Genetic and physiological studies of heat tolerance in hexaploid wheat (*Triticum aestivum* L.)

By

Hamid Shirdelmoghanloo

Thesis submitted for the degree of

Doctor of Philosophy

School of Agriculture, Food and Wine

Discipline of Plant Breeding and Genetics

Australian Centre for Plant Functional Genomics (ACPFG)

November 2014

Table of contents

List of tables

List of figures

List of appendices

Abbreviations

Symbol	Definition
ANOVA	Analysis of variance
ATR-MIR	Attenuated total reflectance mid infrared
AUSC	Area under SPAD curve
ChlC10DAA	Chlorophyll content 10 DAA
ChlC13-16DAA	Chlorophyll content within the range from 13 to 16 DAA
ChlC13DAA	Chlorophyll content 13 DAA
ChlC25DAS	Chlorophyll content 25 DAS
ChlC27DAA	Chlorophyll content 27 DAA
ChlC28DAS	Chlorophyll content 28 DAS
ChlC7-10DAA	Chlorophyll content within the range from 7 to 10 DAA
ChlR13	Chlorophyll loss rate from 10 to 13 DAA
ChlR27	Chlorophyll loss rate from 10 to 27 DAA
CL	Culm length
cM	Centimorgan
D	Drysdale
DAA	Days after anthesis
DAS	Days after sowing
DH	Doubled haploid
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DTA	Days from sowing to anthesis
DTM	Days from anthesis to maturity
DTM	Days from sowing to 95% senescence of spike
FL	Flag leaf length
FLSe	95% flag leaf senescence
Fv/Fm	Flourescence variable/flourescence maximum
FW	Flag leaf width
G	Genotype
G × T	Genotype-by-treatment effect
GFD	Grain-filling duration
GNS	Grain number $spike^{-1}$
GNSp	Grain number $spikelet^{-1}$
g_s	Stomatal conductance
GWS	Grain weight $spike^{-1}$
H^2	Broad-sense heritability
HI	Harvest index
HSI	Heat susceptibility index
KASP	Competitive allele-specific PCR
LHCII	Light harvesting complex proteins associated with PSII
LOD	Logarithm of odds
LSD	Least significant difference
LWP	Leaf water potential
MGR	Maximum grain growth rate
MWSC	Mobilized WSC
PC	Principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
PH	Plant height
PSA	Proportion of senescent area
PSA25DAS	Proportion of senescent area 25 DAS
PSA28DAS	Proportion of senescent area 28 DAS
PSA39DAS	Proportion of senescent area 39 DAS
PSI	Photosystem I
PSII	Photosystem II
QTL	Quantitative trait loci

Symbol	Definition
RFLP	Restriction fragment length polymorphism
RGR	Relative growth rate
RGRAT	Relative growth rate after treatment
RGRBT	Relative growth rate before treatment
RGRDT	Relative growth rate during treatment
$R_{H/C}$	Heat-treated/control ratio
RWC	Relative water content
S.E.	Standard error
SGR	Sustained grain growth rate
SGW	Single grain weight
ShW	Shoot dry weight
SNP	Single nucleotide polymorphism
SpNS	Spikelet number $spike^{-1}$
SSR	Simple sequence repeat
SSS	Soluble starch synthase
T	Treatment
TIP	Time from anthesis to the inflection point
TN	Tiller number
TotChl	Total chlorophyll content (chlorophyll a + b) measured by destructive method
W	Waagan
WSC	Water soluble carbohydrate
WSCME	WSC mobilization efficiency
WUE	Water use efficiency
ZGS	Zadoks' growth stage

Abstract

High temperature is one of the major environmental constraints for wheat production globally. It puts significant pressure on the wheat industry around the world, compromising both the quantity and quality of wheat grain produced. The current study focussed on the impact of brief episodes of very high temperatures during vegetative and grain-filling stages of wheat development using a combined approach of plant physiology and quantitative trait loci (QTL) mapping.

At grain-filling stage, wheat plants were exposed to a brief heat stress (3 days, 37/27 ºC) 10 days after anthesis and the plants evaluated for a number of morphological and physiological traits (Chapters 3, 4, and 6). At the vegetative stage (~ 4 weeks after sowing) plants were challenged with a brief heat treatment (2 days, 40/30 ºC), and growth and senescence related characters were monitored using automated imaging facilities and a SPAD chlorophyll meter (Chapter 7).

In total, 37 bread wheat genotypes were evaluated for different heat responses during the grain-filling stage. Genetic variation was observed among wheat genotypes for various heat responses, particularly for single grain weight, chlorophyll retention, rate and duration of grain-filling, and water soluble carbohydrate content and mobilization (Chapters 3 and 4). Overall, the findings suggested that more than one adaptation process contributed to tolerance. Generally, genotypes with more stable grain weight under heat tended to have particular traits under stress, including the ability to maintain chlorophyll content and rate and duration of grain-filling, and stronger water soluble carbohydrate mobilization efficiency (Chapters 3 and 4). Therefore, these traits may provide appropriate selection criteria for improving heat tolerance in wheat.

A genetic linkage map of a Drysdale/Waagan population was constructed using a 9K SNP array (Chapter 5) and used for QTL analysis (Chapter 6) of heat responses (evaluated using heat susceptibility index) at the grain-filling stage. A region on chromosome 3BS strongly affected heat responses of grain weight, stay-green related traits, grain-filling duration, shoot dry weight and harvest index, explaining 10 to 40% of the phenotypic variation, with Waagan contributing the tolerance allele. Most notably, the results indicated a strong genetic link between stay-green and grain weight maintenance under brief episodes of terminal high temperatures but a lack of a significant association between the *Rht-B1* and *Rht-D1* dwarfing loci and heat tolerance.

Using high-throughput automated imaging facilities in The Plant Accelerator, considerable variation among 77 bread wheat genotypes was observed for growth rate and senescence

responses to a brief heat stress at the vegetative stage (Chapter 7). A subset of 32 genotypes was also screened at the grain-filling stage (Chapter 3) which allowed a comparison of heat responses at these two developmental stages. Growth rate and senescence responses at the vegetative stage showed significant associations with grain weight maintenance and senescence responses at the grain-filling stage. These results suggested a physiological/genetic link between heat responses at the different growth stages, with implications for developing more efficient heat tolerance screening methods.

The present work contributes to the understanding of physiological mechanisms of heat tolerance and its genetic basis in hexaploid wheat, and identifies assays with potential to assist heat tolerance studies and in breeding programs.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Hamid Shirdelmoghanloo, and to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Hamid Shirdelmoghanloo

Signature…… Date……

Acknowledgements

I would like to take this opportunity to acknowledge people who helped me through my PhD and contributed to this work.

I would like to acknowledge Drs. Michael Francki, Dion Bennett, Dan Mullan, Bertus Jacobs, Hugh Wallwork, the Australian Winter Cereals Collection, the Australian Centre for Plant Functional Genomics (ACPFG), and New South Wales Department of Primary Industries (NSW-DPI) Wagga Wagga for providing seeds of lines used in this study. Thanks to Drs. Livinus Emebiri and Peter Martin for providing the Drysdale × Waagan DH mapping population and the 9K array SNP data. I am also grateful to Assoc. Prof. Ken Chalmers (my post-graduate co-ordinator), Dr. Glenn McDonald (my independent advisor), Dr. Monica Ogierman (ACPFG Education Manager), and Mrs. Ruth Harris (ACPFG English tutor). I would also like to thank all staff and researchers at the ACPFG, the molecular marker laboratory and The Plant Accelerator who helped me through this project and provided a friendly working environment.

I would like to express my gratitude to the organizations that provided core funding to the ACPFG (Australian Research Council, Government of South Australia and the Grains Research and Development Corporation, GRDC) and to the University of Adelaide and ACPFG for my scholarship. The GRDC also provided specific project funding for parts of this work (Chapters 3, 4, and 7) and a travel award which allowed me to attend an international conference.

I would like to express my sincerest gratitude to my principal supervisor Dr. Nicholas Collins for providing me the opportunity to do a PhD program and for his excellent guidance, encouragement, and patience during the entire program. In fact, none of this work could be done without his excellent support and there is no way I could ever thank him enough for his immense contribution. I would like also to thank my co-supervisors Prof. Diane Mather and Dr. Boris Parent for their great guidance and support.

Thanks to my parents, brother, and sister who inspired and encouraged me to pursue my PhD. I am very grateful for their unconditional love, encouragement, and support.

Specific contribution to the research

I would like to acknowledge people who contributed specifically to the research presented here. Many thanks to:

Dr. Nicholas Collins for his constructive suggestions for data analysis, interpretation, and suggestions on thesis text.

Prof. Diane Mather for her constructive suggestions for molecular marker genetic map construction (Chapter 5) and suggestions on thesis text.

Dr. Boris Parent for his suggestions how to carry out and analyse the vegetative growth experiments (Chapter 7).

Mr. Iman Lohraseb for his help with the experiment presented in Chapter 3. He sowed the experiment, helped with watering, threshed 188 out of the 648 plants and collected the shoot dry weight data.

Dr. Huwaida Rabie who provided a spatial design for experiments in Chapter 3, 6 and 7 (Experiments 2, 3, 6 and 7) and performed linear mixed model analyses in Chapters 3 and 7 (Experiments 2, 3 and 6).

Dr. Julian Taylor who devoted his precious time to help me with some complex statistical issues and to develop a linear mixed model for data analysis and to provide critical evaluation of thesis text (experimental design and statistical analysis section) in Chapter 6.

Dr. Andy Timmins for providing suggestions for map construction (Chapter 5).

Dr. Daniel Cozzolino who provided access to the ATR-MIR instrument and helped model water soluble carbohydrate content of stem samples (Chapter 4).

Dedication

To my

*father "**Ali**" and mother "**Maryam**"*

Chapter 1: General introduction

Wheat (*Triticum aestivum* L.) plays a critical role in the world's food security and economy. Rapid growth of the world's population, shortage of land and water resources along with biotic and abiotic stresses put significant pressure on wheat production. To increase wheat production at the same pace as the worlds' growing demand, under the aforementioned limitations, increased yield per unit of area will be required.

High temperature is one of the major environmental constraints for wheat production in many wheat growing regions worldwide, compromising both the quantity and quality of wheat grain produced (Cossani and Reynolds 2012; Farooq et al. 2011; Kumar et al. 2013). Although wheat is grown as a winter season crop in Australia, heat stress (>34°C) is frequently experienced during the growing season, and particularly at the grain-filling stage (occurs mainly in the spring), across the Australian wheat belt (Asseng et al. 2011; Blumenthal et al. 1991; Stone and Nicolas 1994; Wardlaw and Wrigley 1994). Higher temperatures that are predicted with climate change are likely to worsen the situation (Hansen et al. 2012; IPCC 2007; Ortiz et al. 2008). Two principal approaches to adapt to these conditions include management strategies (e.g. moving forward the sowing date) and development of genetically superior genotypes (Farooq et al. 2011). The latter may be more helpful, since shifting forward the sowing date increases the risk of frost damage; paradoxically, the frequency of frosts are increasing with climate change in south-eastern parts of the Australian wheat belt (Zheng et al. 2012; http://www.managingclimate.gov.au/). However, breeding for new superior heat tolerant genotypes has been challenging due to the unpredictable nature of heat stress (timing, magnitude, and duration), its co-occurrence with other stresses (e.g. drought), and growth-stage-specific effects (Wardlaw and Wrigley 1994). Therefore, determining mechanisms related to heat tolerance and identifying tolerant sources and reliable screening tools are essential for developing new heat tolerant wheat genotypes.

As heat stress at grain-filling stage is of prime importance to the Australian wheat industry and short episodes of severe heat stress are frequently experienced by the majority of wheat crops in the Australian wheat belt (Asseng et al. 2011; Stone and Nicolas 1994; Wardlaw and Wrigley 1994), the main focus of this study is on a brief episode of heat stress at early grain-filling stage (10 days after anthesis), while the possible utility of the automated plant phenomics facilities (The Plant Accelerator) in heat tolerance studies on earlier stages of wheat growth was also investigated as part of this thesis. Varietal variation for heat tolerance, effects of a brief heat stress on different responses, associations between these responses within/between vegetative and grain-filling stages of wheat development, as well as genetics

of heat tolerance at the grain-filling stage, are presented. Chapter 2 will review the literature. Chapter 3 will report on screening 36 wheat (*Triticum aestivum* L.) genotypes which are mostly parents of available mapping populations, to identify a mapping population suitable for studying the genetics of heat tolerance. Chapter 4 will report on a detailed study of two pairs of parents contrasting for heat tolerance (ability to maintain single grain weight upon heat exposure at the early grain-filling stage) and 6 other genotypes. In these genotypes, responses of grain growth parameters, chlorophyll loss and water soluble carbohydrates are examined and related to the grain weight response. Chapter 5 will report on the construction of a molecular marker linkage map which is a prerequisite for quantitative trait loci (QTL) analysis, including development of a pipeline for processing data generated using the wheat 9K SNP marker array. Chapter 6 will report on QTL mapping of heat responses and other heat related traits in the Drysdale/Waagan doubled haploid mapping population after it was exposed to heat stress at grain-filling. Chapter 7 will examine the utility of high throughput automated phenotyping facilities for heat tolerance evaluation in young wheat plants. Genetic variation for heat responses expressed at this stage, and its correlation with heat tolerance at grain-filling, are reported. The thesis will conclude in Chapter 8, which includes a general discussion, conclusions, summary of contributions to the knowledge, and consideration of future directions.

Chapter 2: Literature review

2.1 Wheat as an important crop

Wheat is one of the most important crops worldwide (http://www.fao.org), and provides the staple food and an abundant source of energy and protein for millions of people (Patnaik and Khurana 2001). In 2012, about 215.5 million ha were devoted to wheat cultivation, and about 670.9 million tons of wheat produced (FAOSTAT 2012). It is the second largest crop in terms of value behind rice (FAOSTAT 2012), and it provides 21% of the world's food (http://www.fao.org). Wheat is also one of the main grain crops in Australia and plays an important role in its economy. About 13.9 million hectares sown to wheat and about 29.9 million tonnes harvested in Australia in 2012 (Australian Bureau of Statistics 2014). This contributed AUD 7 billion to the country's economy in 2012, second only to the beef industry (AUD 7.7 billion) among agricultural commodities (Australian Bureau of Statistics 2014). These statistics show the crucial role of wheat in food security and economy of the world and Australia.

2.2 Impact of heat on the wheat industry

Heat stress occurs when the temperature rises beyond that which is optimal for plant growth and development (Wahid et al. 2007). Global warming due to the increase of greenhouse gases is likely to increase the frequency of heat stress in wheat crops. Over the last four decades, the average daily maximum temperature in Australia has increased steadily, by 0.3 ºC every 10 year (Australian Bureau of Meteorology 2014). The number of days over 34ºC during the grain-filling period has also increased in Australia's wheat growing regions and other wheat major growing regions (Asseng et al. 2011). Moreover, it has been estimated that by 2050 some of the current favourable wheat producing regions will be reclassified as heat-stressed growing regions - for example, more than 50% of Indo-Gangetic Plains where 15% of wheat grain is produced (Ortiz et al. 2008).

Heat stress seriously reduces wheat production, not only in Australia, but also in other areas. Based on field and controlled environment data, it has been estimated that high temperature causes an average of 10 to 15% yield reduction in wheat in Australia and the USA annually (Wardlaw and Wrigley 1994) which equates to ~AUD 400m loss to the Australian economy. In addition, according to a media report (Dowler 2004), in an individual high temperature occasion on 12th of October 2004 in South Australia, a third of the SA wheat production was lost due to severe heat stress. A study on the effect of heat stress on wheat yield in Australia and other major wheat growing regions estimated an average of 5% reduction in grain yield for each day above 34 ºC during the grain-filling stage (Asseng et al.

2011). These reports show the significant effect of heat stress in wheat production, and the urgent need for heat tolerant wheat varieties.

In Australian wheat growing regions, the grain-filling stage (October-November) coincides with rising temperature, and temperatures above the optimum (15 ºC) are frequent (Wardlaw and Wrigley 1994). Most wheat crops experience an average of 4 days above 34 ºC (Stone and Nicolas 1994) and on occasions temperatures can reach 40 ºC (Wardlaw and Wrigley 1994). McDonald et al. (1983) found a 4% reduction in the single kernel mass for each degree above the optimum temperature. Heat stress can also affect grain quality (Blumenthal et al. 1995; Ciaffi et al. 1996; Corbellini et al. 1998; Stone and Nicolas 1996). Specifically, heat stress weakens the dough (Blumenthal et al. 1995; Wrigley 2007). The same situation exists in other major wheat growing areas such as the USA, the Mediterranean region (Graybosch et al. 1995; Mason et al. 2010) and India (Rane and Nagarajan 2004). Where the stress occurs frequently, selection for tolerance to stress could be a worthwhile strategy to increase yield (Rosielle and Hamblin 1981). Thus, selection for heat tolerance may be useful to increase yield under Australian and similar wheat growing environments.

Heat stress at earlier growth stages is a problem for wheat cultivation in other parts of the world such as Pakistan. High temperatures (>35 ºC) occur at wheat seedling emergence in Pakistan which causes early seedling mortality, slows down plant growth, and finally reduces the yield (Mufti 2005). The coleoptile length decreases 1 cm for every 2.6 ºC above 15 ºC (Radford 1987). There is a positive correlation between the duration of the vegetative phase and spikelet number (Rahman and Wilson 1978). High temperatures can shorten the vegetative phase which leads to a smaller number of spikelets per spike (Barnabás et al. 2008; Rahman et al. 1977). Moreover, pre-anthesis reserve pools of photosynthate in vegetative tissues such as stems and sheaths contribute up to 40% of final kernel weight of wheat (Yang and Zhang 2006), and increased temperatures during the 30 days before anthesis can cause a 4% reduction in wheat yield per ºC increase (>15 °C) by shortening the period of assimilate accumulation (Fischer 1985a). Such evidence clearly indicates an important effect of heat stress during vegetative stages of wheat development.

Generally speaking, heat stress can affect all stages of wheat development in different ways from germination to maturity (seed to seed). Nonetheless, the focus of this study will be on (a) the effects of a short duration of heat stress (a few days) on grain size during grain-filling, which is of great importance for the Australian wheat industry and relatively less studied compared to chronic heat stress, and (b) the growth inhibition effects after high temperature in the vegetative stage of wheat development, which has not been studied using

non-destructive, real-time monitoring facilities before. Thus, the following review will focus on these two aspects of heat stress.

2.3 Mechanisms of growth rate responses

High temperatures affect the plant growth rate at all developmental stages. Rising temperatures up to an optimum temperature, which can differ from plant to plant species, increase the rate of physiological processes such as leaf elongation rate, cell division, net photosynthetic rate, etc. Beyond these optima, increasing temperatures negatively impact on these processes (Parent et al. 2010; Parent and Tardieu 2012). The growth-rate-enhancing properties of temperature can be at least partly explained by a general phenomenon of chemical reactions – with high temperatures, molecules in solution have greater kinetic energy, which results in more frequent collisions of molecules with sufficient activation energy. For example, high temperatures have been shown to increase the rate of reactions catalysed by sucrose synthase and acid invertase (Parent et al. 2010).

Negative effects at higher temperature could be caused by various factors. Increased evapotranspiration under high temperatures can lead to water stress, decrease in leaf water potential and perturbation in root hydraulic conductivity, with resulting disturbance of physiological processes (Wahid et al. 2007). The fluidity and permeability of cell membranes increase under high temperatures as a result of increased concentrations of unsaturated fatty acids or protein denaturation (Savchenko et al. 2002). This affects the membrane-based processes photosynthesis and respiration (Barnabás et al. 2008). Thylakoid membranes and photosystem II (PS II) are very sensitive to high temperatures, and their destruction under high temperatures can limit photosynthesis (Ristic et al. 2007). Degradation of chlorophyll *a* and *b*, separation of light harvesting complex II from PS II, dissociation of oxygen evolving complex (OEC) from PS II, reduction of photosynthetic pigments, and reduction in RuBisCO activity, and other changes in photosynthesis machinery due to heat stress, can reduce photosynthetic rate (Wahid et al. 2007). Moreover, high temperatures can adversely affect the mitochondrial electron transport chain by damaging the mitochondrial membrane.

An additional mechanism could be alteration of plant hormone content. It has been shown that the application of gibberellic acid to seeds of a heat tolerant barley can lead to loss of tolerance later in development (Vettakkorumakankav et al. 1999). Exogenous application of cytokinin can increase the number of endosperm cells, grain weight and grain-filling duration under normal temperatures (Alizadeh et al. 2010). On the other hand, reduction in cytokinin (50-80%) due to high temperature (7 days at 35/25 °C) was accompanied by reduced mature grain mass, in wheat (Banowetz et al. 1999). This suggests that cytokinin might have a role in

the heat tolerance response. Transcriptome analysis of heat tolerant and susceptible wheat genotypes following heat treatment suggested that genes for heat shock proteins, transcription factors, calcium signalling and metabolism pathways, and other factors, are involved in responses to heat (Qin et al. 2008). The heat sensitivity of soluble starch synthase (SSS) plays a key role in kernel weight reduction at the grain-filling stage of wheat (Jenner 1994). Therefore, heat sensitivity of SSS in vegetative tissues and/or certain other enzymes may also be heat sensitive and play a crucial role in reducing growth rate at earlier stages in wheat development.

2.4 Mechanism of grain size reduction

High temperature at grain-filling accelerates the grain-filling rate, but also causes truncation of the grain-filling period; the former typically may not be enough to compensate for the latter, depending on genotype, explaining why the overall effect is a reduction of grain size (Jenner 1994). More than two-thirds of the dry grain mass consists of starch. A short period of heat stress affects starch deposition more than it does protein content, so the reduction of grain size due to heat stress mostly comes from reduced starch deposition (Jenner 1994). Thus, understanding the effect of high temperatures on the starch synthesis process is important for development of new heat tolerant varieties. Two hypotheses have been examined as the reason behind the reduced starch deposition under high temperatures: (a) reduction in assimilate supply to the developing grain as the result of accelerated senescence (b) the sensitivity of starch synthesis in the grain.

Manipulating source strength by either reducing assimilate supply to kernels through shading or defoliation, or increasing it by removing all but the assayed tiller, revealed that source-sink balance plays a role; however, this phenomenon seems to have a relatively minor impact in determining sensitivity of grain-filling to heat (Jenner 1994; Wardlaw et al. 1980; Wardlaw and Wrigley 1994). This conclusion receives further support from studies that used sucrose levels as an indicator of the assimilate availability in the grain. When excess sucrose was supplied to the detached head or endosperm cells of wheat, it did not overcome the negative effect of heat stress (Bhullar and Jenner 1983; Jenner 1991a).

