Supplementary Material

Truncation of grain filling in wheat (*Triticum aestivum*) triggered by brief heat stress during early grain filling: association with senescence responses and reductions in stem reserves

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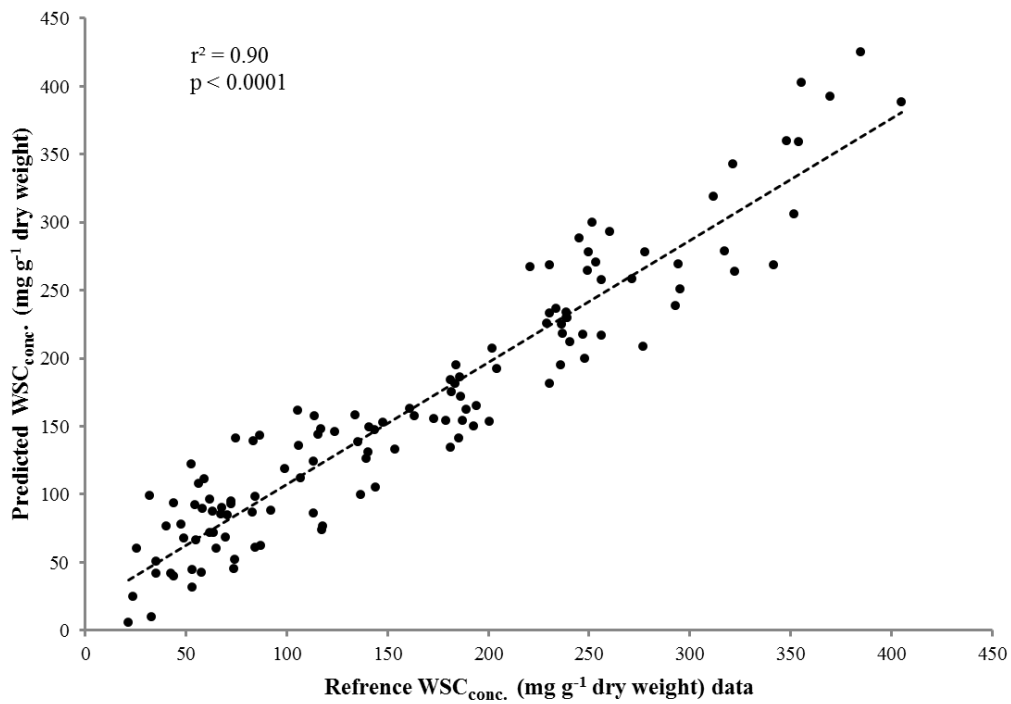[D]Corresponding author. Email: nick.collins@acpfg.com.au

Fig. S1. Water soluble carbohydrate concentration (WSC$_{conc.}$, mg g^{-1} DW) in a chosen reference set of 125 wheat stem samples determined using anthrone method, plotted against WSC content of the samples predicted using attenuated total reflectance midinfrared spectroscopy. Dashed line represents the theoretical regression line.
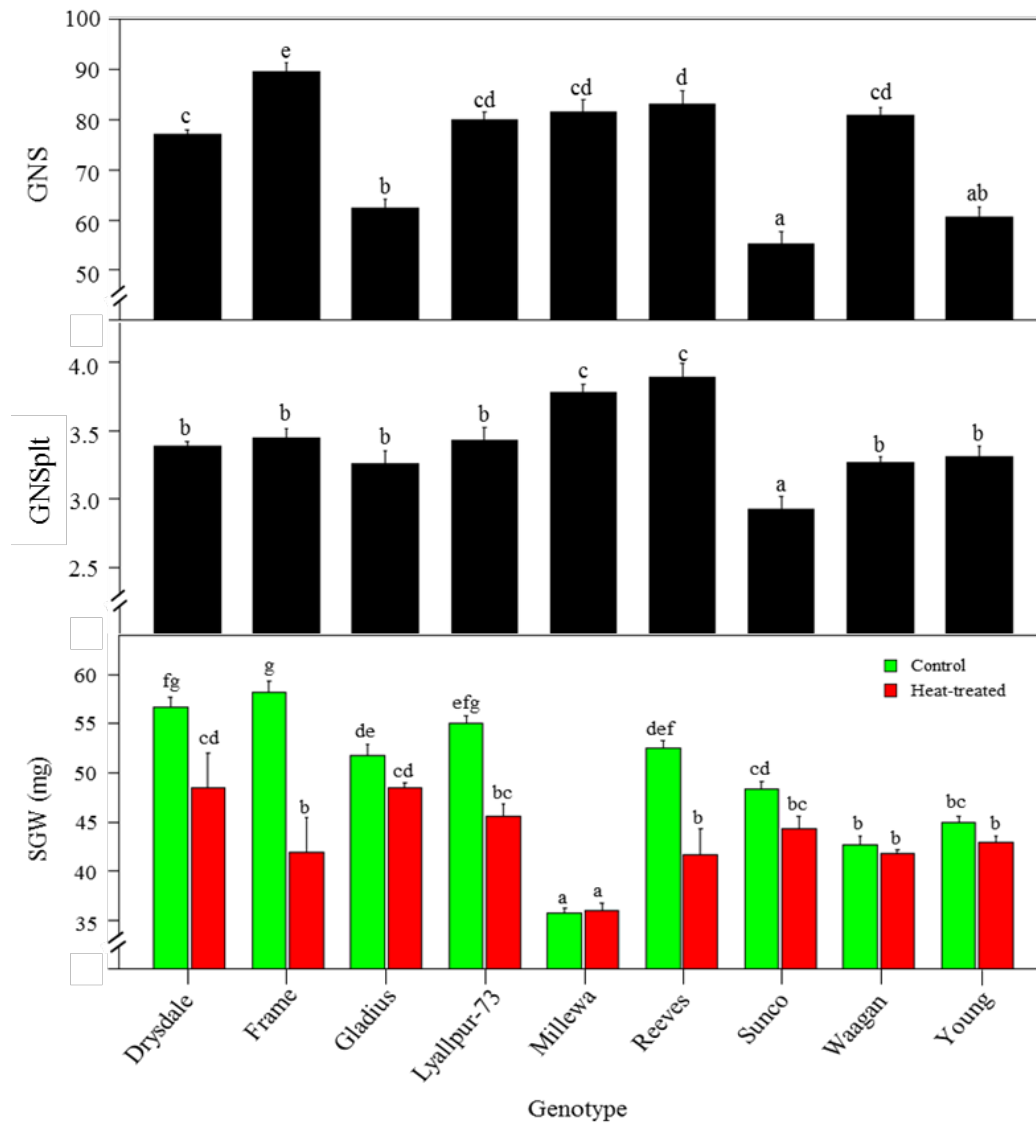
Fig. S2. Means + s.e. for grain number per spike (GNS), grain number spikelet^{-1} (GNSplt) and single grain weight (SGW) from Experiment 1. GNS and GNSplt were not heat responsive, so combined means for control and heat treated plants are shown. Means with the same letter were not significantly different at $P > 0.05$ (LSD test).

