treatment had no significant effects on % UPP. In the *Gpc-B1*+ line WB4-1-6; however, the heat treatment increased % UPP from 39.9 to 42.9%. Despite high grain protein concentration achieved in that line under control conditions (20.0%), % UPP was relatively low (39.9% compared with 48.4% for RS4-11-10, probably due to the Wyalkatchem background). Perhaps in the high-protein condition conferred by the *Gpc-B1* introgression (20.0% compared with 16.8% for WB4-1-8) the heat treatment triggered a change in glutenin polymerisation. In the other *Gpc-B1*+ line, RS4-11-10, the corresponding % UPP values were 48.4% (control) and 49.1% (heat); the smaller difference between these was in the same direction, but not statistically significant ($P = 0.056$). Compared with WB4-1-6, RS4-11-10 may have had less capacity for heat to increase % UPP, given that the control level of % UPP was already high.

In the current study, the use of controlled environment facilities made it possible to expose wheat plants to high temperatures at a specific developmental stage and without the soil moisture deficit that often accompanies heat events in the field. Under the experimental conditions used here, it was not possible to realistically measure bread making quality or grain yield, but data were obtained for indicator variables including grain protein concentration, % UPP, grain number and grain weight. In the experimental conditions used here, the *Gpc-B1* introgression increased not only grain protein concentration but also protein polymerisation. It accelerated senescence yet did not reduce grain number or grain weight. Exposure to heat between 15 and 17 days after anthesis similarly hastened senescence, but reduced grain size. This exposure to heat early in grain filling did not affect % UPP in most lines, and it increased grain protein concentration in only one of the eight lines investigated. Although these effects have not been tested in the field, it is worth noting that the presence of the *Gpc-B1* introgression did not exacerbate the effects of heat stress on any quality or yield-related parameters, in either of the Australian genetic backgrounds used here.

Acknowledgements

This work was supported by the Grains Research and Development Corporation, the Australian Research Council, the South Australian Government and by scholarships awarded to the first author by the University of Adelaide and the Australian Centre for Plant Functional Genomics. We thank Howard Eagles for providing seed of the *Gpc-B1* lines and acknowledge Hugh Wallwork and the late Genet Mekuria for their contributions to the development of those lines. We thank Iman Lohraseb and Hamid Shirdelmoghanloo for technical assistance, Matthew Hayden for SNP genotyping and Marie Appelbee for advice on HPLC analysis for estimating % unextractable polymeric protein.

References

Altenbach SB (2012) New insights into the effects of high temperature, drought and post-anthesis fertilizer on wheat grain development. *Journal of Cereal Science* **56**, 39–50. doi:10.1016/j.jcs.2011.12.012

Appelbee MJ (2007) Quality potential of gluten proteins in hexaploid wheat and related species. PhD Thesis, School of Agriculture, Food and Wine, The University of Adelaide, SA, Australia.

Balla K, Rakszegi M, Li Z, Békés F, Bencze S, Veisz O (2011) Quality of winter wheat in relation to heat and drought shock after anthesis. *Czech Journal of Food Sciences* **29**, 117–128.

Batey IL, Gupta RB, MacRitchie F (1991) Use of size-exclusion high-performance liquid chromatography in the study of wheat flour proteins: an improved chromatographic procedure. *Cereal Chemistry* **68**, 207–209.

Beecher FW, Mason E, Mondal S, Awika J, Dirk Hays D, Ibrahim A (2012) Identification of quantitative trait loci (QTLs) associated with maintenance of wheat (*Triticum aestivum* Desf.) quality characteristics under heat stress conditions. *Euphytica* **188**, 361–368. doi:10.1007/s10681-012-0687-z

Blumenthal CS, Bekes F, Gras PW, Barlow EWR, Wrigley CW (1995) Identification of wheat genotypes tolerant to the effects of heat stress on grain quality. *Cereal Chemistry* **72**, 539–544.

Brevis JC, Dubcovsky J (2010) Effects of the chromosome region including the *Gpc-B1* locus on wheat grain and protein yield. *Crop Science* **50**, 93–104. doi:10.2135/cropsci2009.02.0057

Brevis JC, Morris CF, Manthey F, Dubcovsky J (2010) Effect of the grain protein content locus *Gpc-B1* on bread and pasta quality. *Journal of Cereal Science* **51**, 357–365. doi:10.1016/j.jcs.2010.02.004

Buckee GK (1994) Determination of total nitrogen in barley, malt and beer by Kjeldahl procedures and the Dumas combustion method-collaborative trial. *Journal of the Institute of Brewing. Institute of Brewing* **100**, 57–64.

Butler DG, Cullis BR, Gilmour AR, Gogel BJ (2009) 'ASReml-R Reference Manual.' (ver. 3) (Queensland Department of Primary Industries: Brisbane)

Carceller JL, Aussenac T (1999) Accumulation and changes in molecular size distribution of polymeric proteins in developing grains of hexaploid wheats: role of the desiccation phase. *Australian Journal of Plant Physiology* **26**, 301–310. doi:10.1071/PP99010

Cavanagh C, Taylor J, Larroque O, Coombes N, Verbyla A, Nath Z, Kutty I, Rampling L, Butow B, Ral J-P, Tomoskozi S, Balazs G, Békés F, Mann G, Quail K, Southan M, Morell M, Newberry M (2010) Sponge and dough bread making: genetic and phenotypic relationships with wheat quality traits. *Theoretical and Applied Genetics* **121**, 815–828. doi:10.1007/s00122-010-1352-3