As already mentioned there is a body of literature which points to processes within the grain being important in terms of the response of wheat to heat stress (Bhullar and Jenner 1983; Jenner 1991a; Jenner 1994; Wardlaw et al. 1980; Wardlaw and Wrigley 1994). High temperatures can affect the activity of several enzymes involved in converting sucrose to starch in wheat grains, such as sucrose synthase, ADP-glucose pyrophosphorylase, UDP-glucose pyrophosphorylase and soluble starch synthase (SSS) (Hawker and Jenner 1993).

However, of the enzyme activities known to be involved in the conversion of sugar to starch in the wheat grain, there is an overwhelming body of evidence that SSS is the one that is both rate-limiting and the most heat sensitive (Hawker and Jenner 1993; Jenner 1994; Keeling et al. 1993; Keeling et al. 1994; Zahedi et al. 2003). It has been shown that the activity of SSS is reduced by more than 50% within the first hours of heat stress (Hawker and Jenner 1993; Jenner 1994). Five classes of starch synthase have been discovered in cereals including granule bound starch synthase (GBSS), and 4 isoforms of soluble starch synthase (SSI, SSII, SSIII, and SSIV) (Keeling and Myers 2010; Zeeman et al. 2010). Four of these have been identified in wheat endosperm, GBSS, SSI, SSII and SSIII (Li et al. 2000; Hurkman et al. 2003).

The activity of GSS is not significantly affected by heat stress, whereas SSS enzymes' activities are highly reduced (>60%) under high temperatures (Keeling et al. 1994). Heat stress (37 ºC) also reduces the mRNA levels for SSS more than it reduces the mRNA for the other enzymes in the starch synthesis pathway, and shortens the time to reach the maximum expression levels of the transcripts of starch biosynthetic enzymes (Hurkman et al. 2003). Starch is composed of two types of glucose polymers - amylose (20-30%; linear form with few branches; made by GBSS which is relatively heat tolerant, therefore amylase content is not affected much by heat) and amylopectin (70-80%; highly branched form) (Keeling and Myers 2010). It deposits in three different types of granules, A (large type), B (small), and C (very small), during grain development (Hurkman et al. 2003). The ratio of large and small starch granules changes under heat stress. Heat stress significantly reduces the proportion of B type granules in certain genotypes, whereas other genotypes show no significant reduction or even a slight increase in the proportion of small granules (Blumenthal et al. 1995). The relative size of these granules also changes under heat stress. The size of large granules increases by 25-80%, and the size of small granules decreases by 20-70%, in comparison with regular conditions (Hurkman et al. 2003).

2.5 Mechanisms of dough quality effects

High temperatures (>35 °C) at the grain-filling stage causes a weakening of the dough and consequently a loss of grain quality for bread making (Blumenthal et al. 1991; Blumenthal et al. 1995; Corbellini et al. 1998). Dough strength is regarded as an important component of grain end-use quality. When dough mixes in a mixograph, it gradually strengthens to reach a point of maximum resistance (Rmax), after which it becomes weaker. Strong dough has a longer development time and breaks down slower than weak dough. All of these parameters are affected by heat stress. Temperatures above 35 °C, for as few as 3 days, can cause more

than 50% loss in the Rmax and accelerated breakdown (Blumenthal et al. 1995; Wrigley 2007).

Glutenin and gliadin are the main storage proteins in the wheat grain (Payne 1987). The unique ability of bread wheat (*Triticum aestivum* L.) to produce light and spongy bread comes from glutenins. Glutenins link via disulphide bonds to form high molecular weight polymers which confer the elasticity to dough (Ali et al. 2010; Shewry et al. 2000). Gliadins do not form disulphide linkages. The dough strength reducing effect of heat does appear to derive from a reduction in the disulphide cross linking, and biochemically this is manifested by reduction in the proportion of protein present as high molecular weight glutenin complexes.

There are a number of potential causes for the reduction of glutenin crosslinking in kernels of heat-stressed plants which leads to the weakening of dough.

(a) An increase in the ratio of gliadin to glutenin has been observed after exposure to high temperatures (Blumenthal et al. 1993; Blumenthal et al. 1994). Heat-inducible elements are present in the promoters of gliadin genes, and these are believed to give a greater relative rate of expression of gliadin genes during heat stress (Blumenthal et al. 1993). This may reduce the rate of disulphide bond formation by diluting glutenin.

(b) It is possible that protein disulphide isomerase, an enzyme which catalyses disulphide bond formation, may be heat sensitive (Blumenthal et al. 1998).

(c) A shorter grain-filling period after heat stress may lead to reduced disulphide bound formation due to shortening of the disulphide bond formation process.

(d) Heat-shock proteins (HSP) might have a negative or a positive effect on dough quality. The increase of heat-shock proteins in the heat-stressed wheat grains might be able to change the structure of dough (Bernardin et al. 1994 cited in Blumenthal et al. 1998). HSP and chaperones might also have a role in disaggregating and hydrolysing malformed gluten proteins under heat stress conditions (Blumenthal et al. 1998). Heat-shock proteins (HSP) might have also a positive effect on dough quality by stabilizing the enzymes involved in synthesis or crosslinking of glutenins.

A number of studies have found a relationship between alleles of the *Glu-D1* locus, which encode high molecular weight glutenins, and the stability of dough physical properties after exposure to heat stress (Blumenthal et al. 1991; Stone and Nicolas 1996; Wardlaw et al. 2002). Lines having the *Glu-D1d* allele (5 + 10) are more tolerant than lines carrying the *Glu-D1a* allele (2 + 12) (Wrigley 2007). This has been attributed to earlier polymerization of the protein encoded by the *Glu-D1d* allele than that which is encoded by the *Glu-D1a* allele

(Irmak et al. 2008). Therefore, the *Glu-D1d* allele is able to achieve a higher amount of polymerization before grainfill is terminated.

Moderately high temperatures (< 30 °C) during grain filling can lead to an increase in dough strength (Randall and Moss 1990; Corbellini et al. 1998; Uhlen et al. 1998). High temperatures increase the protein content of wheat grains, as a result of starch reduction, and this in turn may have the effect of concentrating the glutenins, favouring faster and more complete crosslinking (Blumenthal et al. 1995).

2.6 Mechanisms of fertility effects

Heat stress at around meiosis leads to floret sterility while heat within the first three days after pollination can lead to early abortion of grain growth (Saini and Aspinall 1982b; Saini et al. 1983; Tashiro and Wardlaw 1990a; Wardlaw et al. 1989b). Saini and colleagues showed that heat stress can affect both male and female organs in wheat (Saini and Aspinall 1982a; Saini et al. 1983). This was demonstrated by exposing wheat plants during pollen mother cell meiosis to high temperature (30 ºC) followed by reciprocal pollinations with unstressed plants (Saini et al. 1983). However, male sterility due to heat stress is greater than female sterility because male reproduction in plants is more highly sensitive to heat stress (Endo et al. 2009). Starch reserves in pollen grains serve as the energy source for pollen germination and pollen tube growth (Dorion et al. 1996). Heat stress can repress starch accumulation in pollen grains (Dorion et al. 1996; Saini et al. 1984). Adverse effect of heat stress on pollen tube growth has also been reported to be associated with callose plugs (β-1,3-glucan cell wall components) deposition which blocks pollen tubes (Saini et al. 1983). Recently, positive association has been observed between water soluble carbohydrates content/metabolism and floret fertility and grain set in wheat under high temperature conditions (Dreccer et al. 2014).

2.7 Traits and parameters for evaluation of heat tolerance

Various traits have been used for heat tolerance evaluation in wheat. Yield and its components are probably the most important traits, and their improvement is the main goal in every breeding program. Therefore, they have been widely used as heat tolerance indicators in many studies (Bennett et al. 2012; Mason et al. 2013; Pinto et al. 2010; Reynolds et al. 1994; Wardlaw et al. 1989b), but evaluation of these traits is costly in terms of both time and resources. In addition to yield and its components, a range of morpho-physiological traits including cell membrane thermal stability (Blum and Ebercon 1981; Fokar et al. 1998; Reynolds et al. 1994; Saadalla et al. 1990b; Saadalla et al. 1990a), stay-green (Kumari et al. 2007; Lopes and Reynolds 2012; Reynolds et al. 2001), stomatal conductance (Reynolds et al. 1994), photosynthetic rate (Al-Khatib and Paulsen 1989; Al-Khatib and Paulsen 1990;

Reynolds et al. 2000), chlorophyll fluorescence (Peck and McDonald 2010; Sharma et al. 2012), canopy temperature (Amani et al. 1996; Kumari et al. 2007; Reynolds et al. 1994; Reynolds et al. 1998), epicuticular wax (Mason et al. 2010), stem carbohydrate reserves (Blum et al. 1994; Blum 1998; Talukder et al. 2013; Yang et al. 2002a) and grain-filling rate and duration (Stone and Nicolas 1995a; Talukder et al. 2013; Zahedi and Jenner 2003), which are believed to contribute toward heat tolerance in wheat, has been used to discriminate between tolerant and susceptible genotypes. Heat susceptibility index (HSI), which is an estimate of genotypic performance under heat-stress relative to non-stress conditions, adjusted for stress intensity in the particular trial (Fischer and Maurer 1978), has been also calculated for some of the aforementioned traits and was used to define heat tolerance in wheat in some of the previous works (Mason et al. 2010; Mason et al. 2011; Mason et al. 2013; Mohammadi et al. 2008b; Paliwal et al. 2012; Tiwari et al. 2013).

2.8 Stay-green and stress tolerance

Stay-green or delayed senescence can be defined as the ability to maintain green leaf area during grain-filling. Stay-green may be particularly important under stress conditions such as heat and drought (Distelfeld et al. 2014; Thomas and Ougham 2014). Early senescence in response to external environmental factors (e.g. heat, drought, and disease) affects photosynthetic competence and assimilate supply and consequently can negatively impact grain growth and yield. Senescence reduces chlorophyll which in turn affects photosynthesis and photo-assimilate supply. Stay-green prolongs the period that leaves are green and photosynthesizing, and delays senescence. Positive associations have been reported between stay-green and grain yield in sorghum (Borrell et al. 1999; Borrell et al. 2000; Borrell et al. 2014a; Borrell et al. 2014b), maize (Ceppi et al. 1987), soybean (Phillips et al. 1984), sunflower (Cukadar-Olmedo and Miller 1997), and wheat (Kumari et al. 2013; Lopes and Reynolds 2012; Reynolds et al. 1994; Reynolds et al. 1998).

Potential physiological mechanisms of stay-green are manyfold. Stay-green under drought stress in sorghum is associated with increased xylem pressure potential, delayed loss of photosynthetic competence, modification of canopy development, leaf anatomy, root growth, water uptake, and enhanced N uptake (Borrell et al. 2014a; Borrell et al. 2014b; Tuinstra et al. 1998; Vadez et al. 2013). In wheat, stay-green related traits have been found to be highly positively correlated with photosynthesis under high temperature conditions (Al-Khatib and Paulsen 1984; Gutiérrez-Rodriguez et al. 2000; Reynolds et al. 2000) and also to a root architecture that allows water to be extracted from deep in the soil profile post-anthesis under field conditions (Christopher et al. 2008). A positive association between stay-green and high

water use efficiency during grain-filling has been reported in wheat (Gorny and Garczynski 2002). Increased leaf area and grain-filling rate and duration have been observed in a stay-green durum wheat mutant (Spano et al. 2003). In another study, high expression of Rubisco activase, soluble starch synthase and glycine decarboxylase were seen for a longer time in a stay-green durum wheat mutant in comparison with the non-stay-green parent line, which further suggest a positive effect of stay-green in prolonging photosynthesis and grain-filling (Rampino et al. 2006). These studies may suggest that higher yield performance of stay-green genotypes might be due to higher photosynthetic rate, longer photosynthetic competence as well as better overall plant health and prolonged plant canopy survival in particular under stress conditions.

While stay-green could be an advantage under stress conditions there are some reports that it may negatively impact yield under regular conditions (Derkx et al. 2012; Kichey et al. 2007; Kipp et al. 2014; Naruoka et al. 2012). The latter might be because stay-green hampers remobilization of assimilate reserves to grains, resulting in more of the non-structural carbohydrates remaining in the straw (Yang et al. 2000). An alternative explanation for adverse effects of stay-green is prolonged consumption of glucose for continued nitrogen assimilation and protein synthesis by green leaves, which can deprive the grains of assimilate for grain-filling (starch synthesis) (De Vries et al. 1974; Hirel et al. 2007; Kipp et al. 2014).

Although stay-green is an important trait in heat tolerance, it has not been fully exploited in wheat (Kumar et al. 2013). Silva et al. (2001) reported control of stay-green by a single gene with high heritability and partial dominance among crosses of four contrasting genotypes for stay-green. Joshi et al. (2007) found stay-green to be controlled by around four additive genes. They also found that the stay-green trait to be positively associated with spot blotch resistance in two mapping populations made by crossing contrasting spring wheat genotypes, Chirya 3 × Sonalika and Chirya 7 × Sonalika. Kumar et al. (2010) and Vijayalakshmi et al. (2010) observed polygenic inheritance affecting stay-green trait in recombinant inbred lines (RILs) populations under field and controlled environment high temperature conditions.

Chlorosis is the most visible manifestation of senescence. Evaluation of chlorosis in the whole plant is not straightforward due to heterogeneous expression of chlorosis in aboveground organs (Cossani and Reynolds 2012). The flag leaf, which contributes substantially to nitrogen uptake and carbon assimilation and remains green for longer than other leaves, has been suggested to be a good target for senescence investigations (Kipp et al. 2014). There are different methods for monitoring chlorosis including visual scoring (Vijayalakshmi et al. 2010), measuring normalized difference vegetation index (NDVI) with a GreenSeeker sensor (Lopes and Reynolds 2012), and estimating chlorophyll content with a

portable SPAD chlorophyll meter (Vijayalakshmi et al. 2010; Lopes and Reynolds 2012). Indirect measurement of chlorophyll content of the flag leaf with a portable SPAD chlorophyll meter provides a fast, non-invasive, easy, and inexpensive way to monitor senescence over time on a single plant, and an indirect way to assess photosynthesis in wheat, which may also be applied to breeding programs. The SPAD chlorophyll meter measures transmittance of the leaf in the red (650 nm) and infrared (940 nm) wavelengths and uses that to calculate a relative SPAD value which is proportional to the sample's chlorophyll content (Minolta 1989). A strong relationship exist between SPAD readings and chlorophyll concentration in wheat leaves (Uddling et al. 2007), and the SPAD chlorophyll meter has been used in various studies to evaluate heat tolerance in wheat. SPAD readings have been correlated positively with maximum net photosynthetic rate (Gutiérrez-Rodriguez et al. 2000), chlorophyll a florescence which is an indicator of heat damage to thylakoid membranes and the functionality of photosystem II (PS II) (Ristic et al. 2007; Ristic et al. 2008), and grain yield under high temperature conditions (Kumari et al. 2013; Lopes and Reynolds 2012; Reynolds et al. 1994; Reynolds et al. 1998). Therefore, chlorophyll content of the flag leaf (determined either by a SPAD chlorophyll meter or by destructive measurement) is used as a convenient indicator of senescence and its acceleration by a brief heat treatment in this study.

2.9 Quantitative trait loci (QTL) mapping

Many important traits such as yield and tolerance to stresses (e.g. heat and drought) exhibit quantitative variation. Variation in these traits may be influenced by multiple genetic loci, the interaction between these loci and their interactions with the environment. Identification of these loci and their effects can lead to development of markers which can be used for selection of tolerant lines in breeding, or for cloning of the affecting gene(s). Characterization of the cloned genes is likely to shed light on the molecular mechanisms controlling such traits.

QTL mapping tests the association between phenotypic and genotypic variation to identify QTL controlling the target trait(s) (Semagn et al. 2010). A QTL study consists several steps including (1) screening germplasm collections to identify genotypes that contrast for the target trait(s), (2) development of mapping populations such as doubled haploids or recombinant inbred lines from crosses between contrasting genotypes, (3) phenotyping the mapping population for the trait(s) of interest and genotyping the population with molecular markers to construct a genetic linkage map, and (4) associating phenotypic with genotypic data to detect chromosomal regions controlling the trait(s).

2.10 Non-destructive imaging methods for phenotyping

Evaluation of phenotypic performance of individuals is required for mining variation and QTL mapping of target trait(s). Remarkable advances have been made in plant genomics and genetics in recent years. In comparison, the ability to gather phenotypic information has accelerated at a much slower pace, which prevents researchers and breeders from taking full advantage of the available genomic information. Thus, a lack of high-throughput plant phenomics (high-throughput physiology and phenotyping) methods and equipment has become a new bottleneck in genetic studies and breeding (Furbank and Tester 2011). Recently methods for high-throughput phenotyping such as non-destructive, real-time imaging and spectroscopy have been used in plants (Berger et al. 2010; Berger et al. 2012; Furbank and Tester 2011; Golzarian et al. 2011). In Australia an Australian Plant Phenomics Facility (www.plantphenomics.org.au) has been created (Furbank and Tester 2011) and now a High Resolution Plant Phenomics Centre in Canberra and The Plant Accelerator in Adelaide are available to scientists for high-throughput phenotyping (www.plantphenomics.org.au). These platforms provide fully automated facilities in greenhouses/growth chambers with robotics and remote imaging/sensing techniques to assess plant performance (growth/health) non-destructively over time. Similar efforts are underway worldwide in the International Plant Phenomics Initiative (www.plantphenomics.com). In addition to facilities for monitoring plants under control conditions, various field-based approaches have also been developed for *in situ* monitoring crop performance. These ground-based and aerial high-throughput phenotyping platforms use vehicles with a global positioning system (GPS) device and cameras/sensors (e.g. RGB/CIR cameras and thermal-imaging sensor) such as phenomobiles, phenotowers, and unmanned aerial vehicles (e.g. polycopters and airplanes) which allows rapid accurate characterisation of many plots in the field (Araus and Cairns 2014; White et al. 2012; www.plantphenomics.org.au). Moreover, different approaches such as laser imaging detection and ranging (LiDAR, an active remote sensing tool which uses a laser scanner/LiDAR sensor to measure the 3D canopy cover/structure) are beginning to be adapted for high-throughput field phenotyping (Araus and Cairns 2014; Hosoi and Omasa 2009; Weiss and Biber 2011).

Currently, most of the field and controlled environment trials for heat tolerance evaluation are costly, labour-intensive and time consuming. No effort has been made to test the value of the new state-of-art high-throughput phenotyping facilities in wheat heat tolerance studies for accelerating genetic mapping and positional cloning of heat tolerance genes. Therefore, as a part of this thesis, the potential utility of an automated high-throughput plant imaging platform (The Plant Accelerator) in wheat heat tolerance studies was investigated.

2.11 Mapping populations

Populations derived from bi-parental crosses are widely used in QTL mapping studies in particular doubled-haploids (DHs) and recombinant inbred lines (RILs) populations which provide immortal resources (Collard et al. 2005). In these populations allelic variation is limited to the two parental lines; therefore, two alleles at a time can be studied at any given locus (Cavanagh et al. 2008; McMullen et al. 2009). Recently, types of populations have been used, including Multi-parent Advanced Generation Inter-cross (MAGIC) populations which involves crossing multiple parents (Cavanagh et al. 2008) and Nested Association Mapping (NAM) populations which involves crossing a reference line with multiple parental lines (McMullen et al. 2009), to overcome the aforementioned limitations. Other types of populations are natural or breeding populations which are used for genome wide association mapping (Semagn et al. 2010).

2.12 Molecular markers

Molecular markers provide assays for detection of polymorphism at particular genomic locations. They can be used to detect genetic differences between lines or varieties, and can be classified as dominant or co-dominant (discriminating homozygotes from heterozygotes). Various types of molecular markers have been used in wheat, including restriction fragment length polymorphisms (RFLPs) (Botstein et al. 1980), random amplified polymorphic DNA (RAPD) (Williams et al. 1990), simple sequence repeats (SSRs or microsatellites), amplified fragment length polymorphisms (AFLPs) (Vos et al. 1995), and diversity arrays technology markers (DArTs) (Akbari et al. 2006; Semagn et al. 2006; Mantovani et al. 2008; Peleg et al. 2008). Recently, advances in DNA sequencing technologies have facilitated the discovery of large numbers of single nucleotide polymorphisms (SNPs, which provide the most abundant form of DNA sequence variation) (Cavanagh et al. 2013) and have made genotyping-by-sequencing (GBS) feasible in wheat (Poland et al. 2012). Therefore, large amounts of accurate molecular data can now be generated rapidly and at low cost for genetic studies in wheat. Through the efforts of the International Wheat SNP Working Group (http://wheatgenomics.plantpath.ksu.edu), marker arrays containing 9,000 (Cavanagh et al. 2013), and 90,000 features (Wang et al. 2014), scorable using the iSelect technology are available for wheat. Other available SNP platforms can be found at http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB.

2.13 Methods for QTL analysis

Several methods are available for QTL detection (associating genotypic variation with phenotypic variation). Some of the most frequently used methods include single marker

analysis (Edwards et al. 1987) which is a regression based method that evaluates the association of a phenotype at each locus, interval mapping (Lander and Botstein 1989) which uses the genetic linkage map as a framework and evaluates the marker intervals to detect QTL between neighbouring markers considering one marker interval at a time, composite interval mapping (Zeng 1994) which evaluates marker intervals while considering the background effect (variation in other regions), multiple interval mapping (Kao et al. 1999), which assesses multiple marker intervals and associated epistatic effects simultaneously, whole genome average interval mapping (WGAIM) (Verbyla et al. 2007) which considers all intervals on a linkage map simultaneously. Genome wide association mapping has also been used to investigate the association between phenotypic variation and genetic polymorphism in germplasm collections (Flint-Garcia et al. 2003). There are also various pieces of software available for QTL analysis such as GenStat (Payne 2009), R/qtl (Broman et al. 2003), QTL cartographer (Wang et al. 2012b), MapManager (Manly et al. 2001), QTLNetwork (Yang et al. 2008) and QGENE (Nelson 1997).