Fig. S3. Time courses of flag leaf chlorophyll *a* content (Chl*a*) in control (green circles) and heat-treated plants (red triangles) of 9 bread wheat genotypes (mean ± s.e.). Asterisks indicate where there are significant differences between treatments at *P* < 0.05. The red bar at the bottom of the plots represents the period of brief heat treatment.
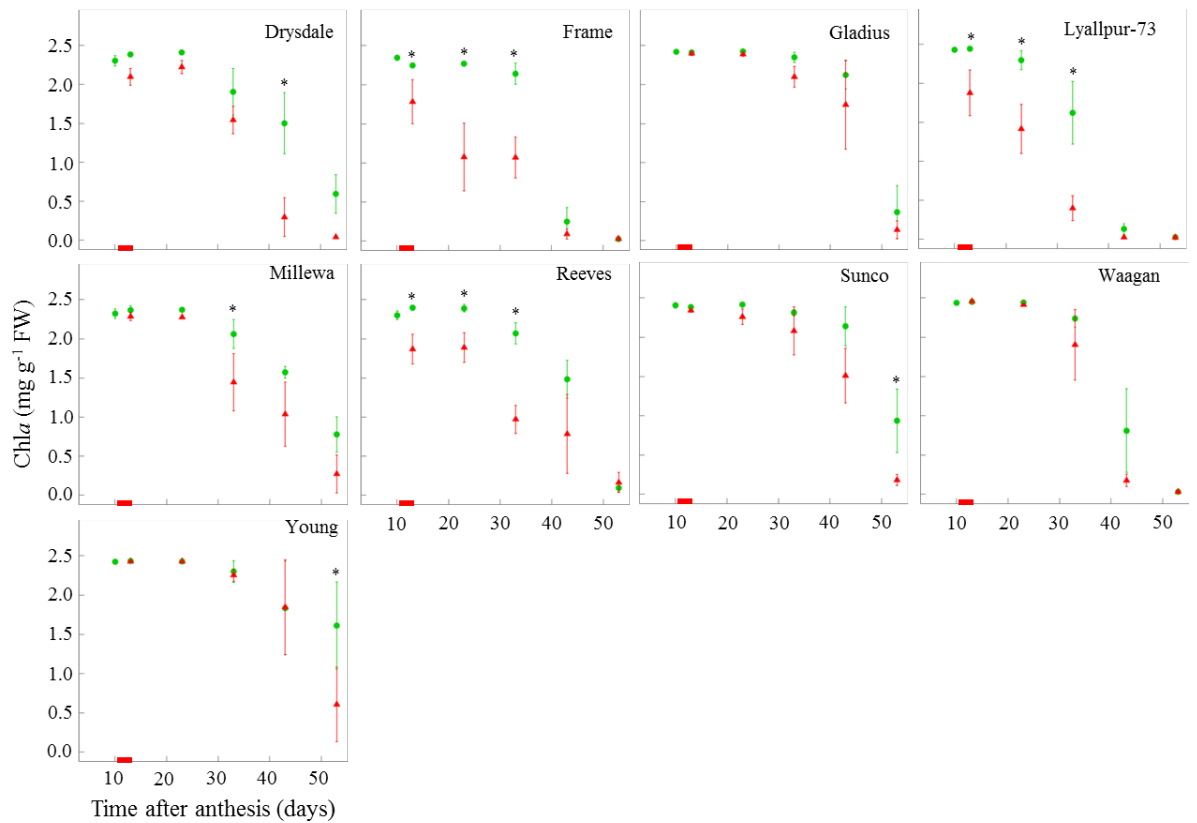
Fig. S4. Time courses of flag leaf chlorophyll *b* content (Chl*b*) in control (green circles) and heat-treated plants (red triangles) of 9 bread wheat genotypes (mean ± s.e.). Asterisks indicate where there are significant differences between treatments at *P* < 0.05. The red bar at the bottom of the plots represents the period of brief heat treatment.