Cavanagh CR, Chao S, Wang S, Huang BE, Stephen S, Kiani S, Forrest K, Saintenac C, Brown-Guedira GL, Akhunova A, See D, Bai G, Pumphrey M, Tomar L, Wong D, Kong S, Reynolds M, da Silva ML, Bockelman H, Talbert L, Anderson JA, Dreisigacker S, Baenziger S, Carter A, Korzun V, Morrell PL, Dubcovsky J, Morell MK, Sorrells ME, Hayden MJ, Akhunov E (2013) Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 8057–8062. doi:10.1073/pnas.1217133110

Distelfeld A, Cakmak I, Peleg Z, Ozturk L, Yazici AM, Budak H, Saranga Y, Fahima T (2007) Multiple QTL-effects of wheat *Gpc-B1* locus on grain protein and micronutrient concentrations. *Physiologia Plantarum* **129**, 635–643. doi:10.1111/j.1399-3054.2006.00841.x

Eagles HA, Hollamby GJ, Gororo NN, Eastwood RF (2002) Estimation and utilisation of glutenin gene effects from the analysis of unbalanced data from wheat breeding programs. *Australian Journal of Agricultural Research* **53**, 367–377. doi:10.1071/AR01074

Fleury D, Jefferies S, Kuchel H, Langridge P (2010) Genetic and genomic tools to improve drought tolerance in wheat. *Journal of Experimental Botany* **61**, 3211–3222. doi:10.1093/jxb/erq152

Gilmour AR, Cullis BR, Verbyla AP (1997) Accounting for natural and extraneous variation in the analysis of field experiments. *Journal of Agricultural Biological & Environmental Statistics* **2**, 269–293. doi:10.2307/1400446

Gupta RB, Masci S, Lafiandra D, Bariana HS, MacRitchie F (1996) Accumulation of protein subunits and their polymers in developing grains of hexaploid wheats. *Journal of Experimental Botany* **47**, 1377–1385. doi:10.1093/jxb/47.9.1377

Irmak S, Naeem HA, Lookhart GL, MacRitchie F (2008) Effect of heat stress on wheat proteins during kernel development in wheat near-isogenic lines differing at *Glu-D1*. *Journal of Cereal Science* **48**, 513–516. doi:10.1016/j.jcs.2007.12.002

Jenner CF (1994) Starch synthesis in the kernel of wheat under high temperature conditions. *Australian Journal of Plant Physiology* **21**, 791–806. doi:10.1071/PP9940791

Li Y, Wu Y, Hernandez-Espinosa N, Peña RJ (2013) The influence of drought and heat stress on the expression of end use quality parameters of common wheat. *Journal of Cereal Science* **57**, 73–78. doi:10.1016/j.jcs.2012.09.014

Olmos S, Distelfeld A, Chicaiza O, Schlatter AR, Fahima T, Echenique V, Dubcovsky J (2003) Precise mapping of a locus affecting grain protein content in durum wheat. *Theoretical and Applied Genetics* **107**, 1243–1251. doi:10.1007/s00122-003-1377-y

Pallotta MA, Graham RD, Langridge P, Sparrow DHB, Barker SJ (2000) RFLP mapping of manganese efficiency in barley. *Theoretical and Applied Genetics* **101**, 1100–1108. doi:10.1007/s001220051585

R Development Core Team (2012) 'R: a language and environment for statistical computing.' (R Foundation for Statistical Computing: Vienna)

Randall P, Moss H (1990) Some effects of temperature regime during grain filling on wheat quality. *Australian Journal of Agricultural Research* **41**, 603–617. doi:10.1071/AR9900603

Stone P, Nicolas M (1995*a*) Effect of timing of heat stress during grain filling on two wheat varieties differing in heat tolerance. I. Grain growth. *Functional Plant Biology* **22**, 927–934.

Stone P, Nicolas M (1995*b*) Comparison of sudden heat stress with gradual exposure to high temperature during grain filling in two wheat varieties differing in heat tolerance. I. Grain growth. *Functional Plant Biology* **22**, 935–944.

Stone PJ, Nicolas ME (1996) Varietal differences in mature protein composition of wheat resulted from different rates of polymer accumulation during grain filling. *Australian Journal of Plant Physiology* **23**, 727–737. doi:10.1071/PP9960727

Tashiro T, Wardlaw IF (1989) A comparison of the effect of high temperature on grain development in wheat and rice. *Annals of Botany* **64**, 59–65.

Uauy C, Brevis JC, Dubcovsky J (2006*a*) The high grain protein content gene *Gpc-B1* accelerates senescence and has pleiotropic effects on protein content in wheat. *Journal of Experimental Botany* **57**, 2785–2794. doi:10.1093/jxb/erl047

Uauy C, Distelfeld A, Fahima T, Blechl A, Dubcovsky J (2006*b*) A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science* **314**, 1298–1301. doi:10.1126/science.1133649

Viswanathan C, Khanna-Chopra R (2001) Effect of heat stress on grain growth, starch synthesis and protein synthesis in grains of wheat (*Triticum aestivum* L.) varieties differing in grain weight stability. *Journal Agronomy & Crop Science* **186**, 1–7. doi:10.1046/j.1439-037x.2001.00432.x

Waters BM, Uauy C, Dubcovsky J, Grusak MA (2009) Wheat (*Triticum aestivum*) NAM proteins regulate the translocation of iron, zinc, and nitrogen compounds from vegetative tissues to grain. *Journal of Experimental Botany* **60**, 4263–4274. doi:10.1093/jxb/erp257

Zahedi M, Jenner CF (2003) Analysis of effects in wheat of high temperature on grain filling attributes estimated from mathematical models of grain filling. *Journal of Agricultural Science* **141**, 203–212. doi:10.1017/S0021859603003411