2.14 Genetics of heat tolerance/responses in wheat

Genetic variation exists among wheat genotypes for the grain size response to high temperature during grain-filling. A wide range of responses among Australian and exotic cultivars, from 10% to more than 60% reduction in individual grain mass, have been reported (Hawker and Jenner 1993; Randall and Moss 1990; Stone and Nicolas 1994; Wardlaw et al. 1989b). This variation has been attributed to differences in grain-filling duration and grain-filling rate (Hunt et al. 1991; Zahedi and Jenner 2003); however, the variation among wheat cultivars for the former has been reported to be much less than for the latter, at least in small samples of wheat genotypes (Hunt et al. 1991; Zahedi and Jenner 2003). Moreover, Australian and exotic wheat varieties show responses ranging from a dramatic reduction in the percentage of starch granules that are of the B-type, to no change in this proportion at all, under high temperature conditions (Blumenthal et al. 1995). Despite this wide variation, the lack of robust selection methods, the unpredictable nature of heat stress in the field, and the quantitative nature of heat tolerance make the breeding of heat tolerant wheat varieties difficult. Additionally, the genetic mechanism of heat tolerance is poorly understood and no wheat heat tolerance gene has been cloned. High resolution mapping of wheat heat tolerance loci can help to provide reliable markers for heat tolerance selection, and to enable the positional cloning of heat tolerance genes. The former would be invaluable for breeding programs, while the latter would improve our knowledge about the molecular mechanism of heat tolerance, and could also give us the opportunity to improve susceptible varieties via gene transformation.

Some studies have mapped chromosomal regions associated with heat tolerance at grain-filling in wheat. Yang et al. (2002b) reported two quantitative trait loci (QTL), associated with grain-filling duration under long-term heat stress (30 °C), on chromosomes 1B and 5A. Kuchel et al. (2007) studied the effect of 11 climatic variables on a doubled haploid mapping population, in 17 environments. The number of days above 30 ºC during June to November had the largest impact on mean grain yield among their studied variables, and a strong interaction was observed at the QTL locus QGyld.agt-1B between yield and the number of days above 30 °C. Mohammadi et al. (2008 a and b) reported QTL for grain-filling duration (GFD) under field high temperature conditions on chromosome 2D and for heat susceptibility index (HSI) of grain weight on chromosomes 1B, 5B, and 7B in response to a brief severe heat stress (3 days of 35/30 ºC applied 7 days after anthesis, DAA) under controlled conditions. Mason et al. (2010) mapped QTL for heat susceptibility index (HSI) of main spike yield components (grain number, grain weight, and single grain weight) and grain-filling duration, in response to a short-term severe heat stress (3 days of 38/18 ºC applied 10 DAA), on chromosomes 1A, 1B, 1D, 2A, 2B, 3B, 4A, 5A, 5B, 6D, and 7A. Mason et al. (2011) detected 14 QTL for organ temperature depression (spike and flag leaf) and HSIs of main spike yield components on 9 chromosomes (1B, 2D, 3B, 4A, 5A, 5B, 6D, 7A, and 7B) in response to 3 days of 38/18 ºC (10 DAA). At 7 of these loci, QTL for organ temperature depression co-localized with HSI QTL, with lower spike/flag leaf temperatures associated with higher tolerance (lower HSI). Seven QTL regions (on chromosomes 1B, 3B, 4A, 5A, 5B, and 6D) in their study were common with those reported by Mason et al (2010). The source of tolerance was similar (Halberd) but the susceptible parent differed (Cutter in Mason et al. 2010 and Karl 92 in Mason et al. 2011). Kumar et al. (2010) reported three QTL for stay-green under field high temperature conditions, on chromosomes 1AS, 3BS, 7DS. Vijayalakshmi et al (2010) found nine QTL for senescence related traits (time to 75, 50, 25% greenness, maximum rate of senescence, time to maximum rate of senescence, percent greenness at maximum senescence, and Fv/Fm) under heat stress, on chromosomes 2A, 3A, 3B, 6A, 6B, and 7A. Naruoka et al. (2012) found QTL associated with longevity of leaf greenness on chromosomes 1B, 2D, 3A, 3B, 4A, 5B, and 7B. At the QTL on chromosome 4A, longer green leaf area duration was associated with higher xylem exudation under hot, dry conditions in several field trials. Paliwal et a. (2012) detected QTL for canopy temperature depression and HSIs of yield, thousand grain weight and grain-filling duration under field conditions, on chromosomes 2B, 7B, and 7D. Higher thousand grain weight heat tolerance was associated with stronger canopy temperature depression on chromosome 2B and 7B in their study. Tiwari et al. (2013) found QTL on chromosome 1B, 1D, 2D, 6B, and

7A for HSIs of grain yield, thousand grain weight, grain-filling duration, and canopy temperature, 7 of which (on chromosomes 1D, 2D, 6B, and 7A) were stable across different trials. They observed co-localization of HSI QTL for thousand grain weight and canopy temperature on chromosome 1D, and co-localization of QTL for thousand grain weight potential (under normal conditions) and HSI of thousand grain weight on chromosome 6B. Mason et al. (2013) reported QTL for yield and its components, HSI of yield and also canopy temperature depression under field high temperature conditions, on 9 wheat chromosomes (2D, 3B, 3D, 4B, 5A, 5B, 5D, 7A, and 7D). They also observed co-localization of QTL for grain yield and canopy temperature depression (on chromosomes 3BL and 5DL).

These studies indicate that heat tolerance in wheat is under the control of multiple loci, located on almost all of the wheat chromosomes, although most of these QTL/genes have been located to the B genome. The studies quoted in the previous paragraph represent a significant advance; however, the numbers of molecular markers (59 to 260) and/or sizes of the population (e.g. 64 in Mason et al. 2010) were too small to allow mapping to a resolution required for identification of potential controlling genes. With few exceptions (Naruoka et al. 2012) studies have only separately looked at mapping stay-green (Kumar et al. 2010; Vijayalakshmi et al. 2010) or grain weight responses (Mason et al. 2010; Mason et al. 2011; Mason et al. 2013; Paliwal et al. 2012; Tiwari et al. 2013) under high temperature conditions.

Wheat genotypes also differ in their degree of responses to high temperatures at earlier stages of development i.e. sowing to emergence, vegetative, spikelet initiation, and stem elongation phases (Slafer and Rawson 1994). However, the genotypic variation seems more evident at the vegetative and stem elongation phases than at the other stages (Slafer and Rawson 1994). Variation has been reported among wheat genotypes for growth rate and recovery of seedlings in response to a severe heat stress (46 ºC) applied after a few hours of acclimation under moderate heat (>30 ºC) (Senthil-Kumar et al. 2007). According to these pieces of evidence, growth rate and recovery of wheat genotypes after a severe heat stress (with or without an acclimation step), applied at a suitable early developmental stage, can be used to identify genetic variation for heat tolerance.

2.15 Aims of the thesis

High temperature is one of the major environmental factors constraining wheat production (both quantity and quality) in most of the world's wheat growing regions. The main aims of this study were to characterize effects of brief episodes of heat stress applied at vegetative (~4-week-old plants) and grain-filling (10 DAA) stages of wheat development, and to

examine the genetic basis for heat tolerance at the grain-filling stage. Therefore, a number of experiments were conducted with the following objectives:

1. To investigate genetic variation for heat tolerance at the grain-filling stage, mainly among parents of available mapping populations, and to identify a suitable mapping population for genetic analysis of heat tolerance at grain-filling stage. Also, to investigate the relationships between different heat responses and heat related traits, and to identify appropriate traits to be targeted for genetic analysis.
2. To undertake detailed analysis of 9 contrasting genotypes for heat tolerance, including two pairs of parents of available mapping populations contrasting for tolerance to heat at the grain-filling stage.
3. To identify chromosomal regions (QTL) affecting heat tolerance and traits related to higher performance, under high temperatures at the grain-filling stage.
4. To investigate associations between grain weight maintenance and stay-green related traits at the genetic level (QTL analysis and varietal comparisons).
5. To investigate whether there is any link between major semi-dwarf loci (*Rht-B1* and *Rht-D1*) and heat tolerance/sensitivity at the grain-filling stage.
6. To investigate genetic variation for heat tolerance at the vegetative stage using of state-of-art automated plant imaging facilities, and to test whether tolerance at vegetative and grain-filling stages are correlated.

Chapter 3: Genetic variation for grain-filling response to a brief post-anthesis heat stress in wheat (*Triticum aestivum* L.): Relationships to flag leaf senescence, plant architecture, and development

3.1 Introduction

Terminal heat stress is a limiting factor in many wheat growing regions including Australia (Asseng et al. 2011; McDonald et al. 1983; Wardlaw and Wrigley 1994). An average of 10 to 15% yield reduction per annum due to high temperatures has been estimated for Australia and USA according to field and controlled environment trials (Wardlaw and Wrigley 1994). This situation is likely to worsen if current trends in climate change continue (Hansen et al. 2012; IPCC 2007; Ortiz et al. 2008) which highlights the importance of breeding new heat tolerant wheat varieties. However, breeding for heat tolerance is challenging due to the unpredictable nature of heat stress (timing, magnitude, and duration), its co-occurrence with drought stress, growth-stage-specific effects, and lack of reliable genetic markers for tolerance genes (Wardlaw and Wrigley 1994). Moreover, many of the physiological and morphological traits believed to be related to heat tolerance are yet to be fully exploited in wheat breeding, and efficient phenotyping assays for them are needed (Kumar et al. 2010; Lopes and Reynolds 2012).

High temperature can affect wheat in various ways, depending on timing during development. It can reduce the number of productive tillers plant^{-1} (Rahman et al. 2009), spikelets spike^{-1} and grain spike^{-1}, and can lower single grain weight (SGW) (Farooq et al. 2011). Early stages of wheat development generally occur under cooler conditions while grain fill coincides with rising temperatures. Temperatures above the maximum grain growth optimum temperature (15 °C) (Chowdhury and Wardlaw 1978) are frequent (Wardlaw and Wrigley 1994), and most of the wheat crops experience a few days (2-5 days) of temperatures above 34°C during grain fill (Asseng et al. 2011; Stone and Nicolas 1994). Pot experiments and simulation modelling suggest that a considerable proportion of wheat yield loss can be attributed to the latter (Asseng et al. 2011; Stone and Nicolas 1994).

A number of physiological and biochemical factors are believed to contribute to grain fill response under high temperatures including accelerated development and senescence, increased respiration, reduced photosynthesis, and decreased starch deposition in developing grains (Bhullar and Jenner 1985; Jenner 1994; Maphosa et al. 2014). Temperatures above 30 ºC reduce grain fill rate and grain fill duration in wheat (Tashiro and Wardlaw 1989; Zahedi and Jenner 2003). The reduced grain fill rate is mainly due to the decreased starch deposition which has been attributed to the adverse effect of high temperatures on the activity of several

enzymes in starch biosynthesis pathway in developing grains (Hawker and Jenner 1993). In particular the soluble starch synthase activity is highly susceptible to high temperatures (Jenner and Hawker 1993; Keeling et al. 1993). This idea gains further support from *in vivo* and *in vitro* studies on source-sink balance which concluded that the high temperature effects on grain fill is likely to be a direct one on the grain itself (sink strength) (Bhullar and Jenner 1983; Bhullar and Jenner 1986; Jenner 1991a; Jenner 1994; Wardlaw et al. 1980). Grain growth also depends on assimilate supply which is provided by photosynthesis during this period as well as accumulated stem carbohydrate reserves (Blum et al. 1994; Yang et al. 2002a). Heat stress limits photosynthetic capacity by altering chloroplast ultrastructure (Peck and McDonald 2010), damaging thylakoid membranes, causing photosystem II destruction, inactivating some of the photosynthetic enzymes such as RuBisCO and RuBisCO activase, and by reducing chlorophyll content (Farooq et al. 2011; Ristic et al. 2007; Wahid et al. 2007). It has been shown that photosynthetic rate (Al-Khatib and Paulsen 1990) and some of the photosynthesis related traits such as chlorophyll fluorescence (Moffatt et al. 1990), stomatal conductance (Reynolds et al. 1994), and stay-green (delayed leaf senescence) (Kumari et al. 2007; Lopes and Reynolds 2012) are positively correlated with a better performance under high temperature conditions. Under optimum conditions, stem reserves contribute less to grain growth compared to the current photosynthesis, while under heat stress conditions when current photosynthetic source is inhibited it can contribute more to grain growth, depending on the genotype (Blum 1998; Yang et al. 2002a). These works suggest that source activity may also play a role in tolerance under heat-stressed conditions.

SGW reduction in response to terminal heat stress which can lead to a significant yield loss is important for the wheat industry in Australia and elsewhere. Testing for associations of the grain fill response with senescence and with plant architectural and developmental traits could shed further light on the biological processes impacting heat tolerance. Accordingly, the present study has been conducted to investigate the responses of 36 spring bread wheat genotypes to a brief period of heat stress at 10 days after anthesis (DAA), and the association of grain fill response to various senescence-related, plant architecture and developmental traits. The observed variation in heat-stress responses among the genotypes can form the basis of future physiological and biochemical studies. Moreover, most of the genotypes presented in this work are parents of available mapping populations, and variation here was hoped to provide basis for follow up genetic analysis.

3.2 Materials and Methods

3.2.1 Plant materials

Thirty six spring bread wheat genotypes (*Triticum aestivum* L.; Table 3.1) were used. The majority were selected because they were parents of available mapping populations (the list of parents is presented in Appendix 1). Lyallpur-73 was selected because it had been reported to be heat susceptible (Mufti 2005; Wardlaw et al. 1989a; Wardlaw et al. 2002). A preference was also given to Australian varieties, and genotypes that were not late flowering. Seeds were obtained from Michael Francki (Department of Agriculture & Food of Western Australia, DAFWA), Dion Bennett (Australian Grain Technologies, AGT), Dan Mullan (Intergrain), Bertus Jacobs (LongReach), Hugh Wallwork (South Australian Research and Development Institute, SARDI), the Australian Winter Cereals Collection and from collections at the Australian Centre for Plant Functional Genomics (ACPFG) and New South Wales Department of Primary Industries (NSW-DPI) Wagga Wagga. Table 1 lists the genotypes together with their countries of origin and years of release.

Table 3. 1 Details of wheat genotypes.

Genotype (origin, year of release)			
Axe (Australia, 2007)	EGA Bonnie Rock (Australia, 2002)	Kukri (Australia, 1999)	Sunstar (Australia, 1983)
Berkut (Mexico, 2002)	Egret (Australia, 1973)	Lincoln (Australia, 2007)	Tammarin Rock (Australia, 2004)
Cadoux (Australia, 1992)	Frame (Australia, 1994)	Lyallpur-73 (Pakistan, 1973)	Tasman (Australia, 1993)
CD87 (Australia, 1989)	Halberd (Australia, 1969)	Millewa (Australia, 1979)	Trident (Australia, 1993)
Correll (Australia, 2006)	Hartog (Australia, 1982)	Molineux (Australia, 1988)	W7985 Synthetic*
Cranbrook (Australia, 1984)	Janz (Australia, 1989)	Opata 85 (Mexico, 1993)	Waagan (Australia, 2009)
Crusader (Australia, 2008)	Katepwa (Canada, 1981)	Reeves (Australia, 1989)	Westonia (Australia, 1997)
Drysdale (Australia, 2002)	King Rock (Australia, 2009)	Sokoll (Mexico, 2002)	Wyalkatchem (Australia, 2001)
EGA Blanco (Australia, 2004)	Krichauff (Australia, 1997)	Sunco (Australia, 1986)	Young (Australia, 2005)

*A synthetic hexaploid wheat line containing A and B genomes from the durum wheat (*T. turgidum* L.) cultivar Altar 84 and the D genome from an accession of *Aegilops tauschii* Coss. (Nelson et al. 2006).

3.2.2 Experimental design, plant growth and heat stress conditions

Pots (8 × 8 cm, 18 cm depth) were filled with a steam-sterilized mixture of 2:1 of coco peat:Waikerie sand (pH 6.0-6.5) containing the following nutrients (mg pot^{-1}): dolomite lime, 202; agricultural lime, 561; hydrated lime, 131; gypsum, 202; superphosphate, 202; iron sulphate, 505; iron chelate, 33.7; trace elements (Micromax), 202; calcium nitrate, 505, and slow-release fertilizer pellets (Mini Osmocote), 2022. Three seeds per pot were sown on 23rd-26th of May 2011, and seven days after sowing, plants were thinned to one healthy seedling per pot. The experiment employed a split-plot design with a randomized complete block design of 9 blocks being used to assign two treatments (control vs. heat) to the 2 main plots in each block. Each main plot was split into 36 subplots to which were assigned 36 genotypes.

Plants were initially grown in two adjacent naturally lit greenhouse compartments (The Australian Plant Accelerator, University of Adelaide, Waite Campus, Adelaide). Measured greenhouse conditions are reported in Table 3.2, and averaged 20/16 ºC day/night. Plants were watered from above every 2 days. As in some previous heat tolerance studies (Tashiro and Wardlaw 1990b; Wardlaw et al. 1989b), plants were pruned back to the single main culm by removing tillers (by cutting them 1 cm above the soil surface with a pair of scissors) as they appeared to enable easier management and better light penetration. A liquid fertilizer was applied to the soil (Thrive all-purpose soluble fertilizer, containing: nitrogen, phosphorus, potassium, sulfur, magnesium, manganese, copper, iron, zinc, boron and molybdenum) fortnightly at the recommended rate from one month after sowing to plant maturity. The anthesis date of each plant was recorded. Each plant that was destined for heat treatment in the experimental design, was transferred to a growth chamber (BDW120, Conviron) at 10 days after anthesis (days after anthesis, DAA), where heat treatment (37/27 ºC day/night temperature) was applied for 3 days, before being returned to the greenhouse. The maximum temperature (37 °C) of the growth chamber was held for 8 hours each day, with 3 hours transition periods used either side to linearly ramp the temperature up and down. Lighting (a mixture of metal halide and tungsten incandescent) was at a maximum of 630 µM m^{-2} s^{-1} at spike height for 10 hours each day, with a 2 hours transition period used either side to step the intensity at 460 µM m^{-2} s^{-1}. Average day/night relative humidity in the chamber was measured at about 60/80%. Pots were placed in trays of water to ~ 2-cm depth while in the chamber to minimize drought stress. Leaf water potential of plants in the chamber, measured using a Scholander pressure chamber, decreased to between -11 and -15 bar by the middle of the day, indicating that there was some foliar dehydration. Turgor then recovered completely during the night.

Table 3. 2 Measured temperatures (ºC) in the greenhouse. Anthesis and physiological maturity were reached during July-August and September-October, respectively.

Month	Average day temperature	Average night temperature	Average minimum temperature	Average maximum temperature	Minimum temperature	Maximum temperature	Days > 30ºC
May	19.1	16.3	15.8	22.7	15.0	24.5	0
June	18.8	16.0	15.5	22.5	14.5	24.5	0
July	18.3	15.4	14.7	22.5	13.2	24.8	0
August	19.4	16.0	15.2	24.0	14.1	26.2	0
September	20.2	16.2	15.4	25.2	13.5	25.0	0
October	21.8	17.8	16.9	26.3	14.6	30.3	1

3.2.3 Data collection

Relative chlorophyll content of the flag leaves was monitored from about 7 to 50 days after anthesis using a portable SPAD chlorophyll meter (SPAD-502; Minolta Co. Ltd., Japan).

Each value was the average of 10 measurements taken along the left hand side of the leaf between the mid-rib and leaf margin.

Details of the traits measured on individual plants are as follows. The same measurements were made at corresponding developmental stages on both control and heat treated plants.

Days to anthesis (DTA): Days from sowing to the day that exerted anthers first became visible.

Chlorophyll content just before the heat treatment, in SPAD units (ChlC7-10DAA): Because SPAD readings were taken on only two days per week, the exact DAA of each measurement (ranging from 7 to 10 DAA) depended on the anthesis date of the plant.

Normalized chlorophyll content just after the treatment period, in SPAD units (ChlC13-16DAA): This value was normalized to the ChlC7-10DAA reading taken on the same plant, to account for variation in starting chlorophyll content between plants and genotypes.

Area under the normalized SPAD progress curve (AUSC): For each plant, SPAD readings continued to be taken after the heat treatment, 2 times per week, for up to ~ 50 DAA, and these values were normalized to the 7-10 DAA value. AUSC for each plant was calculated using the following equation, where X_i is the chlorophyll content (normalized SPAD units) on the i^{th} date, t_i is the date on which the chlorophyll content was measured, and n is the number of dates on which chlorophyll content was recorded (t=~7 to 50 DAA).

$$AUSC = \sum_{i=1}^{n-1} [(\frac{X_i + X_{(i+1)}}{2}) \times (t_{(i+1)} - t_i)]$$

Days from anthesis to complete flag leaf senescence (FLSe): Flag leaves were daily checked after anthesis and recorded as fully senesced when they appeared completely yellow. Days from anthesis to the aforementioned stage (FLSe) was considered as an indicator of stay-green duration.

Grain-filling duration (GFD); time from anthesis to maturity in days: Plants were defined as mature when spikes became ~ 95% senesced and seeds became firm. Time from anthesis to the aforementioned stage was considered as an indication of GFD.

Culm length (CL): Measured at maturity, from the soil surface to 1 cm below the collar.

Shoot weight (ShW): At physiological maturity, each plant was cut off at the soil surface and the shoot (stem + leaves) separated from the spike. Shoots were oven dried at 60 ºC for 3 days before being weighed.

Spikelet number spike^{-1} (SpNS), grain weight spike^{-1} (GWS), grain number spike^{-1} (GNS), grain number spikelet^{-1} (GNSp) and single grain weight (SGW): After counting all spikelets, the spike was threshed, and the grain left in the laboratory at room temperature for ~4 weeks to reach a stable moisture content before being weighed. Grains of all sizes were manually counted. GNSp was determined by dividing GNS by SpNS and SGW was determined by dividing GWS by GNS. Grains from floret positions 1 + 2 (the two most basal positions) and floret positions >2, were initially counted and weighed separately, to check for potential differences in grain set and size between different positions on the spikelets. Basal spikelets that were underdeveloped (small spikelets without grain) were ignored.

Harvest index (HI): Determined for each plant (main tiller) by dividing GWS by above ground biomass (GWS + ShW).

3.2.4 DNA extraction

DNA was extracted from a segment of approximately ~50 mm long sections of leaf, collected from 2 week old seedlings, using the method of Rogowsky et al. (1991), with some modifications (Pallotta et al. 2000). DNA concentrations were determined by absorbance at 260 nm, using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies).