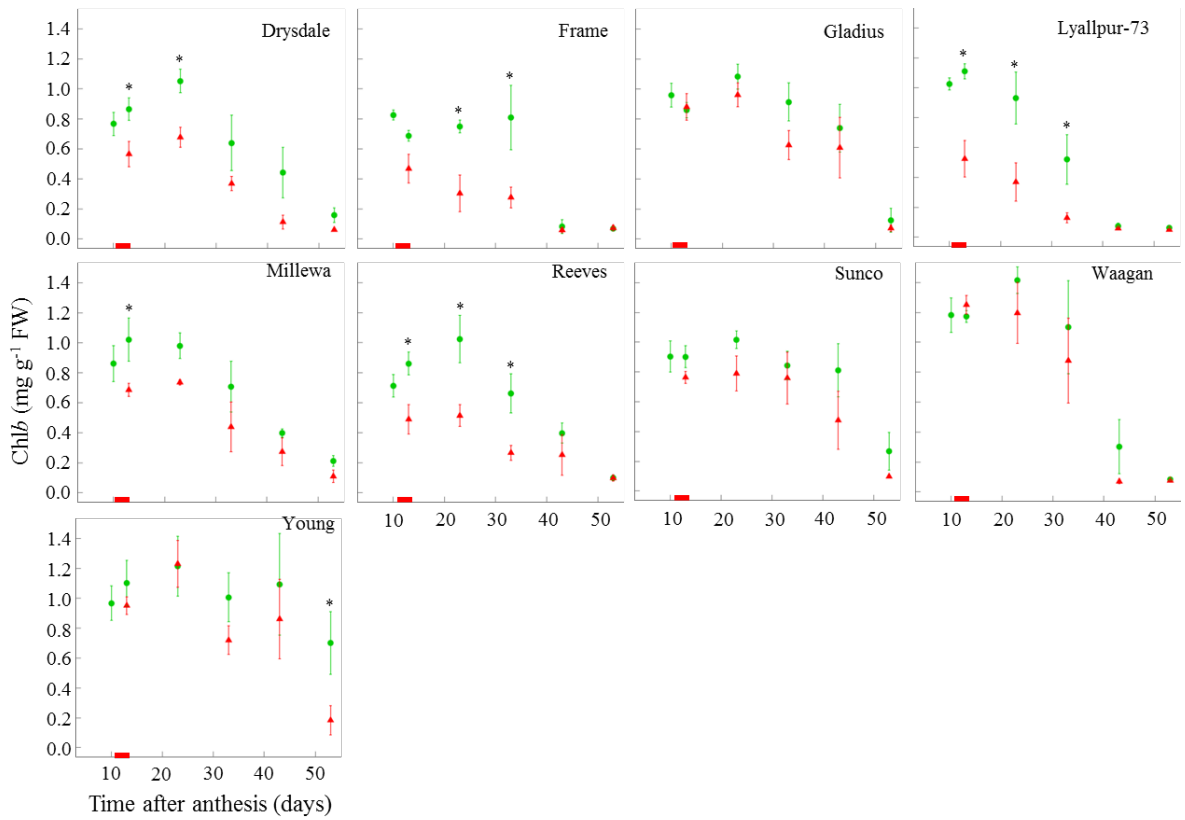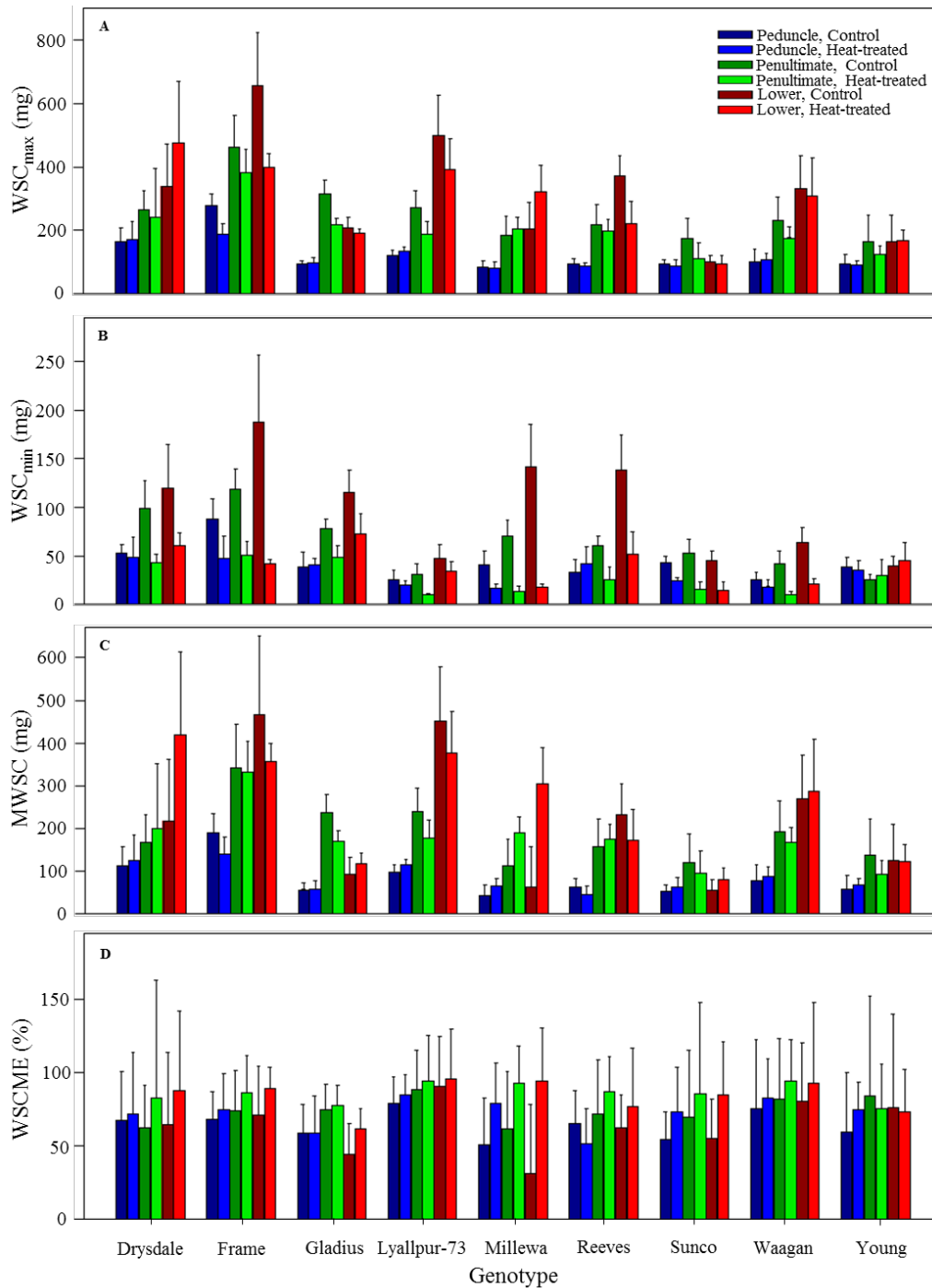
Fig. S5. Maximum water soluble carbohydrate content (WSC$_{max.}$), minimum water soluble carbohydrate content (WSC$_{min.}$), mobilized WSC (MWSC) and WSC mobilization efficiency (WSCME) of different segments of the main stem (peduncle, penultimate and lower internodes) of 9 bread wheat varieties in control and plants that had been heat-treated for 3 days at 37/27 ºC at 10 days after anthesis. Bars indicate mean + s.e. Note that genotype, treatment and genotype × treatment effects were not always significant for these WSC traits (see text for details).
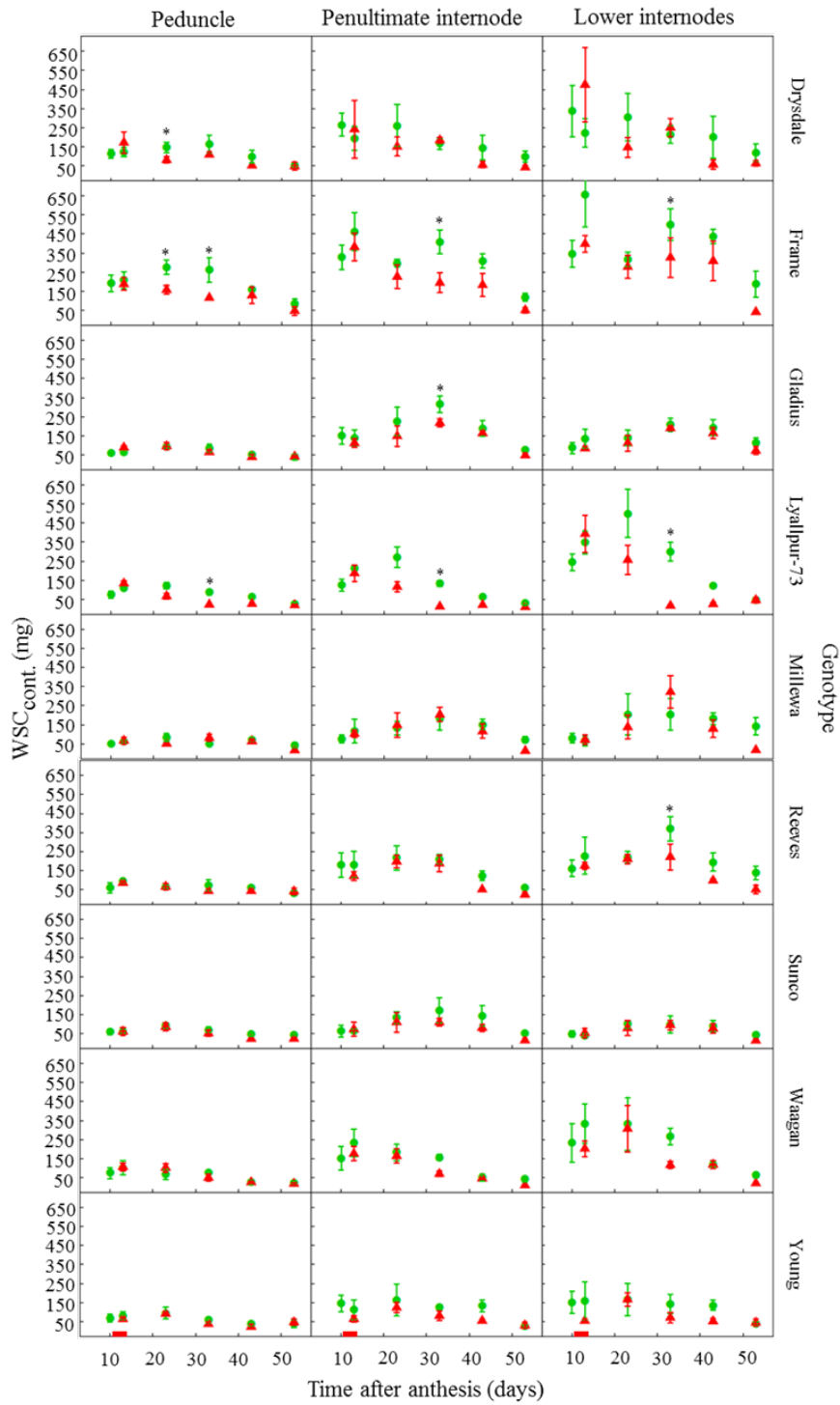
Fig. S6. Time courses of water soluble carbohydrate content (WSC$_{cont.