3.2.5 Markers for *Rht-B1* and *Rht-D1* loci (previously known as *Rht1* and *Rht2*)

Competitive allele-specific PCR (KASP; LGC Genomics, London, UK; www.lgcgenomics.com) markers were used to assay SNPs that were diagnostic of wild type and dwarfing alleles of the *Rht-B1* and *Rht-D1* genes, described by Ellis et al. (2002). Sequences of primers developed by an international consortium (Susanne Dreisigacker, CIMMYT; Gina Brown-Guedira, USDA; Peter Sharp, University of Sydney; Keith Edwards, University of Bristol; Simon Griffiths, Dave Laurie, Cristobal Uauy, JIC; unpublished) were obtained from CIMMYT (Susanne Dreisigacker). In each case, a point mutation changes a CAG codon to a TAG stop codon shortly after the start of translation. Genotyping reactions were performed using 34 cycles in a MJ Research Thermocycler (Waltham MA, USA), followed by 9 cycles in a LightCycler®480Real-Time PCR System for fluorescence detection of the products (Roche Applied Science; www.roche-applied-science.com). Reactions were assembled in a final volume of ~6 µl containing 3 µl of 2× KASP Reaction Mix (LGC Genomics), 0.17 µM of each of two allele-specific competitive forward primers, 0.42 µM of the common reverse primer and 50 ng genomic DNA. The following cycling conditions were

used for the 34 initial cycles: an initial denaturing step for 15 min at 94 ºC, 9 cycles of 20 s at 61 ºC (decreasing by 0.6 ºC every cycle), then 25 cycles of 10 s at 94 ºC and 1 min at 55 ºC, and a final incubation step of 2 min at 25 ºC. The additional 9 cycles in the LightCycler®480 Real-Time PCR System was performed using 10 s at 94 ºC and 1 min at 55 ºC per cycle. Data were analysed using the LightCycler® 480 Software, Version 1.5 (Roche Applied Science; www.roche-applied-science.com).

The marker scores were cross checked against information received from other researchers, where available (Karen Cane, DPI-Vic Horsham, Howard Eagles, The University of Adelaide, and Melissa Garcia, ACPFG, The University of Adelaide).

3.2.6 Data analysis

DTA, GWS, SGW, GNS, GNSp, SpNS, CL, ShW and HI were recorded for each plant, while the other traits were measured just on all plants in just four blocks. The R programming language was used for statistical analyses and to prepare figures (R Development Core Team 2014). Predicted means were obtained using a linear mixed model with ASReml-R software (Butler et al. 2009). Least significant difference (LSD) values were used for mean comparisons. Pairwise Pearson correlation tests and principal component analyses (PCA) were done to study relationships between traits measured under control or heat conditions, and between response ratios of different traits ($R_{H/C}$ = Mean trait $value_{Heat\ treatment}$ / Mean trait $value_{Control}$). Pearson correlation tests and PCAs were performed using Psych (Revelle 2011) and FactoMineR (Lê et al. 2008) packages, respectively.

3.3 Results

There was a strong significant treatment (control vs. heat) effect for most of the traits (Table 3.3), and a (highly significant genotypic effect for all the traits (Table 3.3). Moreover, significant genotype-by-treatment interactions were observed for GWS, SGW, ChlC13-16DAA, AUSC, FLSe, and HI which indicated genotypic variation for the responses of these traits to heat treatment (Table 3.3).

Table 3. 3 P-values for genotype (G), treatment (T) and genotype × treatment (G × T) effects in the linear mixed model analysis.

Trait	G	T	G × T
Days to anthesis (DTA)	< 0.0001	NA*	NA
Grain weight spike^{-1} (GWS)	< 0.0001	0.0260	< 0.0001
Single grain weight (SGW)	< 0.0001	< 0.0001	< 0.0001
Grain number spike^{-1} (GNS)	< 0.0001	0.6663	0.3146
Grain number spikelet^{-1} (GNSp)	< 0.0001	0.9480	0.2960
Spikelet number spike^{-1} (SpNS)	< 0.0001	0.1915	0.0303
Grain-filling duration (GFD)	< 0.0001	0.0003	0.4392
Chlorophyll content 7-10 days after anthesis (ChlC7-10DAA)	< 0.0001	NA	NA
Chlorophyll content 13-16 days after anthesis (ChlC13-16DAA)	< 0.0001	0.0006	< 0.0001
Area under SPAD's progress curve (AUSC)	< 0.0001	0.0620	< 0.0001
Time from anthesis to 95% flag leaf senescence (FLSe)	< 0.0001	< 0.0001	0.0124
Culm length (CL)	< 0.0001	0.4050	0.6850
Shoot weight (ShW)	< 0.0001	0.0300	0.0580
Harvest index (HI)	< 0.0001	0.0001	0.0374

*NA, not applicable; trait measured before heat treatment.

3.4.1 Anthesis date

Days from sowing to anthesis (DTA) varied significantly between genotypes, and ranged from ~63 days (Krichauff and Axe) to ~90 days (Egret and W7985 Synthetic) (Figure 3.1).

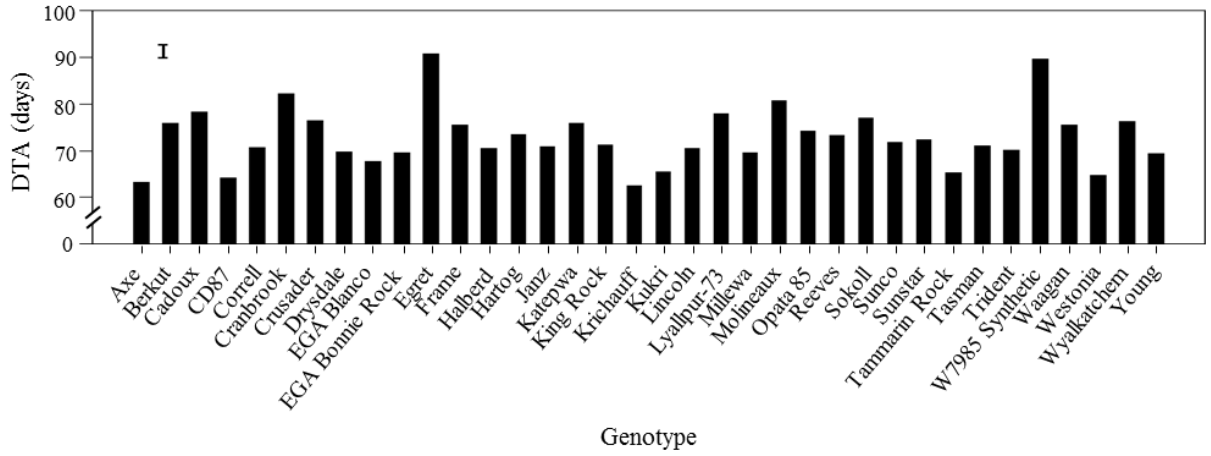


Figure 3. 1 Means for days from sowing to anthesis (DTA). The vertical bar indicates the LSD value ($\alpha = 0.05$) for mean comparisons.

3.3.2 Grain weight spike^{-1} and single grain weight

Single grain weight (SGW) was reduced in the heat-treated plants relative to the control plants, and the effect was significant in 23 of the genotypes (Figure 3.2a). Overall, heat reduced SGW by an average of 14.0%. Genotypes Young, Sunco, Waagan, and EGA-Blanco showed the least response (less than 3.0%) and were therefore the most tolerant of the varieties, while genotypes Reeves, Cadoux, and Crusader showed the greatest responses and were therefore the most intolerant (more than 25.0% reduction in heat-treated plants relative to control; Figure 3.2a). W7985 Synthetic had the largest SGW under both control and heat conditions (69.9 and 54.6 mg, respectively), while Millewa and Katepwa had the smallest

SGW under control and heat conditions, respectively (36.8 and 31.7 mg, respectively). Grains in different floret positions in the spikelets (two most basal positions vs. remainder) showed very similar trends in responses across the genotypes (Figure 3.3). Therefore, the results were described for means over all floret positions together. The trend in response of grain weight spike^{-1} (GWS) across the genotypes was also very similar to that of SGW, which was expected because there was no detectable effect of heat treatment on grain number (Figures 3.2a and b). Sokoll and Egret appeared to have the largest GWS under control and heat conditions, respectively (4.5 and 3.5 g, respectively), while Katepwa showed the smallest GWS under both conditions (0.9 and 0.7, respectively).

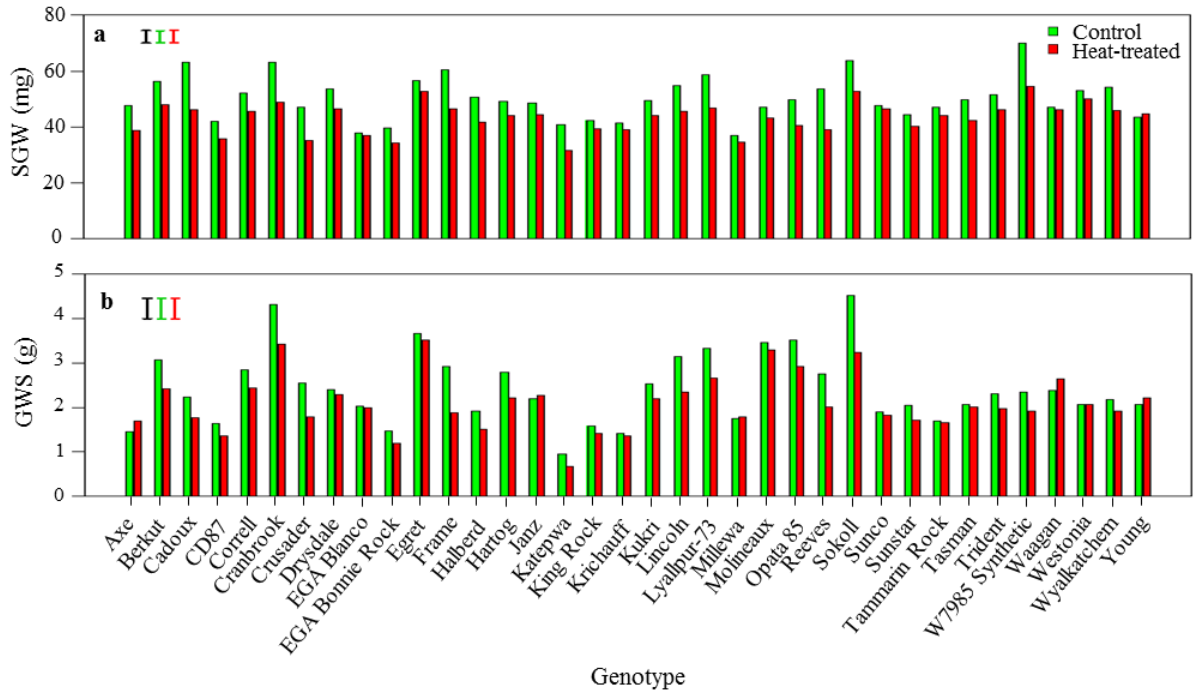


Figure 3. 2 Single grain weight (SGW, a) and grain weight spike^{-1} (GWS, b) in control and heat-treated plants. The vertical bars indicate the LSD values ($\alpha = 0.05$) for within genotype mean comparisons between control and heat-treated plants (black bar), and for mean comparisons between genotypes within control (green bar) or heat (red bar) treatment.

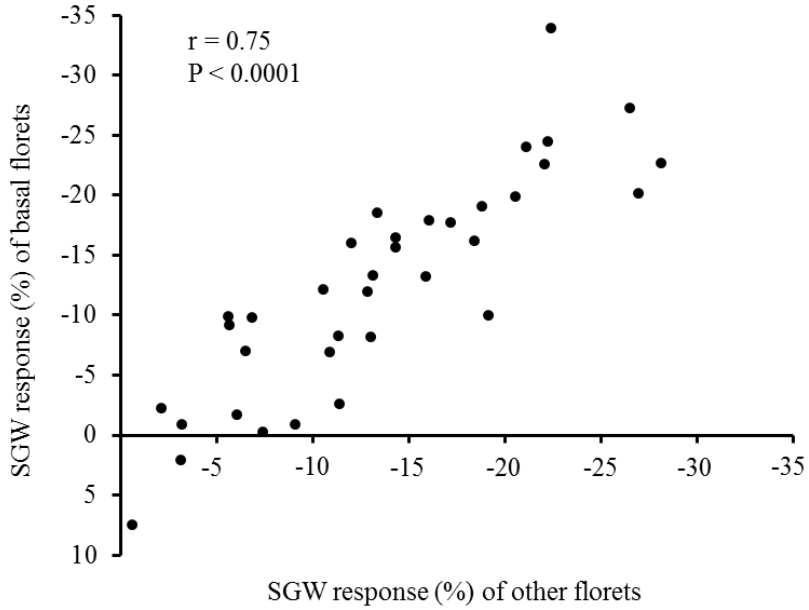


Figure 3. 3 Association between single grain weight (SGW) responses of florets in different positions within the spikelets (basal two florets versus others). Each point represents a genotype.

3.3.3 Spikelet and grain number

A significant genotype-by-treatment interaction was observed for SpNS (Table 3.3; Figure 3.4a), although this seems likely due to chance differences between control and heat-treated plants, since the trait (SpNS) would have been set prior to anthesis. It is noteworthy that there was no significant difference between controlled and heat-treated plants for the number of undeveloped spikelet per spike (data not shown). This further suggests that these results are unlikely to be derived from the heat effect on spikelet development. There was no significant difference between control and heat-treated plants for overall means of grain number spike^{-1} and grain number spikelet^{-1} (Table 3.3, and Figure 3.4b and c). The absence of a heat effect on grain number spikelet^{-1} also held true at different floret positions within the spikelets (two most basal positions vs. remainder; data not shown). These results indicate that the heat treatment at 10 days after anthesis did not affect the frequency of grain set.

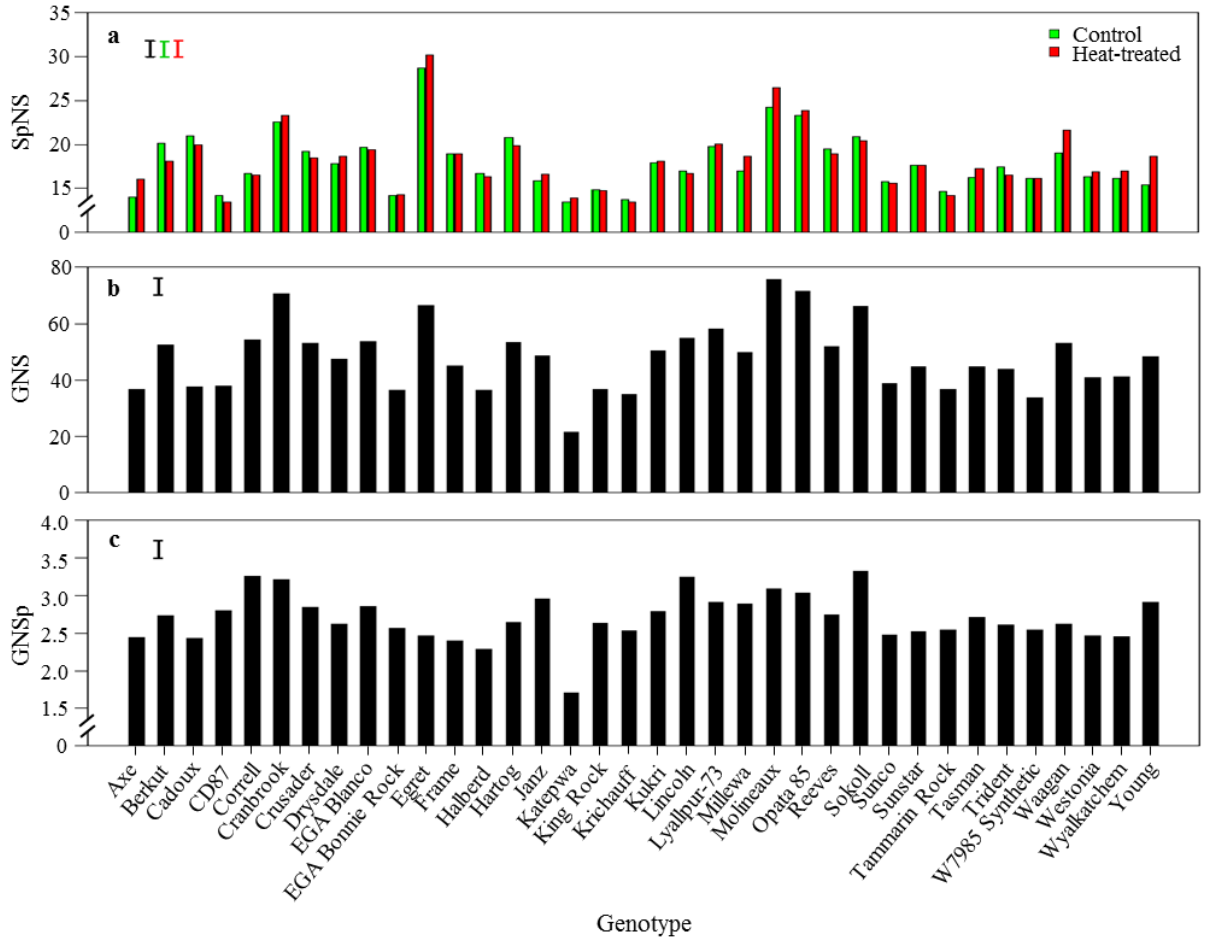


Figure 3. 4 a) Means of each genotype for control and heat-treated plants for spikelet number spike^{-1} (SpNS; **a**). Genotype-by-treatment interaction was significant for this trait; the vertical bars indicate the LSD values (α = 0.05) for within genotype mean comparisons between control and heat-treated plants (black bar), and for mean comparisons between genotypes within control (green bar) or heat (red bar) treatment. **b** and **c**) Means for grain number spike^{-1} (GNS; **b**) and grain number spikelet^{-1} (GNSp; **c**). These traits were not significantly affected by heat, so the combined means of control and heat-treated plants are shown; the vertical bars indicate the LSD values (α = 0.05) for mean comparisons.

3.3.4 Chlorophyll responses

Significant variation was observed for chlorophyll content among genotypes before the treatment, with genotypes varying by up to seven SPAD units (Figure 3.5a). The heat treatment accelerated the rate of chlorophyll loss in the flag leaves beyond the rate observed in the control plants undergoing natural senescence. Most of the genotypes showed a two-phase response of chlorophyll loss to heat, as illustrated by the examples shown in Figure 3.6a and b (for all of the genotypes, see Appendix 3.1). Chlorophyll content (SPAD units) decreased rapidly during the treatment. Then after the treatment, it decreased at a slower rate, although generally more rapidly than in the control plants at the corresponding developmental stage, indicating that some of the effect of heat persisted after the stress was relieved. The trait 'ChlC13-16DAA' represents the first phase of the response, and is the proportion of chlorophyll retained during the treatment period relative to just before the treatment. It was reduced in the heat-treated plants relative to control in 17 genotypes (Figure 3.5b). Losses

ranged from 0.24% (Egret) to 62.63% (Reeves). Across all genotypes, heat reduced ChlC13-16DAA by an average of 17.88%. The trait AUSC captured both phases of the response, and summarized the amount of chlorophyll retained during the treatment period plus the time up to ~40 days after the end of the treatment. AUSC was decreased by heat in all genotypes, and the effect was significant in 17 genotypes (Figure 3.5c). On average, heat decreased AUSC by 23.09%. The period from anthesis to complete flag leaf senescence (FLSe) was also shortened by heat, by an average of 13.57 days, consistent with a phenomenon of heat-accelerated chlorophyll loss. A shortening of this interval under heat conditions was observed in all varieties except Egret, and the reduction was significant for 12 of the genotypes (Figure 3.5d).

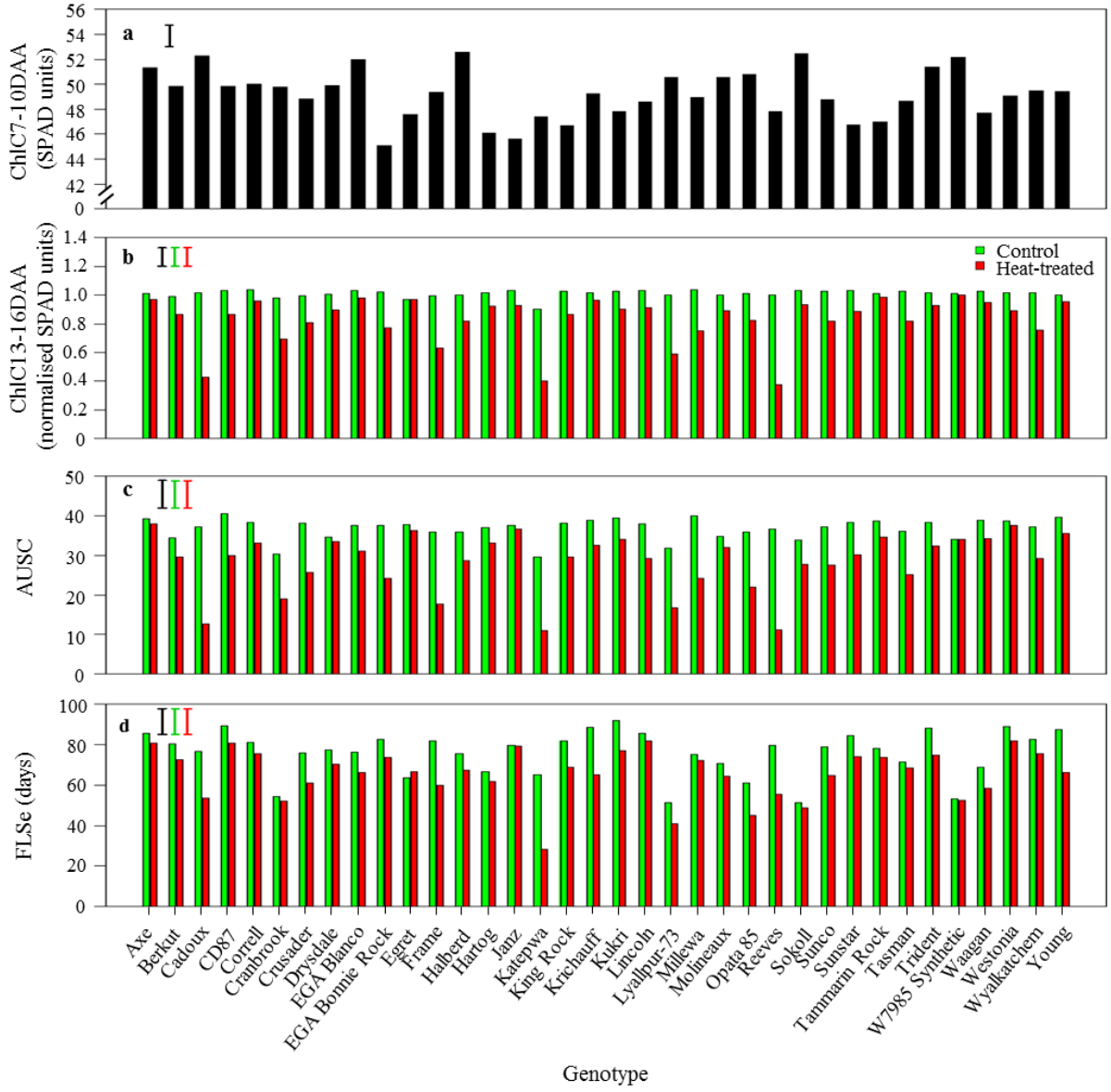


Figure 3. 5 Means for chlorophyll content 7-10 days after anthesis (ChlC7-10DAA; **a**). The trait was measured before heat treatment, so the combined means of control and heat-treated plants are shown; the vertical bar indicate the LSD value (α = 0.05) for mean comparisons. Means for control and heat-treated plants for chlorophyll content 13-16 days after anthesis (ChlC13-16DAA; **b**), area under SPAD curve (AUSC; **c**), and days from anthesis to 95% flag leaf senescence (FLSe; **d**). ChlC13-16DAA, AUSC, and FLSe showed significant genotype-by-treatment effects. Bars indicate the LSD values (α = 0.05) for within genotype mean comparisons

between control and heat-treated plants (black bar), and for mean comparisons between genotypes within control (green bar) or heat (red bar) treatment (**b**, **c**, and **d**).

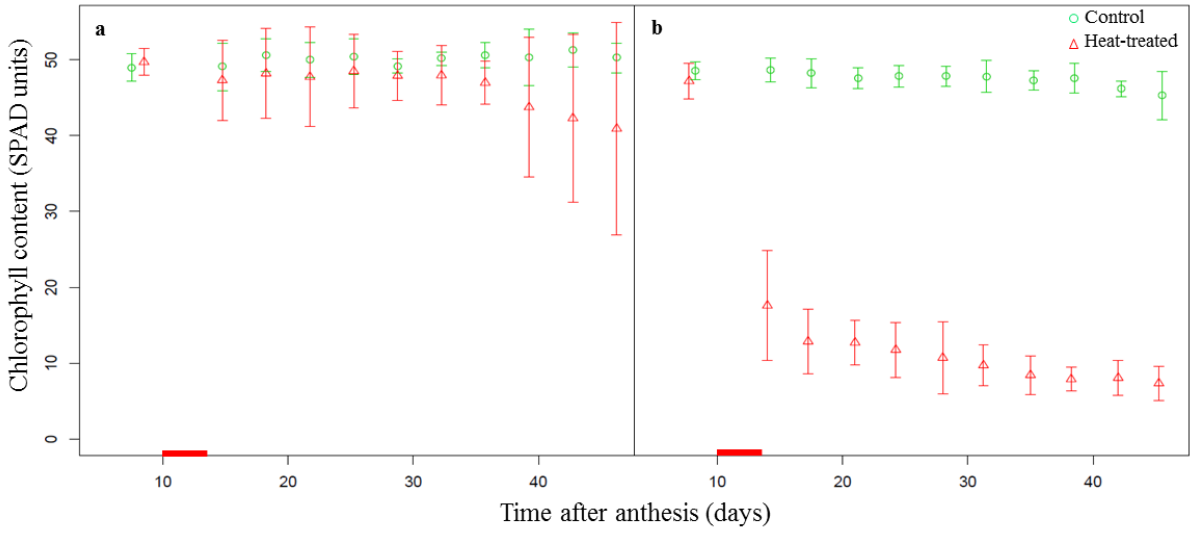


Figure 3. 6 Relative chlorophyll content of flag leaves (means and 95% confidence intervals of SPAD readings; n=4) in heat-treated and control plants, before the period of brief heat treatment (represented by the horizontal red bar) and thereafter, in a representative tolerant variety **a**) Young and intolerant variety **b**) Reeves.

3.3.5 Grain-filling duration

Overall, heat significantly shortened grain-filling duration (GFD), and the average reduction was 13.0%. However, GFD showed no genotype-by-treatment interaction, indicating that genotypes did not vary significantly in this heat response. Under control conditions Krichauff and W7985 Synthetic had the longest and the shortest grain-filling duration, while under heat-stress conditions Janz and Lyallpur-73 appeared to have the longest and the shortest grain-filling duration, respectively (Figure 3.7).

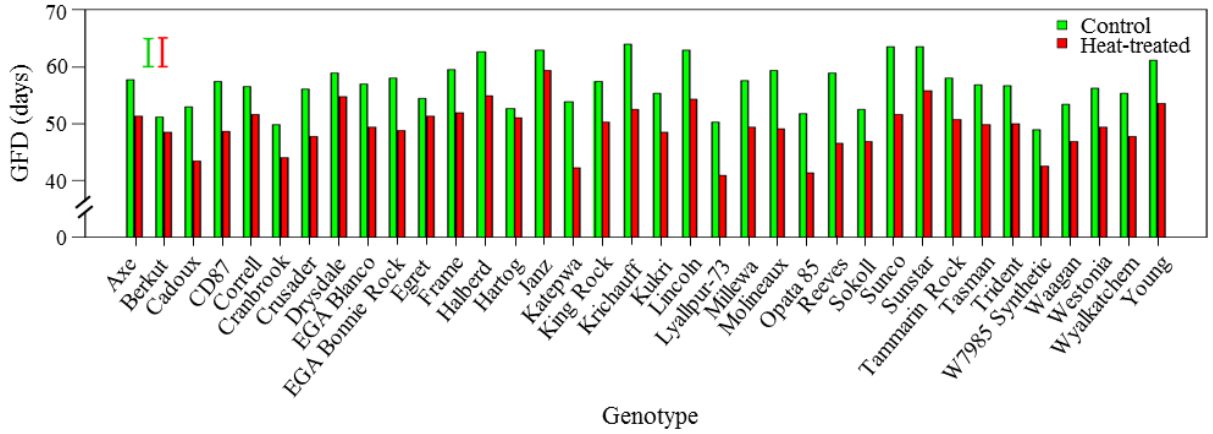


Figure 3. 7 Means for grain-filling duration (GFD) in control and heat-treated plants. Bars indicate LSDs (α = 0.05) for mean comparisons between genotypes within control (green) or heat (red) treatment.

3.3.6 Culm length, shoots weight, and harvest index

There was no significant effect of heat treatment on culm length (CL) (Table 3.3; Figure 3.8a). However, shoot weight (ShW) was significantly reduced by the treatment (by an

average of 4.81%; Table 3.3). ShW showed no genotype-by-treatment interaction, indicating the ranking of genotypes held very similar under both control and heat conditions for this trait. Egret and Krichauff had the largest and the smallest ShW, respectively, under both control and heat conditions (Fig. 3.8b). Overall, heat stress significantly reduced harvest index (HI) (by 3.83%; Table 3.3) - a result of heat causing a greater reduction in grain weight than in shoot weight (13.5 vs. 4.8% overall reduction). Thirty genotypes showed a reduction in HI while 6 genotypes showed an increase. The effect was significant in 7 genotypes (Figure 3.8c; one genotype increased).

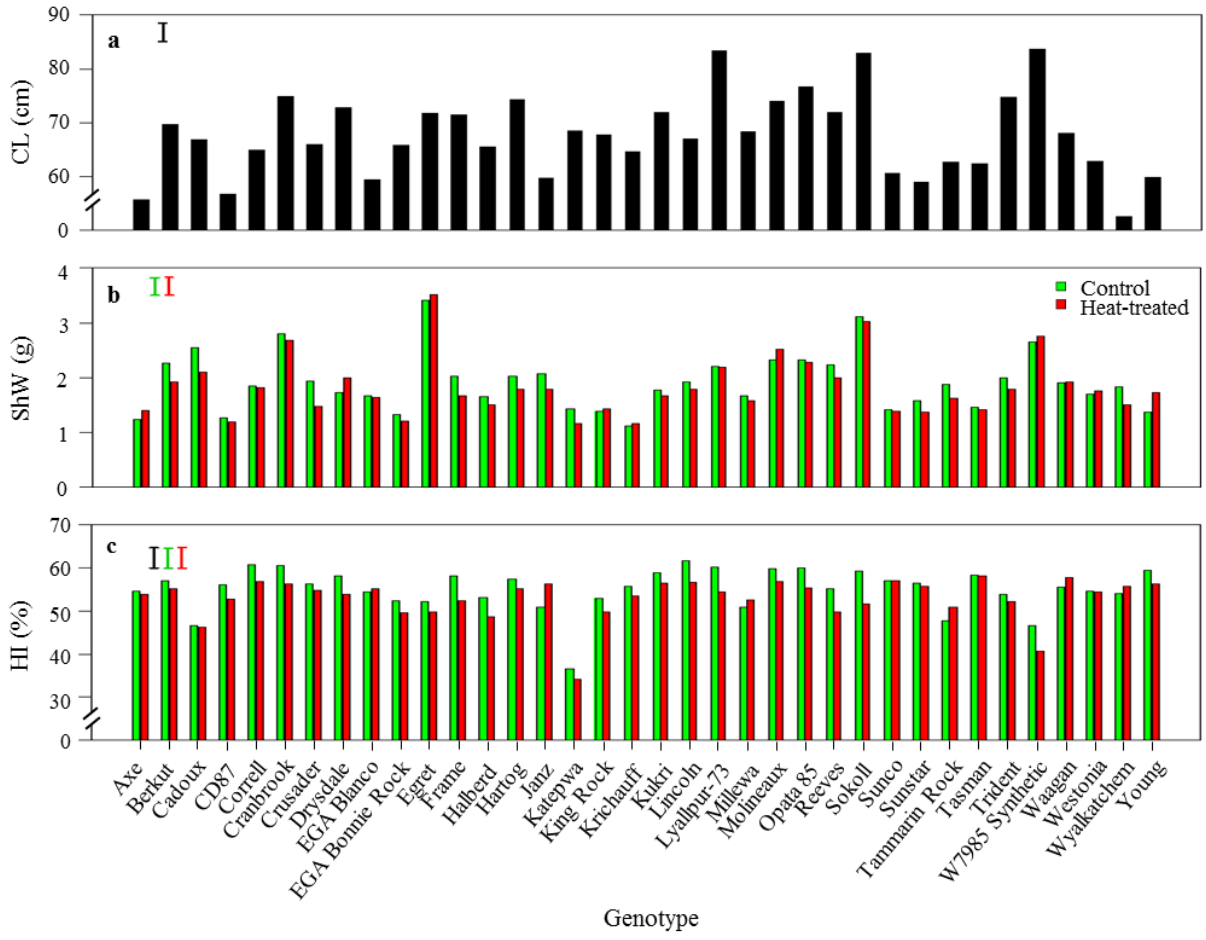


Figure 3. 8 Means for culm length (CL; a). CL was not significantly affected by the heat treatment, so the combined means of control and heat treated plants are shown; the vertical bar represents LSD ($\alpha = 0.05$) for mean comparisons. Means for shoot dry weight (ShW; b) and harvest index (HI; c). For ShW, G and T but not genotype-by-treatment effects were significant and LSDs ($\alpha = 0.05$) are shown for mean comparison between genotypes within control (green vertical bar) or heat treatment (red vertical bar). HI showed a significant genotype-by-treatment interaction and therefore the LSD (vertical bar; $\alpha = 0.05$) is shown for within genotype mean comparisons between control and heat-treated plants (black bar), and for mean comparisons between genotypes within control (green bar) or heat (red bar) treatment.

3.3.7 Associations of traits within each treatment

To explore relationships between the traits within each treatment, principal component analysis (PCA) and pairwise correlation tests were performed (Table 3.1; Figure 3.9a and b).

The first and second principal components (PCs) together explained 68% of the total variance under control conditions and 65% of the variance under heat conditions (Figures 3.9a and b).

Under either condition (control or heat), the duration from sowing to anthesis (DTA) was positively associated with all grain and vegetative productivity components except GNSp (i.e., GWS, SGW, GNS, CL, and ShW) but tended to be negatively associated with grain-filling duration (GFD) and indicators of flag leaf chlorophyll retention (i.e., ChlC13-16DAA, AUSC and FLSe), although the association with ChlC13-16DAA and AUSC was not significant under heat (Figure 3.9a and b; Table 3.4). Therefore, later flowering was associated with greater vegetative and grain biomass including a greater single grain weight, but was (perhaps unexpectedly) associated with faster flag leaf senescence and a shorter grain-filling period. In line with these relationships to DTA, grain/vegetative productivity components showed positive relationships to each another, as did senescence and grain-filling duration indicators to each another (Figure 3.9a and b; Table 3.4). Conversely, where there were significant relationships between the traits across the two different classes, these relationships were almost always negative (the exception being ChlC13-16DAA vs. GNSp under control conditions, which showed a significant positive correlation) (Table 3.4).

Under either condition, harvest index (HI) was correlated positively with GWS but showed no significant correlation with ShW, indicating that variation in HI was driven mainly by variation in GWS.

With the exception of a positive correlation with SGW in control plants (Table 3.4), the amount of flag leaf chlorophyll just before the treatment (ChlC7-10 DAA) showed no significant correlations with any other trait.

Table 3. 4 Genotypic means correlation between traits in control plants (above diagonal) and heat-treated plants (below diagonal). DTA, days from sowing to anthesis; GWS, grain weight spike^{-1}; SGW, single grain weight; GNS, grain number spike^{-1}; GNSp, grain number spikelet^{-1}; SpNS, spikelet number spike^{-1}; GFD, grain-filling duration; ChlC7-10DAA, chlorophyll content 7-10 days after anthesis (corresponding to the measurement before treatment in heat treated plants); ChlC13-16DAA, chlorophyll content 13-16 days after anthesis (corresponding to first measurement after treatment in heat treated plants; normalized for the 1st SPAD measurement); AUSC, area under SPAD curve (SPAD data were normalized for the first measurement); FLSe, days from anthesis to 95% flag leaf senescence; CL, culm length; ShW, shoot dry weight; HI, harvest index.

Traits	DTA	GWS	SGW	GNS	GNSp	SpNS	GFD	ChlC7-10DAA	ChlC13-16DAA	AUSC	FLSe	CL	ShW	HI
DTA	-	0.57***	0.62***	0.40*	0.08	0.66***	-0.54***	0.15	-0.41*	-0.56***	-0.72***	0.57***	0.79***	-0.12
GWS	0.52**	-	0.65***	0.90***	0.69***	0.80***	-0.43**	0.27	-0.02	-0.41*	-0.56***	0.63***	0.83***	0.57***
SGW	0.49**	0.64***	-	0.29	0.19	0.42*	-0.49**	0.43**	-0.10	-0.47**	-0.48**	0.59***	0.74***	0.14
GNS	0.38*	0.92***	0.31	-	0.79***	0.82***	-0.26	0.14	0.10	-0.2	-0.42*	0.48**	0.64***	0.68***
GNSp	-0.02	0.61***	0.17	0.71***	-	0.36*	-0.04	0.16	0.46**	0.02	-0.17	0.27	0.34*	0.76***
SpNS	0.64***	0.85***	0.39*	0.85***	0.29	-	-0.41*	0.19	-0.17	-0.27	-0.51**	0.53**	0.82***	0.31
GFD	-0.43**	-0.12	-0.03	-0.13	0.01	-0.21	-	-0.26	0.27	0.48**	0.66***	-0.57***	-0.56***	0.11
ChlC7-10DAA	0.15	0.21	0.29	0.15	0.16	0.15	-0.31	-	0.04	-0.21	-0.24	0.25	0.26	0.14
ChlC13-16DAA	-0.21	0.23	0.27	0.16	0.32	0.00	0.49**	0.02	-	0.64***	0.32	-0.22	-0.18	0.45**
AUSC	-0.26	0.16	0.28	0.06	0.19	-0.03	0.57***	-0.06	0.94***	-	0.72***	-0.56***	-0.41*	0.12
FLSe	-0.54***	-0.12	-0.01	-0.11	0.19	-0.28	0.71***	-0.2	0.56***	0.67***	-	-0.65***	-0.66***	0.06
CL	0.53***	0.48**	0.40*	0.39*	0.22	0.42*	-0.49**	0.24	-0.10	-0.24	-0.61***	-	0.67***	0.09
ShW	0.76***	0.84***	0.71***	0.68***	0.36*	0.79***	-0.3	0.31	0.10	0.04	-0.33*	0.65***	-	0.07
HI	-0.3	0.46**	0.14	0.54***	0.59***	0.23	0.38*	-0.08	0.43**	0.40*	0.50**	-0.23	-0.04	-

Values are Pearson correlation coefficients among genotype means, with asterisks showing significance levels: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

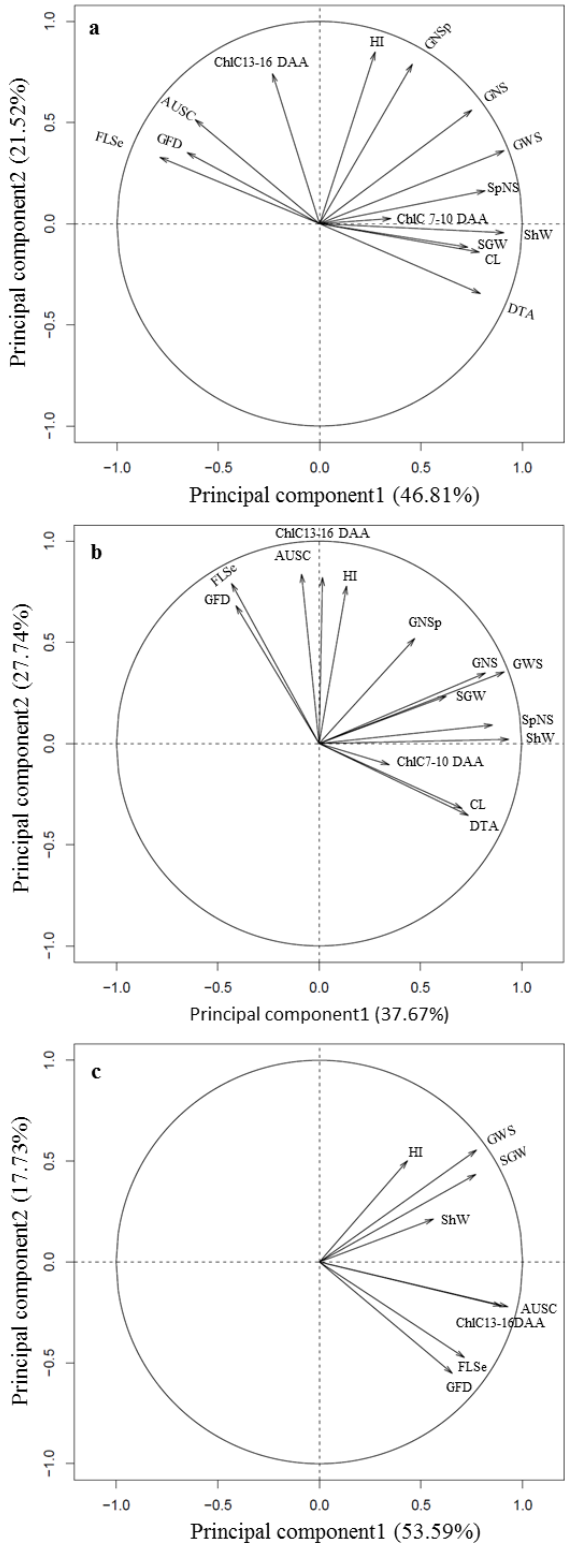


Figure 3. 9 Projection of trait variables in principal component analysis (PCA), showing traits in control plants (a), traits in heat-treated plants (b) and heat/control ratios of traits (c). DTA, days from sowing to anthesis; GWS, grain weight spike^{-1}; SGW, single grain weight; GNS, grain number spike^{-1}; GNSp, grain number spikelet^{-1}; SpNS, spikelet number spike^{-1}; GFD, grain-filling duration; ChlC7-10DAA, chlorophyll content 7-10 days after anthesis (corresponding to the measurement before treatment in heat treated plants); ChlC13-16DAA, chlorophyll content 13-16 days after anthesis (corresponding to first measurement after treatment in heat treated plants; normalized for the 1st SPAD measurement); AUSC, area under SPAD curve (SPAD data were normalized for the first measurement); FLSe, days from anthesis to 95% flag leaf senescence; CL, culm length; ShW, shoot dry weight; HI, harvest index.

Table 3. 5 Variables' loadings for the first three principal components of the principal component analyses (PCA) under control and heat stress conditions, and for heat-treated/control trait ratios. Values in parenthesis are the percentages of the variation explained by the corresponding principal component. DTA, days from sowing to anthesis; GWS, grain weight spike^{-1}; SGW, single grain weight; GNS, grain number spike^{-1}; GNSp, grain number spikelet^{-1}; SpNS, spikelet number spike^{-1}; GFD, grain-filling duration; ChlC7-10DAA, chlorophyll content 7-10 days after anthesis (corresponding to the measurement before treatment in heat treated plants); ChlC13-16DAA, chlorophyll content 13-16 days after anthesis (corresponding to first measurement after treatment in heat treated plants; normalized for the 1st SPAD measurement); AUSC, area under SPAD curve (SPAD data were normalized for the first measurement); FLSe, days from anthesis to 95% flag leaf senescence; CL, culm length; ShW, shoot dry weight; HI, harvest index.

Variable	PC1			PC2			PC3		
	Control (46.81)	Heat (37.67)	$R_{H/C}$ (53.59)	Control (21.52)	Heat (27.74)	$R_{H/C}$ (17.73)	Control (8.08)	Heat (10.51)	$R_{H/C}$ (15.59)
DTA	0.31	0.32	-	-0.20	-0.18	-	-0.14	0.22	-
GWS	0.36	0.40	0.37	0.21	0.18	0.47	-0.06	-0.08	-0.01
SGW	0.29	0.27	0.37	-0.07	0.12	0.36	0.39	0.45	-0.02
GNS	0.29	0.36	-	0.32	0.18	-	-0.26	-0.34	-
GNSp	0.18	0.21	-	0.45	0.26	-	0.00	-0.37	-
SpNS	0.32	0.37	-	0.09	0.05	-	-0.27	-0.11	-
GFD	-0.26	-0.18	0.32	0.20	0.35	-0.46	-0.16	0.11	0.24
ChlC7-10DAA	0.14	0.15	-	0.01	-0.05	-	0.74	0.16	-
ChlC13-16DAA	-0.09	0.01	0.43	0.43	0.42	-0.18	0.30	0.31	-0.10
AUSC	-0.24	-0.04	0.45	0.30	0.42	-0.19	0.02	0.37	-0.07
FLSe	-0.31	-0.19	0.34	0.19	0.40	-0.40	0.02	0.05	0.16
CL	0.31	0.31	-	-0.08	-0.16	-	0.08	0.11	-
ShW	0.36	0.41	0.27	-0.03	0.01	0.18	-0.03	0.21	-0.68
HI	0.11	0.06	0.21	0.49	0.39	0.42	0.02	-0.38	0.66

3.3.8 Associations between heat responses of traits

To examine the relationships between the heat responses of different traits, PCA and pairwise correlation tests were performed for those traits that showed significant T or G x T effects (Table 3.6; Figure 3.9c). In the PCA, the first and second principal components (PCs) explained 71% of the total variance. PC1 was mainly explained by responses for SGW, ChlC13-16DAA, and AUSC, while PC2 was mainly explained by responses for GWS, GFD, and FLSe (Figure 3.9c; Table 3.5). ShW and HI mainly contributed to PC3, which explained 16% of the total variance. Heat responses of indicators of flag leaf chlorophyll retention (ChlC13-16 DAA, AUSC and FLSe) showed strong positive correlations with one another (Table 3.6; Figure 3.9c), indicating that the patterns of these heat responses across the genotypes tended to be similar. Heat responses of GWS and SGW were also positively correlated with the responses of these flag leaf chlorophyll retention traits. In other words, the more tolerant genotypes which were better able to maintain single grain weight under heat

(relative to control) also tended to maintain flag leaf chlorophyll content under heat. This relationship in response ratios also reflects the fact that GWS and SGW *per se* were negatively associated with ChlC13-16 DAA, AUSC and FLSe under control conditions, and that these traits were not associated under heat conditions (Table 3.4).