}$) of the peduncle, penultimate internode and remaining lower internodes of the main stem from control (green circles) and heat-treated plants (red triangles) of 9 bread wheat genotypes (mean ± s.e.). Asterisks indicate where there is a significant difference between treatments at $P < 0.05$. The red bar at the bottom of the plots represents the period of brief heat treatment.
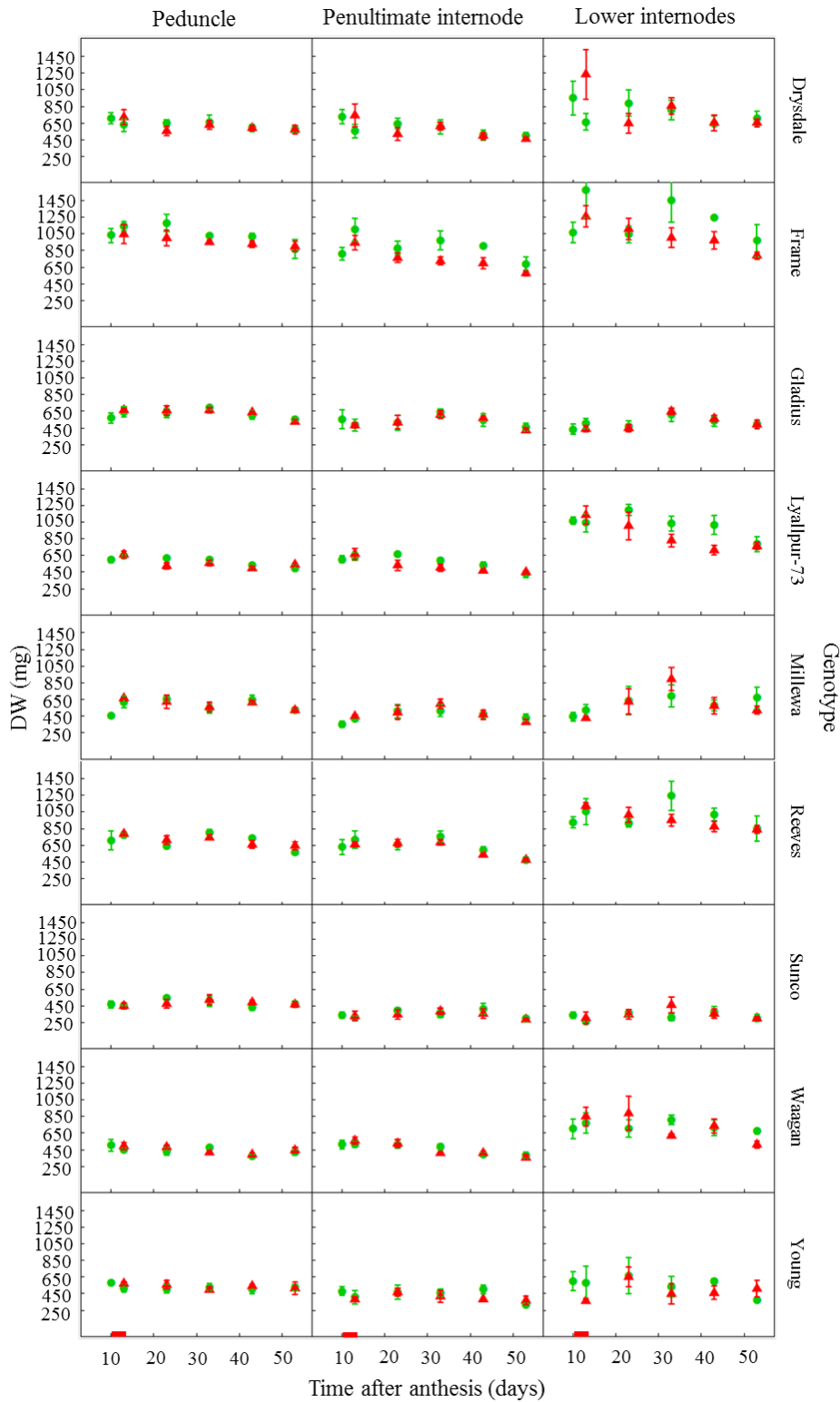
Fig. S7. Time courses of stem dry weight (DW) of the peduncle, penultimate internode and remaining lower internodes of the main stem from control (green circles) and heat-treated plants (red triangles) of 9 bread wheat genotypes (mean ± s.e.). Asterisks indicate where there is a significant difference between treatments at *P* < 0.05. The red bar at the bottom of the plots represents the period of brief heat treatment.

Table S1. Measured temperatures (ºC) and relative humidity (RH; %) in the greenhouse. Anthesis and maturity occurred during October and November-December, respectively.

Conditions/Month	August	September	October	November	December
Ave. day temp.	21.3	20	19.7	20.1	21.2
Ave. night temp.	17.9	16.5	16.1	16.4	16.9
Ave. min. temp.	16.9	15.6	15.2	15.3	15.8
Ave. max. temp.	23.4	22.7	22.3	22.6	23.9
Min. temp.	14.7	14.8	14.5	14.7	14.4
Max. temp.	25.1	27.8	28.1	25.7	29.7
Days > 30 ºC	0	0	0	0	0
Ave. max. RH	87.0	90.7	89.4	89.4	89.6
Ave. min. RH	55.0	66.3	60.9	66.9	66.7

Table S2. *P*-values for genotype, treatment and genotype × treatment (G × T) effects in the linear mixed model analysis of grain growth data from Experiment 2[a], and average and range of heat effects.

Trait	Genotype	Treatment	G × T	Ave. heat effect (%)	Range in effect (%)[b]
MGR	<0.001	ns[c] (0.057)	ns (0.258)	-3.6	+8.8 to -15.7
SGR	<0.001	ns (0.235)	<0.001	-2.3	+18.3 to -16.8
TIP	<0.001	<0.001	<0.001	-16.0	-5.8 to -25.8
GFD	<0.004	<0.001	<0.05	-13.7	-2.1 to -24.2
SGW$_{pred}$	<0.001	<0.001	<0.001	-14.6	-2.4 to -28.8
SGW[a]	< 0.001	< 0.001	< 0.001	-11.2	+0.7 to -28.1

[a] SGW from Experiment 1 also shown for comparison to SGW$_{pred}$.

[b] difference in means of heat vs. control represented, whether differences were significant or not.

[c] ns, not significant ($P > 0.05$).