Table 3. 6 Genotypic correlations between response ratios of traits (Mean trait value$_{\text{Heat treatment}}$/Mean trait value$_{\text{Control}}$) that showed significant treatment or genotype-by-treatment effects. Spikelet number spike^{-1} (SpNS) showed a significant genotype-by-treatment effect but was not included since this trait is determined pre-anthesis, before the treatment period. GWS, grain weight spike^{-1}; SGW, single grain weight; GFD, grain-filling duration; ChlC13-16DAA, chlorophyll content 13-16 days after anthesis (corresponding to first measurement after treatment in heat treated plants; normalized for the 1st SPAD measurement); AUSC, area under SPAD curve (SPAD data were normalized for the first measurement); FLSe, days from anthesis to 95% flag leaf senescence; ShW, shoot dry weight; HI, harvest index.

Trait	GWS	SGW	GFD	ChlC13-16DAA	AUSC	FLSe	ShW	HI
GWS	-							
SGW	0.71***	-						
GFD	0.22	0.28	-					
ChlC13-16DAA	0.51**	0.63***	0.59***	-				
AUSC	0.57***	0.60***	0.67***	0.94***	-			
FLSe	0.35*	0.27	0.67***	0.65***	0.68***	-		
ShW	0.62***	0.48**	0.10	0.47**	0.49**	0.21	-	
HI	0.63***	0.48**	0.20	0.19	0.24	0.22	-0.20	-

Values are Pearson correlation coefficients, with significance levels indicated by asterisks: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

3.3.9 Relationships between trait potentials and heat responses of traits

Correlations between the potentials of traits (under control conditions) and the heat responses of traits were also examined (Table 3.7). GWS and SGW responses showed significant negative correlations with the values of the same traits under control conditions (Table 3.3). In other words, genotypes with larger grains under non-stress conditions tended to lose a greater proportion of their grain weight due to heat stress. Heat responses of ChlC13-16DAA and AUSC were positively correlated with their trait potentials, indicating that genotypes that normally had a smaller senescence rates also lost their chlorophyll more slowly upon heat exposure. GWS and SGW responses tended to show positive associations with ChlC13-16DAA, AUSC, FLSe and GFD but negative associations with CL, ShW and DTA (Table 3.7). That is, the heat tolerant genotypes (smaller SGW and GWS responses) tended in the absence of heat stress to have slower senescence rates, be shorter and have less vegetative biomass and to flower earlier, than the less tolerant genotypes.

Table 3. 7 Association between trait potentials (value under control conditions) and response ratios of traits (Mean trait value$_{\text{Heat treatment}}$ / Mean trait value$_{\text{Control}}$). Trait potentials and response ratios are listed on horizontal and vertical axes, respectively. DTA, days from sowing to anthesis; GWS, grain weight spike^{-1}; SGW, single grain weight; GNS, grain number spike^{-1}; GNSp, grain number spikelet^{-1}; GFD, grain-filling duration; ChlC7-10DAA, chlorophyll content 7-10 days after anthesis (corresponding to the measurement before treatment in heat treated plants); ChlC13-16DAA, chlorophyll content 13-16 days after anthesis (corresponding to first measurement after treatment in heat treated plants; normalized for the 1st SPAD measurement); AUSC, area under SPAD curve (SPAD data were normalized for the first measurement); FLSe, days from anthesis to 95% flag leaf senescence; CL, culm length; ShW, shoot dry weight; HI, harvest index.

Trait	DTA	GWS	SGW	GNS	GNSp	GFD	ChlC7-10DAA	ChlC13-16DAA	AUSC	FLSe	CL	ShW	HI
GWS	-0.34*	-0.35*	-0.40*	-0.19	-0.16	0.25	-0.09	0.28	0.48**	0.25	-0.51**	-0.32	-0.01
SGW	-0.35*	-0.26	-0.55***	-0.01	0.06	0.39*	-0.28	0.34*	0.52**	0.33*	-0.40*	-0.33*	0.13
GFD	0.06	0.21	0.17	0.17	0.12	-0.07	-0.16	0.20	0.16	0.05	-0.04	0.22	0.19
ChlC13-16DAA	-0.16	0.03	-0.14	0.14	0.23	0.16	0.02	0.40*	0.42*	0.14	-0.16	-0.05	0.29
AUSC	-0.12	0.01	-0.04	0.07	0.12	0.14	0.00	0.33*	0.34*	0.16	-0.17	-0.02	0.21
FLSe	0.09	0.29	0.20	0.29	0.37*	-0.01	0.00	0.52**	0.26	-0.05	-0.04	0.27	0.34*
ShW	-0.11	0.02	-0.08	0.09	0.17	0.10	0.21	0.12	0.14	0.00	-0.03	-0.13	0.32
HI	-0.33*	-0.41*	-0.44**	-0.26	-0.25	0.25	-0.37*	0.27	0.46**	0.34*	-0.63***	-0.28	-0.25

Values are Pearson correlation coefficients, with significance levels indicated by asterisks: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

3.3.10 Relationship to *Rht* genes (*Rht-B1* and *Rht-D1*)

Initially, we noticed that the tall varieties Cadoux and Reeves (lacking a GA-insensitive mutation at either *Rht-B1* or *Rht-D1*) were particularly susceptible to heat-induced chlorophyll loss and SGW reduction. Similarly, the tall genotypes W7985 Synthetic, Sokoll and Lyallpur-73 were also among the most intolerant for SGW reduction. To explore the relationship between *Rht* genes and heat susceptibility further, we scored all genotypes with *Rht-B1* and *Rht-D1* diagnostic markers (Table 3.8). All of the genotypes were semi-dwarfs (mutation in either *Rht-B1* or *Rht-D1*), except Cadoux, Halberd, Katepwa and Reeves, which were tall genotypes having wild-type versions of both *Rht-B1* and *Rht-D1*.

Table 3. 8 *Rht-B1* and *Rht-D1* alleles carried by genotypes according to analysis with diagnostic KASP markers and information obtained from other researchers (Karen Cane, DPI-Vic Horsham, Howard Eagles, The University of Adelaide, and Melissa Garcia, ACPFG, The University of Adelaide).

Genotype	*Rht-B1*	*Rht-D1*	Comment
Axe	a*	b*	
Berkut	a/b	b/a	mixture at both loci¥
Cadoux	a	a	
CD87	b	a	
Correll	a	b	
Cranbrook	b	a	
Crusader	b	a	
Drysdale	a	b	
EGA Blanco	b	a	
EGA Bonnie Rock	b	a	
Egret	b	a	
Frame	a	b	
Halberd	a	a	
Hartog	a	b	
Janz	b	a	
Katepwa	a	a	
King Rock	b	a	
Krichauff	b	a	
Kukri	a	b	
Lincoln	a	b	
Lyallpur-73	a	b	
Millewa	b	a	
Molineux	a/b	b/a	mixture at both loci
Opata 85	b	a	
Reeves	a	a	
Sokoll	b	a	
Sunco	b	a	
Sunstar	b	a	
Tammarin Rock	b	a	
Tasman	a/b	b/a	mixture at both loci
Trident	a/b	b/a	mixture at both loci
W7985 Synthetic	b	a	
Waagan	b	a	
Westonia	a	b	
Wyalkatchem	a	b	
Young	b	a	

* a and b represent the wild type and dwarfing alleles, respectively.
¥ individual plants were either aabb or bbaa genotype.

3.4 Discussion

In the present study, genotypes varied from showing no response to losing more than 27 and 60% of their SGW and chlorophyll content in response to a brief episode of heat stress, respectively. These results suggest a marked effect of brief episodes of high temperatures on wheat performance and also a considerable scope for yield improvement under high

temperature conditions, which is in accordance with earlier studies (Stone and Nicolas 1994; Stone and Nicolas 1995b; Wardlaw et al. 1989b).

Pruning plants back to the single main culm was a procedure used in some previous heat tolerance studies (Tashiro and Wardlaw 1990b; Wardlaw et al. 1989b). This approach helps to avoid water stress and reduces variation in light penetration to the lower leaves (Tashiro and Wardlaw 1990b; Wardlaw et al. 1989b), along with enabling easier management of the experiment (e.g. disease and pest management). Moreover, in an experiment with and without pruning in our laboratory (data not shown) pruned and non-pruned plants showed similar genotype rankings for tolerance in the main tiller. Therefore, in the current study and the other experiments at the grain-filling stage that reported in following chapters, plants were pruned back to main culm.

Heat stress at around meiosis leads to floret sterility while heat within the first three days after pollination can lead to early abortion of grain growth (Saini and Aspinall 1982b; Saini et al. 1983; Tashiro and Wardlaw 1990a; Wardlaw et al. 1989b). Florets on the wheat spike develop asynchronously, with development proceeding from the base upwards on each spikelet, and from the middle outwards on each spike (Percival 1921). Genotypes with larger spikes (i.e. more spikelets and grains spikelet^{-1}) may be expected to have less synchronous floret development and hence, with the heat treatment applied at 10 days after the appearance of the first extruded anthers, may be expected to have more florets that were heat-treated much earlier than 10 DAA, compared to genotypes with smaller spikes. However, there was no significant relationship between SGW response and GNS or GNSp potentials (traits value under control conditions; Table 3.7), nor were there any significant overall effects of the heat treatment on GNS and GNSp (Table 3.3), indicating that anthesis, fertilization and establishment of grain growth was completed in all or the vast majority of the florets of the assayed spikes by the time of the heat treatment. This is also in accordance with the findings of other studies where heat was applied at ≥10 DAA (Bhullar and Jenner 1985; Stone and Nicolas 1995b; Tashiro and Wardlaw 1990a; Tashiro and Wardlaw 1990b).

High temperatures during grain-filling affect both rate (may increase or decrease) and duration (decrease) of grain-filling, depending on stress intensity and genotype, resulting in a net decrease in final SGW (Hunt et al. 1991; Sofield et al. 1977; Stone and Nicolas 1995a; Zahedi and Jenner 2003). Genetic variation has been reported for both grain-filling rate and duration among wheat genotypes in response to elevated temperatures. Several studies reported that genotypic differences for grain weight response under high temperatures during grain-filling (30 ºC) were largely explained by difference in grain-filling rate rather than

grain-filling duration (Hunt et al. 1991; Sofield et al. 1977; Wardlaw and Moncur 1995; Zahedi and Jenner 2003; Zahedi et al. 2003). However, Stone and Nicolas (1995a) observed a significant difference between two genotypes differing in heat tolerance, for both grain-filling rate and duration in response to a brief severe heat stress (40 ºC for 5 days at different stages of grain-filling) and a stronger association of tolerance with response of grain-filling duration than response of grain-filling rate. Recently, Talukder et al. (2013) found a significant difference among bread wheat genotypes for grain growth rate response to a single day of severe heat stress (35 ºC, at 7-10 DAA) in both field and control environments. As done by others (Mason et al. 2010; Stone and Nicolas 1995b; Yang et al. 2002b), we defined the end of grainfill as the point at which the spike became ~ 95% senesced and seeds became hard. The times from anthesis to this point (GFD trait) were shortened by the heat treatment by an average of 13%, but this response did not show any significant G x T interaction or a significant association with SGW or GWS responses. This could mean that differences in tolerance (SGW response) were driven primarily by differences in the responsiveness of grainfill rate, rather than duration. On the other hand, it may also reflect the difficulty of measuring grainfill duration accurately by this subjective method. Establishing which factor is the more important in determining tolerance variation will require destructive sampling of grains over time for dry weight determination, in a selection of tolerant and intolerant genotypes.

Senescence is a genetically determined phenomenon which interacts with environmental factors such as biotic and abiotic stresses and results in chlorophyll loss, reduced photosynthesis, and remobilization of reserves to younger or reproductive parts of the plant (Vijayalakshmi et al. 2010). Assimilates derived from current photosynthesis and mobilized stem reserves both contribute to grain growth, but their relative contribution depends on the environment (Blum 1997; Hossain et al. 1990). Although stem reserves contribute less under non-stressed conditions to the grain growth, under stressed conditions it can make a major contribution to grain growth, depending on the genotype (Blum 1998; Yang et al. 2002a).

Delayed senescence can reduce yield, by hindering remobilization of stored reserves to the reproductive parts of the plant. Heavy application of nitrogen fertilizer can be one cause of delayed senescence associated with reduced mobilization to the grain and reduced yield (Yang et al. 2000; Yang and Zhang 2006). An alternative explanation for adverse effects of stay-green is prolonged consumption of glucose for continued nitrogen assimilation and protein synthesis by green leaves and grains, which can deprive the grains of assimilate for grain-filling (starch synthesis) (De Vries et al. 1974; Hirel et al. 2007; Kipp et al. 2014). In

control plants in the current study, FLSe and AUSC showed significant negative relationships with GWS and SGW (Table 3.1), indicating there may have been a yield penalty for stay-green genotypes. The control plants were well watered and fertilized and not subjected to heat stress – conditions that may have resulted in slower than optimal rate of senescence (for grain yield) in some of the genotypes.

Accelerated senescence caused by biotic or abiotic stress can have both positive and negative consequences for crop yield. It can help yield by increasing remobilization of stem reserves to the grain during late grain-filling, but it can also reduce the capacity for late generation of assimilates that normally contribute significantly to grain yield particularly in wheat (Lopes and Reynolds 2012; Rosyara et al. 2010a; Yang et al. 2000; Yang and Zhang 2006). It is reasonable to use chlorophyll content (SPAD readings) to infer declining photosynthetic capacity under terminal stress because a strong association has been observed between SPAD readings and PS II efficiency and maximum net photosynthetic rate under high temperature conditions (Gutiérrez-Rodriguez et al. 2000; Ristic et al. 2007; Ristic et al. 2008). In the current study, stay-green (SPAD based traits) was positively associated with grain yield (GWS and SGW) under heat conditions (Table 3.4). Moreover, chlorophyll content responses to heat during grain-filling were significantly positively associated with the GWS and SGW responses (Table 3.6) – suggesting that under these conditions, genotypes that responded with less chlorophyll loss under heat were also better able to maintain grain weight. Those genotypes were likely to have been able to better maintain photosynthetic competence under heat, which could have contributed positively to grain-filling. An advantage of stay-green in wheat under biotic and abiotic stress conditions has been reported in earlier studies (Lopes and Reynolds 2012; Reynolds et al. 1994; Reynolds et al. 2000; Rosyara et al. 2009; Rosyara et al. 2010b). Alternatively, stay-green and grain-filling may have been independently affected by heat stress, rather than being directly related by cause-effect. Nevertheless, this information suggests that a portable SPAD chlorophyll meter may provide an easy and inexpensive tool to indirectly select stress tolerant varieties and stay-green trait may relate to a better performance under heat stress conditions.

A few of the genotypes did not conform well to the overall relationship between stay-green and heat tolerance for grain weight, suggesting that there may have been other factors at play. For example, Axe and W7985-Synthetic were relatively heat tolerant for chlorophyll retention but heat susceptible for SGW. Such genotypes may represent cases of ‘cosmetic’ stay-green, where photosynthetically inactivated chlorophyll is allowed to be retained, due to damaged chlorophyll catabolism (Kumar et al. 2010; Thomas and Howarth 2000). High temperatures

can also restrict sucrose conversion to starch in the developing wheat grain by affecting several enzyme activities of the starch synthesis pathway (Hawker and Jenner 1993), particularly soluble starch synthase (SSS) (Hawker and Jenner 1993; Jenner and Hawker 1993; Jenner 1994). The adverse effect of high temperature on SSS can continue for some time after temperatures are returned to normal (Jenner 1991b). Thus, Axe and W7985-Synthetic may have had forms of SSS that were particularly heat-sensitive, less abundant, or less able to recover, preventing these genotypes from being able to convert carbohydrates afforded by the stay-green trait from being efficiently converted to grain mass (starch synthesis).

King Rock, Millewa, and Sunco were relatively poor for stay-green but good for SGW maintenance under heat. It is possible that these genotypes had particularly high levels of stem reserves and efficient mechanisms of carbon remobilization, which buffered them against the disadvantage afforded by their low stay-green. Verifying stay-green-independent mechanisms of heat tolerance in these 'outlying' genotypes will require genetic mapping of stay-green and tolerance traits in populations derived from these parents.

There were correlations between some of the trait responses and trait potentials (absolute trait values under control conditions; Table 3.7). Genotypes with larger GWS and SGW in the control showed stronger responses of these traits to the heat treatment, i.e. less tolerance. In another experiment performed in our laboratory using a larger wheat genotype panel (60 genotypes; data not shown) the same trend was observed. Yang et al. (2002a) also reported a positive association between heat susceptibility index and yield potential which indicated a stronger response for genotypes with larger yield potential. Grain number per unit area as well as SGW contributes to yield, so larger grained varieties do not always yield more than smaller grained varieties. However, larger SGW generally contributes positively to processing quality, as it is correlated with lower % screenings and higher % flour extraction (milling yield). Whether selection of small grains provides a sensible strategy for increasing heat tolerance would therefore depend on the frequency of shock events in the target environment, whether its effect on yield could be compensated for by increased grain number, and whether the minimum required quality attributes (for low % screenings and high flour extraction) could still be met, under stress and/or non-stress conditions.

DTA also positively correlated with GWS and SGW under non-stressed conditions, and therefore negatively correlated with tolerance (smaller SGW and GWS responses). In contrast to grain weight effects, chlorophyll loss during heat treatment (ChlC13-16DAA response), and AUSC response to heat were positively correlated with their potentials ($p < 0.05$),

indicating that genotypes with higher senescence rate potential tended to experience higher acceleration of senescence in response to heat (Table 3.7). This tends to suggests that heat mainly affected senescence by accelerating senescence processes that occurred under non-stress conditions, rather than by causing damage that was heat-specific in nature. Importantly, starting chlorophyll content was not related to the rate of chlorophyll loss under control or heat (Table 3.4 and 3.7), indicating that processes determining chlorophyll *per se* at this developmental stage were independent to those determining rate of chlorophyll loss under control or heat. As expected from the aforementioned relationships, tolerance (smaller SGW response) was also positively correlated with smaller senescence rate potential (higher AUSC under control conditions). Curiously, under control conditions, SGW was negatively correlated with GFD, which implies that shorter GFD was associated with (and over-ridden by) higher grain-filling rate. On the other hand, SGW response (tolerance) was positively associated with GFD response and GFD potential (GFD under control conditions; Tables 3.6 and 3.7). Genotypes with a shorter GFD and higher grain-filling rate under control conditions therefore tended to respond more to heat.

Tolerance (smaller SGW response) was also negatively associated with potential plant size (CL and ShW under control conditions; $p < 0.05$). That is, smaller genotypes (with shorter CL and smaller ShW) tended to be more tolerant. *Rht-B1* and *Rht-D1* are the main loci affecting plant height and size in wheat and the wild-type (tall) alleles act via gibberellic acid (GA) signalling (Blum and Sullivan 1997; Gale et al. 1985). According to our marker analysis and cross checks with information from other researchers (Table 3.8) Cadoux, Halberd, Katepwa and Reeves were the only 'double tall' genotypes in this study (carrying *Rht-B1a* and *Rht-D1a* wild-type alleles; Table 3.8). On average, these 'double tall' genotypes showed 11 and 35% larger SGW and AUSC response than the semi-dwarfs. W7985-Synthetic, Sokoll and Lyallpur-73 were the tallest, although they each contained an *Rht* dwarfing allele, and they were also among the most intolerant for grainfill. This may suggest that intolerance was favoured by a tall stature and/or greater vegetative mass, rather than by GA-insensitive *Rht* alleles *per se*. However, these ideas should be treated with caution. The pedigrees of the four double-tall varieties include some common varieties and Cadoux and Reeves are particularly closely related (not shown), increasing the chances that separate genes for dwarfing and heat susceptibility occurred together in the sampled genotypes. Some of the semi-dwarf genotypes such as Crusader, Cranbrook, Frame and Lyallpur-73 were also quite susceptible.

There are contradictory reports linking GA-insensitive dwarfing alleles to reduced abiotic stress tolerance. Law et al. (1981) and Law and Worland (1985) observed a marked sensitivity

to heat and/or drought stress at booting stage in genotypes carrying GA-insensitive alleles (*Rht-B1b*, *Rht-D1b* and *Rht-B1c*). Smaller plants (carrying dwarfing allele *Rht-B1b*, *Rht-D1b* or *Rht-B1c*) appeared to be more tolerant to top-root drying in terms of reduction in tillering, plant height, shoot and root biomass (Blum and Sullivan 1997). Plants with smaller size (carrying dwarfing gene/s) and smaller growth rate potentials showed higher tolerance to water deficit and heat and lower response to ABA, measured as the plant growth rate response in seedlings (Blum et al. 1997). Alghabari et al. (2014) concluded that GA-insensitivity does not necessarily confer higher sensitivity to heat and/or drought stress at booting and anthesis developmental stages. Butler et al. (2005) reported a grain yield and grain weight advantage of tall lines, in comparison with semi-dwarf lines, under stress conditions. Yield benefits associated with dwarfing genes can be affected by various factors such as growth habit (spring vs. winter), genetic background, environmental factors, etc (Alghabari et al. 2014; Bush and Evans 1988) which may at least partly explain the conflict between the findings of the aforementioned studies, and between some of these and the current study.

Overall, the association of tolerance (smaller SGW response) with slower rates of senescence and grain-filling and smaller plant size (shorter CL and smaller ShW) suggests a penalty for genotypes with higher trait potentials and bigger plant size. This may indicate a hindrance for breeding varieties that will be high yielding in both non-stressed and heat-stressed environments. The observed associations between traits such as plant size, flowering time, grain yield spike^{-1}, SGW, flag leaf senescence, and tolerance (smaller SGW response) might be due to control of these traits by common gene(s). On the other hand, some of these associations could have merely reflected chance association of genes within the small sample of genotypes. Distinguishing these possibilities will require QTL mapping of the traits and responses.