MGR, maximal grain growth rate; SGR, sustained grain growth rate; TIP, time to inflection point of grain growth; GFD, grain filling duration; SGW$_{pred}$, final single grain weight predicted from the logistic models. SGW, final single grain weight, measured directly.

Table S3. Correlations between % heat responses.

Trait	SGW_pred	GFD	TIP	MGR	SGR	TotChl_av	Chla_av	Chlb_av	Peduncle WSCmax	Peduncle WSCmin	Peduncle WSCav	Peduncle MWSC	Peduncle WSCME	Peduncle DWav	Penult. WSCmax	Penult. WSCmin	Penult. WSCav	Penult. MWSC	Penult. WSCME	Penult. DWav	Lower WSCmax	Lower WSCmin	Lower WSCav	Lower MWSC	Lower WSCME	Lower DWav
SGW_pred	-																									
GFD	0.87**	-																								
TIP	0.95***	0.95***	-																							
MGR	0.35	-0.15	0.13	-																						
SGR	0.49	0.01	0.24	0.95***	-																					
TotChl_av	0.93***	0.86**	0.85**	0.17	0.39	-																				
Chla_av	0.94***	0.85**	0.85**	0.19	0.41	0.99***	-																			
Chlb_av	0.90***	0.86**	0.83**	0.09	0.31	0.98***	0.96***	-																		
WSCmax (Ped)	0.24	0.22	0.26	0.04	0.05	0.3	0.33	0.23	-																	
WSCmin (Ped)	-0.12	-0.03	-0.1	-0.23	-0.24	0.01	0.05	-0.06	0.23	-																
WSCav (Ped)	0.71*	0.64	0.61	0.09	0.24	0.82**	0.80**	0.81**	0.44	0.24	-															
MWSC (Ped)	0.52	0.33	0.47	0.47	0.49	0.39	0.42	0.34	0.56	-0.56	0.24	-														
WSCME (Ped)	0.48	0.26	0.41	0.54	0.56	0.3	0.31	0.27	0.05	-0.82**	0.02	0.86**	-													
DWav (Ped)	0.77*	0.81**	0.78*	-0.01	0.13	0.80**	0.83**	0.73*	0.55	0.32	0.79*	0.37	0.1	-												
WSCmax (Pen)	-0.16	-0.41	-0.34	0.43	0.37	-0.23	-0.21	-0.3	-0.09	-0.13	0.05	0.2	0.27	-0.06	-											
WSCmin (Pen)	0.2	0.27	0.19	-0.08	0.07	0.31	0.37	0.21	-0.05	0.4	-0.03	-0.12	-0.11	0.29	-0.26	-										
WSCav (Pen)	0.31	-0.04	0.06	0.62	0.65	0.28	0.27	0.23	0.01	-0.06	0.52	0.24	0.26	0.26	0.78*	-0.29	-									
MWSC (Pen)	-0.08	-0.39	-0.26	0.56	0.46	-0.21	-0.2	-0.26	-0.04	-0.31	0.07	0.33	0.4	-0.1	0.95***	-0.48	0.81**	-								
WSCME (Pen)	-0.03	-0.34	-0.16	0.58	0.46	-0.21	-0.23	-0.22	-0.03	-0.44	0.04	0.35	0.43	-0.17	0.74*	-0.73*	0.73*	0.91***	-							
DWav (Pen)	0.53	0.3	0.43	0.45	0.48	0.5	0.52	0.43	0.78*	0.13	0.69*	0.59	0.22	0.67*	0.24	-0.23	0.56	0.35	0.4	-						
WSCmax (Low)	0.36	0.01	0.17	0.70*	0.76*	0.3	0.33	0.22	0.46	-0.36	0.27	0.80**	0.67*	0.29	0.54	-0.11	0.63	0.63	0.58	0.70*	-					
WSCmin (Low)	0.09	0.23	0.16	-0.23	-0.12	0.21	0.27	0.12	0.36	0.45	-0.07	0.03	-0.18	0.31	-0.46	0.86**	-0.54	-0.63	-0.81**	-0.06	-0.1	-				
WSCav (Low)	0.29	-0.09	0.1	0.72*	0.74*	0.23	0.21	0.21	0.24	-0.17	0.27	0.35	0.28	0.12	0.27	-0.36	0.64	0.45	0.63	0.66	0.71*	-0.39	-			
MWSC (Low)	0.31	-0.07	0.12	0.75*	0.71*	0.16	0.18	0.11	0.19	-0.52	0.24	0.74*	0.76*	0.1	0.72*	-0.35	0.73*	0.83**	0.78*	0.53	0.85**	-0.43	0.53	-		
WSCME (Low)	0.29	-0.08	0.11	0.74*	0.67*	0.11	0.11	0.08	0.03	-0.54	0.22	0.62	0.72*	0	0.70*	-0.44	0.73*	0.84**	0.82**	0.42	0.69*	-0.57	0.47	0.97***	-	
DWav (Low)	0.46	0.21	0.4	0.53	0.53	0.36	0.35	0.34	0.44	0	0.41	0.37	0.18	0.39	0	-0.36	0.45	0.21	0.46	0.79*	0.54	-0.27	0.88**	0.34	0.28	-

Pearson's correlation coefficients are shown, together with significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Cells are coloured green and orange to highlight significant positive or negative correlations, respectively.

SGW_pred, predicted final grain weight; GFD, grain filling duration; TIP, time to inflection point of grain growth; MGR, maximal grain growth rate; SGR, sustained grain growth rate; TotChl_av, total flag leaf chlorophyll a and b averaged over all time points, Chla_av, flag leaf chlorophyll a averaged over all time points; Chlb_av, flag leaf chlorophyll b averaged over all time points; WSC, main stem water soluble carbohydrates; WSCmax, peak WSC value; WSCmin, minimum WSC value; WSCav, WSC content averaged over all time points; MWSC, mobilized WSC; WSCME, WSC % mobilization efficiency; DWav, main stem dry weight averaged over all time points.

Table S4. Correlations for trait potentials vs. % heat responses.

	Trait	SGW$_{pred}$	GFD	TIP	MGR	SGR	TotChl$_{av}$	Chla$_{av}$	Chlb$_{av}$	WSC$_{max}$ (Ped)	WSC$_{min}$ (Ped)	WSC$_{av}$ (Ped)	MWSC (Ped)	WSCME (Ped)	DW$_{av}$ (Ped)	WSC$_{max}$ (Pen)	WSC$_{min}$ (Pen)	WSC$_{av}$ (Pen)	MWSC (Pen)	WSCME (Pen)	DW$_{av}$ (Pen)	WSC$_{max}$ (Low)	WSC$_{min}$ (Low)	WSC$_{av}$ (Low)	MWSC (Low)	WSCME (Low)	DW$_{av}$ (Low)
	SGW$_{pred}$	-0.87**	-0.18	-0.43	-0.83**	-0.84**	0.89**	0.85**	0.79*	-0.68*	-0.38	-0.68*	-0.76*	-0.65	-0.68*	-0.62	-0.39	-0.62	-0.62	-0.11	-0.82**	-0.91***	-0.43	-0.85**	-0.87**	-0.48	-0.92***
	GFD	-0.71*	-0.48	-0.56	-0.49	-0.5	0.79*	0.63	0.89**	-0.63	-0.51	-0.66	-0.63	-0.27	-0.70*	-0.59	-0.64	-0.62	-0.48	0.3	-0.74*	-0.70*	-0.64	-0.68*	-0.57	-0.03	-0.71*
	TIP	-0.79*	-0.35	-0.54	-0.66	-0.66	0.84**	0.76*	0.83**	-0.72*	-0.5	-0.73*	-0.76*	-0.49	-0.75*	-0.70*	-0.6	-0.72*	-0.63	0.1	-0.86**	-0.86**	-0.63	-0.85**	-0.75*	-0.25	-0.86**
	MGR	-0.39	0.52	0.16	-0.73*	-0.73*	0.31	0.53	-0.09	-0.2	0.18	-0.14	-0.35	-0.83**	-0.07	-0.24	0.35	-0.16	-0.45	-0.80**	-0.29	-0.53	0.26	-0.48	-0.69*	-0.87**	-0.53
	SGR	-0.5	0.53	0.2	-0.83**	-0.83**	0.47	0.64	0.12	-0.19	0.21	-0.13	-0.34	-0.83**	-0.12	-0.21	0.35	-0.13	-0.42	-0.74*	-0.34	-0.6	0.22	-0.5	-0.75*	-0.85**	-0.63
	TotChl$_{av}$	-0.78*	-0.15	-0.3	-0.71*	-0.74*	0.85**	0.76*	0.85**	-0.59	-0.38	-0.6	-0.63	-0.45	-0.63	-0.46	-0.34	-0.49	-0.43	0.04	-0.69*	-0.78*	-0.4	-0.70*	-0.74*	-0.34	-0.82**
	Chla$_{av}$	-0.79*	-0.13	-0.29	-0.73*	-0.77*	0.89**	0.79*	0.88**	-0.62	-0.41	-0.63	-0.67*	-0.47	-0.65	-0.51	-0.38	-0.53	-0.48	0.04	-0.72*	-0.81**	-0.43	-0.73*	-0.77*	-0.34	-0.83**
	Chlb$_{av}$	-0.73*	-0.21	-0.31	-0.62	-0.65	0.76*	0.65	0.80*	-0.5	-0.32	-0.51	-0.54	-0.37	-0.58	-0.34	-0.29	-0.4	-0.31	0.08	-0.63	-0.69*	-0.37	-0.61	-0.65	-0.29	-0.77*
Peduncle	WSC$_{max}$	-0.24	0.01	-0.08	-0.22	-0.25	0.25	0.15	0.36	-0.70*	-0.83**	-0.71*	-0.6	0.18	-0.80**	-0.57	-0.58	-0.73*	-0.48	0.17	-0.64	-0.42	-0.61	-0.49	-0.27	0.08	-0.4
Peduncle	WSC$_{min}$	0.37	0.3	0.35	0.26	0.21	0.11	0.12	0.08	-0.25	-0.33	-0.24	-0.2	0.22	-0.02	-0.08	-0.13	-0.04	-0.04	0.19	0.1	-0.04	-0.03	-0.07	-0.03	0.17	0.12
Peduncle	WSC$_{av}$	-0.64	-0.23	-0.35	-0.52	-0.55	0.53	0.45	0.55	-0.65	-0.55	-0.66	-0.65	-0.25	-0.58	-0.4	-0.24	-0.45	-0.4	-0.08	-0.54	-0.59	-0.14	-0.53	-0.61	-0.4	-0.56
Peduncle	MWSC	-0.73*	-0.17	-0.42	-0.70*	-0.69*	0.41	0.37	0.4	-0.53	-0.4	-0.52	-0.56	-0.4	-0.68*	-0.62	-0.4	-0.69*	-0.61	-0.17	-0.74*	-0.58	-0.44	-0.58	-0.51	-0.32	-0.62
Peduncle	WSCME	-0.73*	-0.2	-0.44	-0.71*	-0.67*	0.34	0.35	0.27	-0.2	0.04	-0.18	-0.29	-0.59	-0.32	-0.38	-0.13	-0.37	-0.43	-0.3	-0.5	-0.44	-0.17	-0.4	-0.44	-0.43	-0.51
Peduncle	DW$_{av}$	-0.68*	-0.22	-0.36	-0.57	-0.58	0.79*	0.68*	0.84**	-0.82**	-0.72*	-0.83**	-0.81**	-0.25	-0.82**	-0.79*	-0.63	-0.76*	-0.72*	0.1	-0.78*	-0.77*	-0.55	-0.74*	-0.68*	-0.12	-0.68*
Penultimate internode	WSC$_{max}$	-0.21	0.06	-0.09	-0.31	-0.25	-0.2	-0.09	-0.34	0.05	0.18	0.1	-0.01	-0.31	0.23	-0.08	0.37	0.1	-0.26	-0.65	0.19	0.08	0.63	0.17	-0.12	-0.49	0.22
Penultimate internode	WSC$_{min}$	-0.08	0.22	0.24	-0.16	-0.22	0.58	0.51	0.59	-0.07	0.04	-0.05	-0.1	-0.15	-0.01	-0.12	-0.26	-0.03	-0.04	0.39	-0.06	-0.24	-0.27	-0.18	-0.18	0.2	-0.17
Penultimate internode	WSC$_{av}$	-0.46	0.2	-0.03	-0.61	-0.57	0.17	0.3	-0.06	-0.17	0.09	-0.13	-0.27	-0.55	-0.03	-0.17	0.44	-0.03	-0.4	-0.83**	-0.11	-0.32	0.54	-0.19	-0.54	-0.78*	-0.26
Penultimate internode	MWSC	-0.27	0.06	-0.14	-0.37	-0.31	-0.22	-0.08	-0.4	0	0.16	0.05	-0.07	-0.38	0.13	-0.12	0.4	0.02	-0.32	-0.77*	0.07	0	0.59	0.07	-0.2	-0.6	0.09
Penultimate internode	WSCME	-0.21	0.09	-0.12	-0.33	-0.25	-0.27	-0.1	-0.47	0.01	0.15	0.04	-0.05	-0.35	0.05	-0.11	0.43	-0.03	-0.32	-0.84**	-0.03	-0.05	0.48	-0.01	-0.21	-0.6	-0.03
Penultimate internode	DW$_{av}$	-0.52	0.15	-0.1	-0.61	-0.61	0.43	0.45	0.33	-0.74*	-0.64	-0.73*	-0.74*	-0.29	-0.76*	-0.66	-0.25	-0.70*	-0.72*	-0.39	-0.72*	-0.69*	-0.28	-0.67*	-0.68*	-0.45	-0.64
Lower internodes	WSC$_{max}$	-0.56	0.3	0.03	-0.75*	-0.72*	0.34	0.4	0.2	-0.31	-0.11	-0.27	-0.37	-0.47	-0.42	-0.44	0.07	-0.39	-0.58	-0.59	-0.48	-0.5	-0.02	-0.41	-0.56	-0.53	-0.51
Lower internodes	WSC$_{min}$	0.05	0.17	0.23	0.01	-0.06	0.45	0.33	0.56	-0.24	-0.3	-0.24	-0.19	0.15	-0.27	-0.23	-0.53	-0.26	-0.07	0.62	-0.22	-0.21	-0.59	-0.24	-0.04	0.44	-0.16
Lower internodes	WSC$_{av}$	-0.21	0.61	0.38	-0.52	-0.49	0.2	0.35	-0.07	-0.1	0.1	-0.07	-0.18	-0.41	-0.23	-0.16	0.43	-0.13	-0.39	-0.81**	-0.31	-0.43	0.15	-0.35	-0.53	-0.59	-0.5
Lower internodes	MWSC	-0.63	0	-0.3	-0.72*	-0.68*	0.13	0.23	-0.05	-0.28	-0.03	-0.23	-0.36	-0.61	-0.21	-0.34	0.15	-0.29	-0.49	-0.67*	-0.34	-0.37	0.25	-0.31	-0.5	-0.74*	-0.35
Lower internodes	WSCME	-0.6	-0.09	-0.38	-0.66	-0.62	0.04	0.17	-0.16	-0.23	0.04	-0.19	-0.33	-0.66	-0.09	-0.24	0.22	-0.2	-0.4	-0.69*	-0.25	-0.31	0.36	-0.27	-0.47	-0.81**	-0.3
Lower internodes	DW$_{av}$	-0.27	0.41	0.18	-0.49	-0.46	0.36	0.46	0.14	-0.42	-0.27	-0.42	-0.46	-0.34	-0.55	-0.45	0.06	-0.46	-0.59	-0.59	-0.59	-0.62	-0.21	-0.61	-0.63	-0.45	-0.66