Among 18 pairs of parents of mapping populations Young and Wyalkatchem, Janz and Frame, Sunco and Tasman, Drysdale and Waagan, and Sokoll and Krichauff showed the highest level of contrast (ranging from ~11 to 19%) for SGW response (Appendix 3.2a). These pairs of parents also showed a considerable level of contrast for stay-green (except Sokoll and Krichauff; Appendix 3.2b). These populations may be suitable for genetic analysis of heat tolerance/susceptibility. In subsequent chapters of this thesis, the Drysdale/Waagan population was chosen for genetic study of heat tolerance, for a number of additional reasons which are listed in Chapter 5. Targeted crosses could be attempted between some of the most tolerant and the most intolerant genotypes (Appendix 3.2) to produce new populations for heat tolerance studies.

3.5 Concluding remarks

The present work demonstrates significant variation among wheat genotypes for stay-green (maintenance of source activity) under a brief episode of severe high temperatures and a positive association between chlorophyll loss response and both SGW and GWS under these conditions. Although SGW response should be considered to be the key element in wheat breeding programs for heat tolerance after anthesis, the stay-green trait might provide a useful way of indirectly selecting tolerance. Moreover, a portable SPAD chlorophyll meter provides an easy, inexpensive, and effective way to evaluate stay-green in wheat. It is not immediately apparent whether low trait potentials and early flowering are necessary for tolerance to brief episodes of heat stress. However, results of this study suggest that more than one adaptation process may be required for environments with terminal heat stress, and that there may be a conflict in breeding for yield under non-stressed and heat-stressed environments. QTL mapping of the traits and responses will help to distinguish these possibilities. The Drysdale/Waagan mapping population, studied in subsequent chapters, may provide a means of addressing some of these issues.

Chapter 4: Effects of a brief episode of post-anthesis heat stress on grain growth, chlorophyll loss and stem water soluble carbohydrates in wheat (*Triticum aestivum* L.)

4.1 Introduction

Brief episodes of heat during grain-filling are common in many wheat growing regions (Asseng et al. 2011). In Australia, most of the wheat crops experience a few very hot days (≥4 days of >34 ºC) during grain-filling (Stone and Nicolas 1994; Wardlaw and Wrigley 1994). The frequency of such events is likely to increase as a consequence of climate change (Pittock 2003). As wheat has a low optimum temperature (15 ºC) for maximum grain growth (Chowdhury and Wardlaw 1978), temperatures above 34 °C can significantly reduce its yield as demonstrated in previous studies using controlled environment experiments (Stone and Nicolas 1994; Stone and Nicolas 1995b), field trials (Talukder et al. 2013) and simulation modelling (Asseng et al. 2011).

Grain yield reduction in response to heat stress >10 days after anthesis (DAA) mainly derives from single grain weight reduction (Bhullar and Jenner 1985; Stone and Nicolas 1995a; Tashiro and Wardlaw 1990b; Wardlaw and Wrigley 1994) rather than grain set reduction (Tashiro and Wardlaw 1990a). Grain growth has two components, namely grain-filling rate and duration, in which both are independently affected by high temperature (Jenner 1994; Zahedi and Jenner 2003). High temperature during grain-filling accelerates the grain-filling rate, but also truncates the grain-filling period (Stone and Nicolas 1995a; Tashiro and Wardlaw 1989; Zahedi and Jenner 2003). The latter shortens the assimilate utilization time for yield formation. The former (rate) may not increase enough to fully compensate for the latter (duration), explaining why the overall effect is a reduction of grain weight (Jenner, 1994). It has been shown that even a short period (1-7 days) of high temperature (35 °C) can significantly reduce grain growth (Jenner 1991a; Jenner 1991b; Jenner 1994; Talukder et al. 2013) likely due to its adverse effect on several enzymes in the starch synthesis pathway, in particular soluble starch synthase, which leads to a reduced starch accumulation rate (Bhullar and Jenner 1985). Genetic variation among wheat genotypes has been reported for the high temperature responses of both grain-filling rate and duration (Hunt et al. 1991; Sofield et al. 1977; Stone and Nicolas 1995a; Talukder et al. 2013; Wardlaw and Moncur 1995; Zahedi and Jenner 2003; Zahedi et al. 2003).

Studying grain growth characteristics is not straightforward since there is no non-invasive method available for its evaluation. Grain sampling over time and application of various mathematical models such as linear regression, logistic, Gompertz and polynomial curves

have been used to evaluate grain growth characteristics in wheat (Bruckner and Frohberg 1987; Darroch and Baker 1990; Gebeyehou et al. 1982; Loss et al. 1989; Zahedi and Jenner 2003). Grain-filling data analysis using mathematical growth models can shed further light in understanding high temperature effects on grain growth dynamics and the relative contribution of responses of different grain growth parameters to heat tolerance in wheat. Here, an ordinary logistic model and linear regression, described in previous works (Loss et al. 1989; Zahedi and Jenner 2003), were used to study grain growth.

Grain-filling relies on two major carbon sources - assimilate supplied by photosynthesis, from leaves and to a lesser extent from spikes, and mobilization of water-soluble carbohydrates previously stored in leaves, spike and stem tissue (Blum et al. 1994). However, the relative contribution of these sources of carbon to final grain mass can change depending on environment and genotype. Under high temperature, demands for assimilates increase as the result of accelerated grain growth and increased respiration. However, under these conditions, photosynthesis declines due to the adverse effects of high temperature on various aspects of the photosynthesis machinery (Farooq et al. 2011; Peck and McDonald 2010; Ristic et al. 2007; Wahid et al. 2007). Thus, disturbed photosynthesis may not be able to fully meet the demands of increased rates of grain growth and respiration. However, variation among wheat genotypes has been reported for photosynthesis and photosynthesis related traits (e.g. chlorophyll content and chlorophyll fluorescence) under high temperature conditions (Al-Khatib and Paulsen 1990; Kumari et al. 2007; Lopes and Reynolds 2012; Moffatt et al. 1990; Reynolds et al. 1994; Sharma et al. 2012). WSC reserves can play an important role in supporting grain growth under stress conditions when the availability of the current photoassimilates declines as a result of disturbed photosynthesis (Blum et al. 1994; Yang et al. 2002a). WSCs in wheat mainly consist of fructan (fructosyl-oligosaccharides) and to a lesser extent sucrose, glucose and fructose (Wardlaw and Willenbrink 1994). Under optimum conditions WSCs have been estimated to contribute to between 10 to 20% of final grain weight (Austin et al. 1977; Borrell et al. 1993; Wardlaw and Porter 1967), while under stress conditions (e.g. low light, heat and drought) its contribution to grain growth may reach 100% depending on the genotype and the stress magnitude, which can significantly buffer grain growth against stress (Blum 1998 and references cited therein). Thus, genotypes with high levels of WSCs and the ability to utilize that are expected to better withstand the stress and maintain grain weight.

In Chapter 3, 36 bread wheat genotypes were screened for their responses to a brief episode of heat stress at an early grain-filling stage. Genotypes varied significantly for single

grain weight (SGW) and chlorophyll loss responses. In that experiment, samples were collected at maturity and flag leaf chlorophyll content was monitored using a SPAD chlorophyll meter, so the effect of heat on grain growth components and different types of chlorophyll could not be quantified. Moreover, a general correlation between stay-green in the flag leaf and the ability to maintain SGW was observed among the 36 studied genotypes, but there were some genotypes that diverged from this general trend. For instance, Millewa and Sunco showed a considerable response in chlorophyll content but not in SGW. The ability of these genotypes to maintain SGW despite a considerable chlorophyll loss might be due to larger WSC mobilisation efficiency under stress conditions. In general, effects of brief episodes of very high temperatures have not been studied as much as those caused by chronic heat (Talukder et al. 2013). Therefore, the present study was conducted to investigate the effects of a brief episode of very high temperatures (37 ºC, 3days), at early stage of grain-filling (10 days after anthesis), on grain growth components, chlorophyll *a* and *b*, chlorophyll fluorescence and stem water-soluble carbohydrates, and to quantify correlations between heat responses of these traits and the final grain weight response, in Millewa, Sunco, and seven other genotypes. This was intended to give further insights into the physiological bases of tolerance of grain-filling to brief episodes of very high temperatures in various genotypes, and to identify traits that may be useful for selecting heat tolerance in breeding programs and for conducting genetic studies of heat tolerance.

4.2 Materials and methods

Two experiments were conducted concurrently in the same environment to study effects of a brief heat stress at grain-filling stage on grain growth, chlorophyll loss, chlorophyll fluorescence and stem water-soluble carbohydrates. In the first experiment, chlorophyll fluorescence was measured non-destructively during and shortly after the heat treatment and plants were harvested at maturity. In the second experiment, grain growth, chlorophyll *a* and *b*, total chlorophyll content, and water-soluble carbohydrates were measured using destructive methods starting 10 DAA and concluding 58 DAA as described in following sections.

4.2.1 Plant material

Nine bread wheat varieties (Drysdale, Frame, Gladius, Lyallpur-73, Millewa, Reeves, Sunco, Waagan, and Young) were used. These varieties differed in their responses (single grain weight and chlorophyll loss) to a brief heat stress at grain-filling stage in other experiments (Chapter 3 and Appendix 6.1). Gladius, Millewa, Sunco, Waagan and Young were relatively heat tolerant while Drysdale, Frame, Lyallpur-73 and Reeves were heat susceptible. Lyallpur-73 has been also reported to be heat susceptible in other studies

(Wardlaw et al. 1989a; Wardlaw et al. 2002). Drysdale & Waagan, and Gladius & Drysdale are pairs of parents of available mapping populations and were included to evaluate potential of those populations for genetic studies of heat tolerance related traits. Seeds were obtained from various sources as described in Chapter 3.

4.2.2 Experimental design, plant growth and heat stress conditions

The experiments were set up in a split-plot (Experiment 1, 6 blocks) and split-split-plot (Experiment 2, 4 blocks) designs. In Experiment 1 each block was split into 9 main plots (genotypes) and 2 sub-plots (control vs. heat) and in Experiment 2 each block was split into 7 main plots (Time of sampling), 9 sub-plots (genotypes) and 2 sub-sub-plots (control vs. heat). The first sampling time in Experiment 2 (10 DAA) was before the treatment, and the same set of plants (four plants per genotype) was used to define 'control' and 'heat' values at this time-point. Plant growth and heat stress conditions were similar to those described in Chapter 3. In brief, seed were sown early August 2013 and plants grown in a naturally lit greenhouse (The Australian Plant Accelerator, University of Adelaide, Waite Campus, Adelaide). Plants were pruned back to the single main culm by removing tillers as they appeared. Measured greenhouse conditions were approximately 20/17 ºC, 14/10 h day/night (Table 4.1). For each plant, anthesis date was recorded, and each plant destined for heat treatment in the experimental design, was transferred to a growth chamber (BDW120, Conviron) at 10 days after its anthesis date (days after anthesis, DAA), where they were exposed to a brief heat stress (37/27 ºC day/night temperature for 3 days), before being returned to the greenhouse. Pots were placed in trays of water to ~ 2-cm depth while in the chamber to minimize drought stress.

Table 4. 1 Measured temperatures (ºC) in the greenhouse. Anthesis and maturity occurred during October and November-December, respectively.

Conditions/Month	August	September	October	November	December
Average day temperature	21.3	20	19.7	20.1	21.2
Average night temperature	17.9	16.5	16.1	16.4	16.9
Average minimum temperature	16.9	15.6	15.2	15.3	15.8
Average maximum temperature	23.4	22.7	22.3	22.6	23.9
Minimum temperature	14.7	14.8	14.5	14.7	14.4
Maximum temperature	25.1	27.8	28.1	25.7	29.7
Days > 30ºC	00.0	00.0	00.0	00.0	00.0

4.2.3 Data collection

Chlorophyll fluorescence: The maximum quantum efficiency of photosystem II (PSII; *F*v/*F*m) was monitored using a portable chlorophyll fluorometer MINI-PAM (Walz, Effeltrich, Germany). Measurements were taken on the left hand side of the flag leaves between the mid-rib and leaf margin, halfway between the base and the tip, after dark-adapting of the sampled leaf segment for 30 minutes. Measurements were taken at around

midday, on a daily basis, from 10 to 15 DAA. This trait was not measured in the variety Frame because it was the latest flowering variety, and the MINI-PAM was not available at the right time.

Chlorophyll *a, b* (Chl*a* and Chl*b*) and total chlorophyll content (TotChl): Total chlorophyll pigments were extracted using the dimethyl sulfoxide (DMSO) method described by Hiscox & Israelstam (Hiscox and Israelstam 1979). This method was chosen mainly because of its speed (no grinding and centrifuge steps are required) and the higher stability of chlorophyll pigments in DMSO compare to other solvents such as acetone and ethanol (Richardson et al. 2002). In brief, leaf samples (~100 mg FW) were collected from the flag leaves at 10, 13, 23, 33, 43 and 53 DAA, from the same plants as were used in the grain growth study. The collected samples were transferred to the lab and placed in glass centrifuge vials containing 7 ml DMSO. Then, samples were heated at 65 ºC for ~1 h in a water bath to extract the total chlorophyll pigments. Samples were then removed from the water bath and the content of each vial topped up to 10 ml with DMSO. Samples were left at room temperature to cool down. Then 1 ml of each extract was transferred to a disposable polystyrene cuvette and the absorbance of each sample measured at 645 and 663 nm using a Shimadzu UV-VIS spectrophotometer (model UV-160A). The concentrations of chlorophyll *a*, *b* and total chlorophyll content was estimated from these absorbances according to Arnon's equations (Arnon 1949). The concentrations of Chl*a*, Chl*b* and TotChl was presented over time and also as an average across all harvest times (Chl$a_{av.}$, Chl$b_{av.}$ and TotChl$_{av.}$).

Grain growth: Grain samples were collected at 10 DAA (corresponding to directly before treatment), 13 DAA (corresponding to directly after treatment), and at 5-day intervals thereafter up to 58 DAA. At each date, 10 grains spike^{-1} were collected from the two basal floret positions of spikelets located in the middle of the spike to minimize variation due to floret position. At 10, 13, and 58 DAA each plant was sampled at only one time point, taking 5 grains from each side of the spike from the central spikelets. For 18 to 53 DAA, individual plants were used for consecutive pairs of sampling time points due to limitations of growth space (i.e. samplings at 18 & 23, 28 & 33, 38 & 43, and 48 & 53 DAA), taking 10 grains from one and then the other side of the spike at the first and the second time point, respectively. The collected grains were oven-dried for 3 days at 85 ºC before being weighed.

Measurements of grain biomass over time were subjected to the following logistic equation, to estimate grain growth characteristics, where *W(t)* is single grain weight (mg) at time t (day) after anthesis, c estimates the theoretical final single grain weight (FGW; mg), b is the slope parameter which controls the steepness of the curve, m (day) is the time from

anthesis to the inflection point (point of maximum grain growth rate, MGR), and *e* is Napier's number (mathematical constant with an approximate value of 2.718281828).

$$W(t) = \frac{c}{1+e^{(-b(t-m))}}$$

MGR and grain-filling duration (GFD) was obtained using the following equations, as described by Zahedi et al. (2003).

$$\text{MGR (mg day}^{-1}) = \frac{bc}{4}$$

$$\text{GFD (days)} = \frac{bm+2.944}{b}$$

To estimate sustained grain growth rate (SGR), data during the linear phase of grain growth was subjected to linear regression analysis as described in previous studies (Loss et al. 1989; Zahedi and Jenner 2003).

Water soluble carbohydrates (WSC) measurements: Stem samples (including leaf sheaths), were collected at 10, 13, 23, 33, 43 and 53 DAA, from the same plants as were used in the grain growth study. At each date, stems were cut at the soil surface, the leaf blades and the spike removed, and the stem divided into three segments: peduncle (the first internode below the spike), penultimate internode (the internode below the peduncle) and the lower internodes (remaining portion). Samples were then snap frozen in liquid nitrogen and freeze-dried for ~ 20 h. Each segment was then weighed, chopped into ~5 mm segments, placed in a 10 ml Falcon tube containing two 5 mm ball bearings and reduced to a fine powder using a Geno/Grinder® high-throughput plant & tissue homogenizer (SPEX SamplePrep, New Jersey, USA). Then samples were scanned using a platinum diamond attenuated total reflectance (ATR) single reflection sampling module cell mounted in a Bruker Alpha instrument (Bruker Optics GmbH, Ettlingen, Germany), using air for normalization. The attenuated total reflectance mid infrared (ATR-MIR) spectra were recorded on OPUS software version 7.0 provided by Bruker Optics and then exported to Unscrambler X software (version 10.1, CAMO ASA, Oslo, Norway) for analysis.

Spectra were recorded for all harvested stem samples. WSC was measured directly in a subset of 125 samples using the anthrone method (see below), and the spectra/values from this subset used to derive a calibration model for predicting WSC quantities in all of the samples. Firstly, the 125 samples for developing the model were chosen on the basis that they represented the maximum spectral variability. This was done using principal component analysis (PCA), with the Mahalanobis distance (H) being applied as a measure of variability.

WSC were then measured in the 125 samples by extraction in 80% ethanol solution and then 100% water (Van Herwaarden et al. 1998b), followed by quantification using the anthrone method (Yemm and Willis 1954), using absorbance at 620 nm on a Shimadzu UV-VIS spectrophotometer (model UV-160A) and fructose as the standard. Some of the samples had to be re-measured because they did not fit within the linear part of the relationship between absorbance and concentrations as based on the standards. In other words, for the samples with the absorbance values < 0.2 or >1.83, WSC of the samples were extracted in a smaller volume or diluted by 3× before re-measuring, respectively. The spectra and WSC measurements were then used to develop the model by partial least squares (PLS) regression with full cross validation. The optimum number of terms in the PLS calibration models was defined as the lowest number of factors that gave the minimum value of the prediction residual error sum of squares (PRESS) in cross validation, in order to avoid over fitting. The resulting calibration equations were evaluated using the coefficient of determination in calibration (R^2) and the standard error in cross validation (SECV). The ratio of standard deviation (SD) to SECV, which is called the residual predictive deviation (RPD), was used to test the accuracy of the calibration models. RPD demonstrates how well the calibration models perform in predicting the reference data, with values >3 considered adequate for analytical purposes in most ATR-MIR applications (Dr. Daniel Cozzolino, personal communication). The RPD value for the calibration model in this study was 4. The developed calibration model was then used to predict WSC values for all of the samples. A comparison of the WSC concentration (mg g^{-1} dry weight) determined by the anthrone method and ATR-MIR predicted data in the subset of 125 samples is shown in Appendix 4.1.

Water soluble carbohydrate concentration ($WSC_{conc.}$, mg g^{-1} dry weight) was presented over time for each stem segment. WSC content ($WSC_{cont.}$, mg) was calculated as $WSC_{conc.}$ multiplied by stem dry weight for each stem segment. Average $WSC_{cont.}$ across all harvest times was also calculated ($WSC_{cont.av.}$, mg). Maximum WSC content (WSC_{max}, mg) was defined as the highest value of the $WSC_{cont.}$ among the harvest times for each stem segment. The amount of mobilized WSC (MWSC) in each stem segment (peduncle, penultimate and lower internodes) was calculated as the difference between maximum and minimum WSC content of the segment. WSC mobilization efficiency (WSCME) for each stem segment was calculated as the proportion (%) of the maximum WSC content of the segment that was mobilized $[\text{WSCME (\%)} = \frac{\text{MWSC}}{\text{WSCmax}} \times 100]$. Dry weight of each stem segment over time (DW) and on average across all of the harvest times ($DW_{av.}$) were also presented.

Grain number spike^{-1} (GNS) and single grain weight (SGW) at maturity: The collected spikes were oven-dried for 3 days at 85 ºC. Then, the spikes were threshed and grains of all sizes were manually counted and weighed. SGW was calculated as GWS/GNS.

4.2.4 Data analysis

Analysis of variance (ANOVA) was carried out for each studied trait using GenStat 16 (http://www.vsni.co.uk/genstat). LSD tests ($\alpha = 0.05$) were used for mean comparisons. R language (R Development Core Team, 2012) was used for model regressions, pairwise Pearson correlation tests and to prepare figures. In Experiment 2, data for the sampling time 10 DAA (before treatment) was collected on a common set of plants for both control and heat.

4.3 Results

4.3.1 Grain number spike^{-1} (GNS) and grain number spikelet^{-1} (GNSp) at maturity

There was no significant difference between unheated control and heat-treated plants for grain number spike^{-1} (GNS, p=0.054) and grain number spikelet^{-1} (GNSp, p=0.556) which is in accordance with the results presented in Chapter 3. This suggests that the heat treatment at 10 days after anthesis did not affect the frequency of grain set. The effect of genotype was significant for both GNS and GNSp ($p<0.001$). Frame and Sunco showed the largest and smallest GNS, respectively (Figure 4.1A). Millewa and Reeves showed the largest GNSp while Sunco had the smallest value for the same trait (Figure 4.1B).

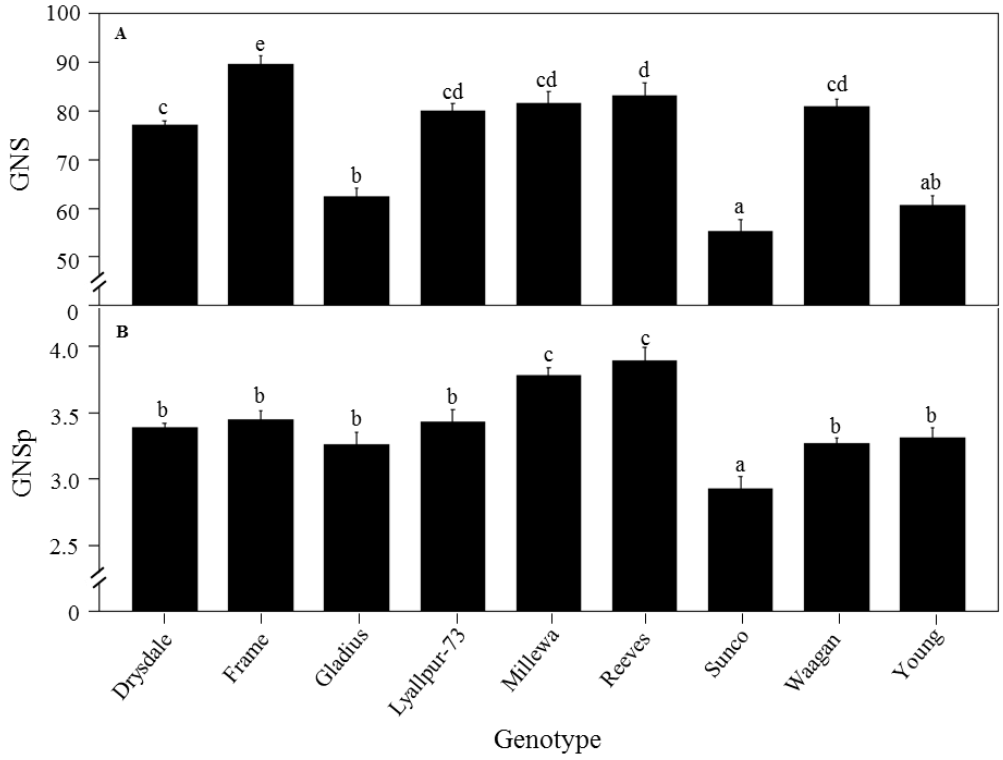


Figure 4. 1 Grain number spike^{-1} (GNS; A), and grain number spikelet^{-1} (GNSp; B;) at maturity in 9 bread wheat genotypes. Bars indicate mean + S.E. Means with the same letter were not significantly different at $p > 0.05$ (LSD test).