Column groups: columns 9–14 = Stem segment / Peduncle; columns 15–20 = Penultimate internode; columns 21–26 = Lower internodes. Row groups under "Trait % heat response" / "Stem segment": Peduncle, Penultimate internode, Lower internodes.

Pearson's correlation coefficients are shown, together with significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Cells are coloured green and orange to highlight significant positive or negative correlations, respectively.

Positive association mean higher value in control was associated with less negative response (stability).

Negative assocation mean higher value in control was associated with more negative response (instability).

Greyed out values represent comparisons between arithmetically related variables where the direction of the correlation (significant or not) is consistent with the possibility that the correlation was a mathematical artefact.

SGW$_{pred}$, predicted final single grain weight; GFD, grain filling duration; TIP, time to inflection point of grain growth; MGR, maximal grain growth rate; SGR, sustained grain growth rate; TotChl$_{av}$, total flag leaf chlorophyll *a* and *b* averaged over all time points, Chla$_{av}$, flag leaf chlorophyll *a* averaged over all time points; Chlb$_{av}$, flag leaf chlorophyll *b* averaged over all time points; WSC, main stem water soluble carbohydrates; WSC$_{max}$, peak WSC value; WSC$_{min}$, minimum WSC value; WSC$_{av}$, WSC content averaged over all time points; MWSC, mobilized WSC; WSCME, WSC % mobilization efficiency; DW$_{av}$, main stem dry weight averaged over all time points.