4.3.2 Single grain weight at maturity (SGW)

The effects of genotype and treatment, and genotype-by-treatment interaction were all highly significant ($p<0.001$) for SGW measured at maturity. SGW was reduced in the heat-treated plants relative to the controls in all genotypes (except Millewa; Figure 4.2). Gladius, Millewa, Sunco, Waagan and Young showed the least response (~ +1 to -8%) and were therefore relatively tolerant, while Drysdale, Frame, Lyallpur-73 and Reeves showed the highest sensitivity (~ -14 to -28%; Figure 4.2). SGW responses of these genotypes were similar to those observed in the Chapter 3 experiment ($r=0.87$; $p=0.002$).

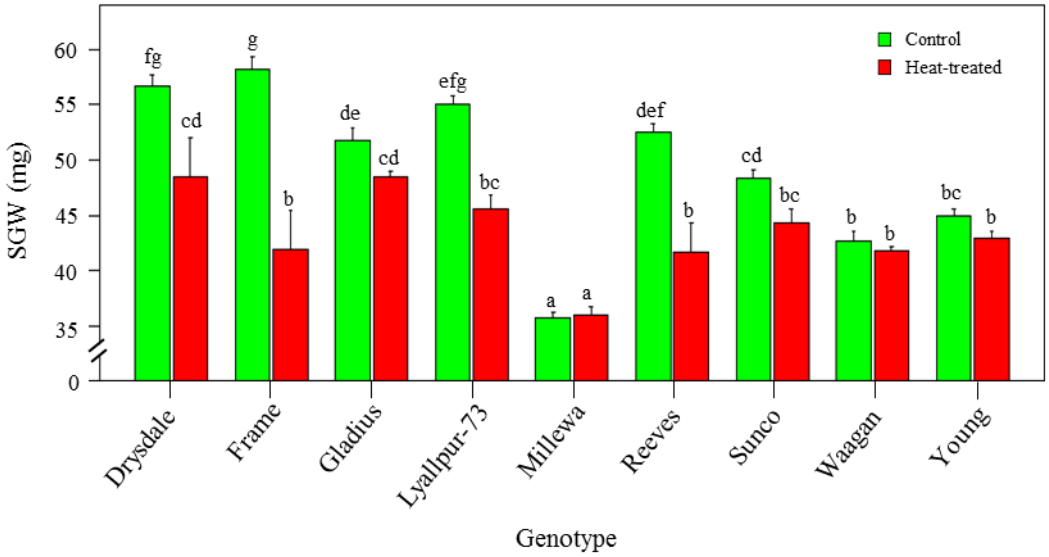


Figure 4. 2 Single grain weight (SGW) of control and heat-treated plants of nine wheat varieties at maturity. Bars indicate mean + S.E.. Means with the same letter were not significantly different at $p > 0.05$ (LSD test).

4.3.3 Grain growth attributes

Grain dry weights observed over time and the fitted logistic models for each genotype, for both control and heat-treated plants, are illustrated in Figure 4.3. Sunco showed no detectable change, while Frame, Lyallpur-73 and Reeves showed the greatest reduction in grain weight in response to the heat treatment. Generally, single grain weight was larger in heat-treated plants than control plants directly after treatment, indicating that grain-filling rate was enhanced during heat exposure.

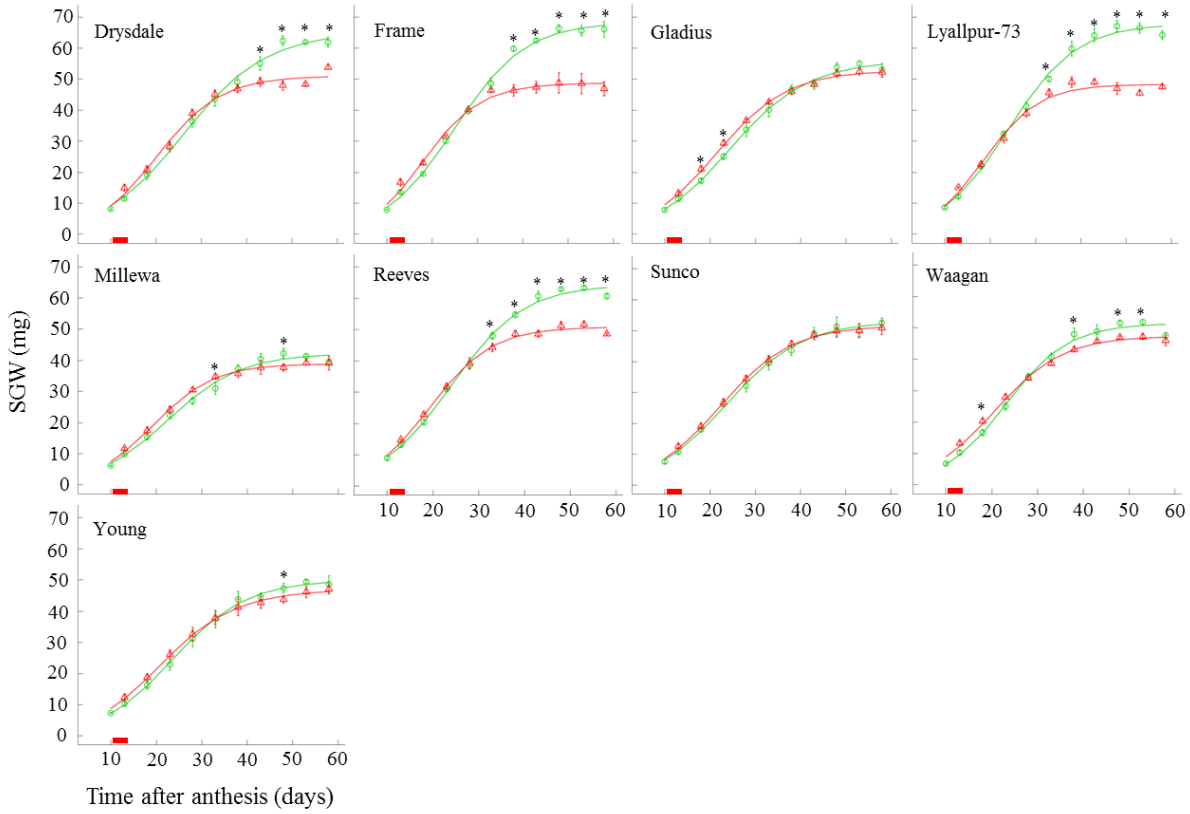


Figure 4. 3 Time courses of single grain weight (SGW) of control (green circles) and heat-treated plants (red triangles) of 9 bread wheat genotypes (mean ± S.E.). Asterisks indicate a significant difference between treatments at $p < 0.05$. Lines represent logistic regressions with 3 parameters (c, b, m) on control (green) and heat-treated plants (red). The red bar on the x axis represents the period of heat treatment.

There was a significant genotypic effect on the absolute values of all of the attributes estimated using the logistic models ($p<0.001$ for theoretical final single grain weight, FGW, maximum growth rate, MGR, and time to inflection point, TIP, and $p<0.004$ in the case of grain-filling duration, GFD) and linear models ($p<0.001$ for sustained grain growth rate, SGR). The effect of heat treatment was also significant for FGW, TIP and GFD ($p<0.001$) while it was insignificant in the case of SGR ($p=0.235$) and MGR ($p=0.057$). Moreover, the genotype-by-treatment interactions was significant for FGW, SGR, TIP ($p<0.001$) and GFD ($p<0.019$).

Grain growth characteristics of control and heat-treated plants are summarised in Figure 4.4. SGR was significantly increased by heat in Drysdale, Gladius, and Millewa and significantly reduced in Lyallpur-73, Reeves and Waagan. Sunco, Young and Frame showed an insignificant increase/decrease for the same trait. Lyallpur-73 and Millewa showed the largest heat effect for SGR, -17 and +18%, respectively. MGR was larger in heat-stressed plants of Drysdale, Gladius, Millewa and Sunco but smaller in heat-stressed plants of Frame, Lyallpur-73, Reeves, Waagan and Young. The effect was only significant in Waagan (-16%) in an LSD test. Gladius and Millewa showed the smallest (1%) and the largest (9%) MGR increase in response to heat stress, respectively. Time from anthesis to the MGR (time to the

inflection point, TIP) was decreased in heat-treated plants of all of the genotypes. On average, heat shortened TIP by 16%. The reduction in TIP was significant in all but Sunco. Drysdale, Frame and Lyallpur-73 showed the largest TIP response (> -21%) while Sunco had the smallest response (-6%). Grain-filling duration (GFD) was also shortened in all of the genotypes in response to heat stress and the reduction was significant in Drysdale, Frame, Gladius, Lyallpur-73, Millewa and Reeves. Drysdale, Frame and Lyallpur-73 showed the highest GFD response (> -21%) while Waagan showed the smallest response (-2%).

Theoretical final single grain weight (FGW), estimated by the logistic model, was strongly correlated ($r=0.84$; $p<0.01$) with SGW measured at maturity which indicates a good estimation of the FGW at maturity by the fitted logistic model. Sunco showed the smallest FGW reduction (~-2.5) while Lyallpur-73 and Frame showed the highest (> -28%). In Frame, Lyallpur-73, and Reeves the observed large FGW reduction was caused by the large negative impact of heat stress on both grain growth rate and duration. In Drysdale, heat stress increased grain growth rate; however, this did not compensate for the reduced grain-filling duration in this genotype, which led to a large significant reduction in the FGW. In Gladius, Millewa and Sunco the increased grain-filling rate in response to the heat treatment did compensate for the reduced GFD and FGW of these genotypes was not significantly affected by heat treatment. In Young, heat stress did not have any detectable effect on grain growth rate during the linear phase (SGR) and had a low impact on grain-filling duration, leading to an insignificant FGW reduction in this genotype. Waagan behaved differently from the rest of genotypes. In this genotype, FGW was only moderately affected by heat treatment; it showed a large reduction in grain growth rate but a small decrease in GFD.

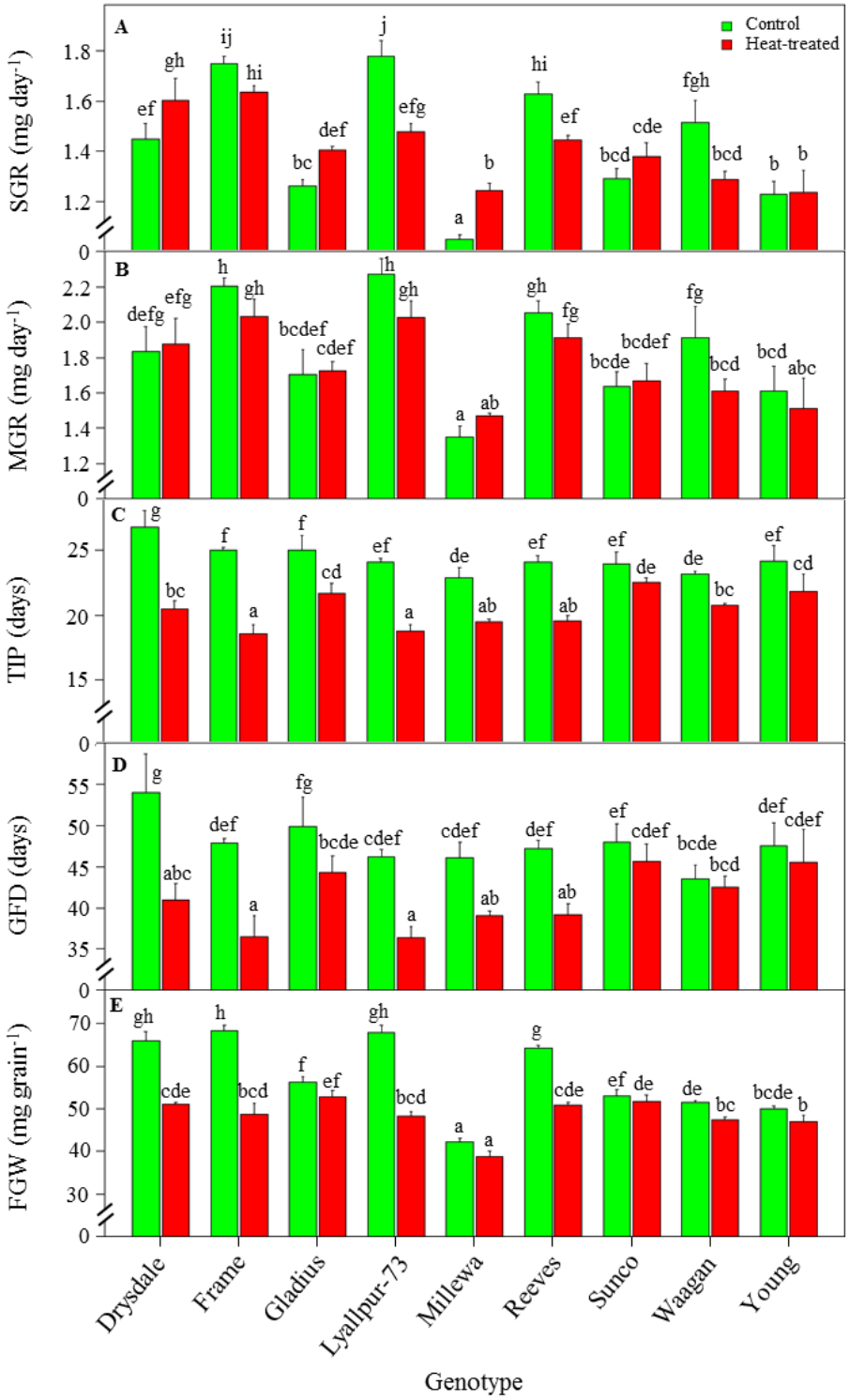


Figure 4. 4 Grain growth characteristics of control and heat-treated plants of nine wheat varieties. Sustained grain growth rate (SGR; A), maximum growth rate (MGR; B), time to inflection point (TIP; C), grain-filling duration (GFD; D), and final grain weight (FGW; E). SGR was estimated using linear regression while the other parameters were estimated using a logistic model. Bars indicate mean + S.E. Means with the same letter were not significantly different at $p > 0.05$ (LSD test).

4.3.4 Chlorophyll fluorescence

A significant genotype, treatment, and genotype-by-treatment effect ($p<0.001$) was observed for chlorophyll fluorescence in a combined ANOVA across all measurements at 10-15 DAA. Prior to exposure to heat stress, there was no significant difference in *Fv*/*Fm* ratio

between control plants and those assigned for later heat treatment. The varieties showed a roughly similar value prior to heat stress except Millewa which was noticeably lower than the rest (Figure 4.5). Upon heat exposure, the *Fv*/*Fm* ratio significantly and immediately decreased in all varieties except Sunco, which showed a non-significant decrease within the first day of heat stress. Further into the heat treatment, there was a further decline, or no more reduction in the case of Drysdale, Gladius and Waagan. Lyallpur-73 and Reeves showed the largest reduction while Gladius, Sunco and Waagan showed the least (Figure 4.5). By the time of the first measurement after relief of heat stress, the *Fv*/*Fm* ratio recovered in all of the varieties, but to different extents. In Gladius, Sunco, Waagan and Young the *Fv*/*Fm* ratio recovered to levels statistically indistinguishable to those seen in control plants. Millewa also recovered completely, but only by the second time point after relief from stress. By contrast, the *Fv*/*Fm* ratio in Drysdale, Lyallpur-73, Millewa and Reeves recovered poorly and remained significantly lower than the control plants for both time points after relief from stress (Figure 4.5).

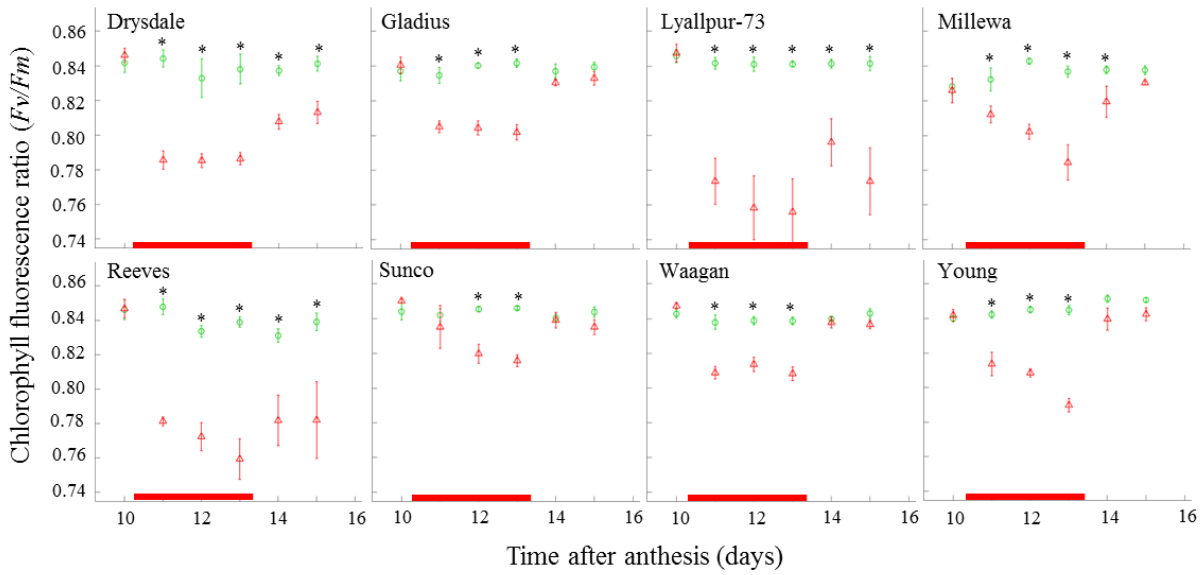


Figure 4. 5 Chlorophyll fluorescence ratio (*Fv/Fm*) of flag leaves (mean ± S.E.) in heat-treated (red triangles) and control plants (green circles), before, during and after a period of brief heat treatment (red bar), in 8 bread wheat genotypes. Asterisks indicate a significant difference between treatments at $p < 0.05$.

4.3.5 Chlorophyll content

Significant genotype and treatment effects ($p<0.001$) were observed for total chlorophyll content. However, the genotype-by-treatment effect was insignificant ($p=0.281$) and the ranking of genotypes was the same for both treatments (Figure 4.6). In other words, genotypes with larger chlorophyll content under control conditions also tended to maintain larger chlorophyll content under heat conditions. Young, Sunco and Gladius showed the largest total chlorophyll content while Frame and Lyallpur-73 had the lowest (Figure 4.6).

The magnitude of the response ranged from less than 12% decrease (Gladius, Waagan and Young) to greater than 32% decrease (Frame and Lyallpur-73).

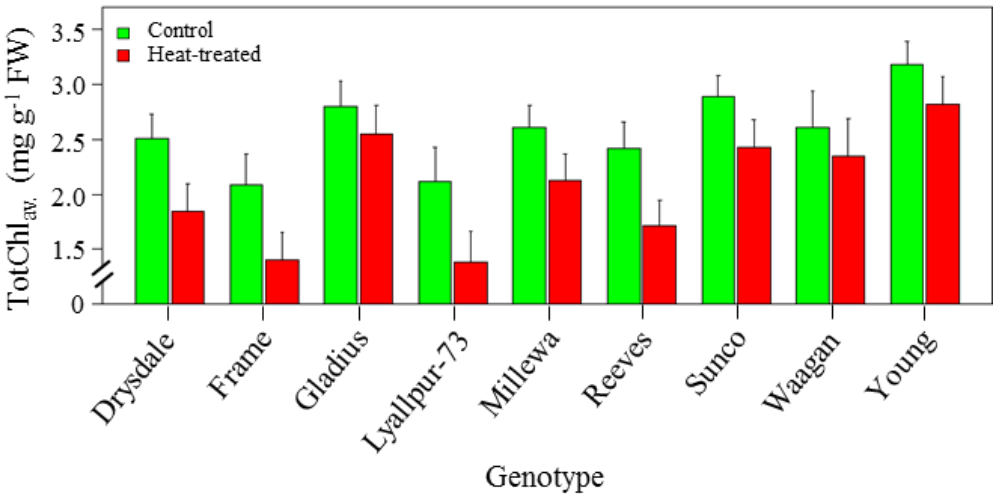


Figure 4. 6 Total chlorophyll content (TotChl$_{av.}$, mg g^{-1}FW) averaged over all time points in control and heat-treated plants of 9 bread wheat genotypes. Bars indicate mean + S.E.

Time courses of flag leaf total chlorophyll content (mg g^{-1}FW; chlorophyll *a* + *b*) in control and heat-treated plants are illustrated in Figure 4.7. Similar to *Fv*/*Fm*, the extent of the chlorophyll loss and its pattern over time differed between genotypes. Drysdale, Frame, Lyallpur-73, Millewa and Reeves showed a large immediate decline in chlorophyll during the treatment, while Gladius, Sunco, Waagan and Young showed either no response or a smaller response during the treatment. After heat treatment, control and heat-treated plants of a given variety tended to gain/lose chlorophyll similarly. For instance, directly after the period of heat treatment, chlorophyll increased in both control and heat-treated plants of Drysdale and Reeves. In Lyallpur-73, chlorophyll loss of heat-treated plants continued at a larger rate than in other varieties after heat treatment, and control plants of this variety also tended to senesce at a larger rate than the other varieties. However in Frame, enhanced chlorophyll loss due to heat stress was evident after the treatment, up to 23 DAA. In general, the difference between sensitive and tolerant varieties was most evident from directly after treatment up to 33 DAA. This period roughly coincides with the log phase of grain growth before grain-filling begins to decelerate in control plants. In other words, heat-treated plants of susceptible varieties suffered the biggest losses in chlorophyll content, and most probably photosynthesis (as showed by a significantly lower *Fv/Fm* in heat-treated plants of the susceptible genotypes shortly after treatment), when there was normally greatest photo-assimilate demand for grain-filling.

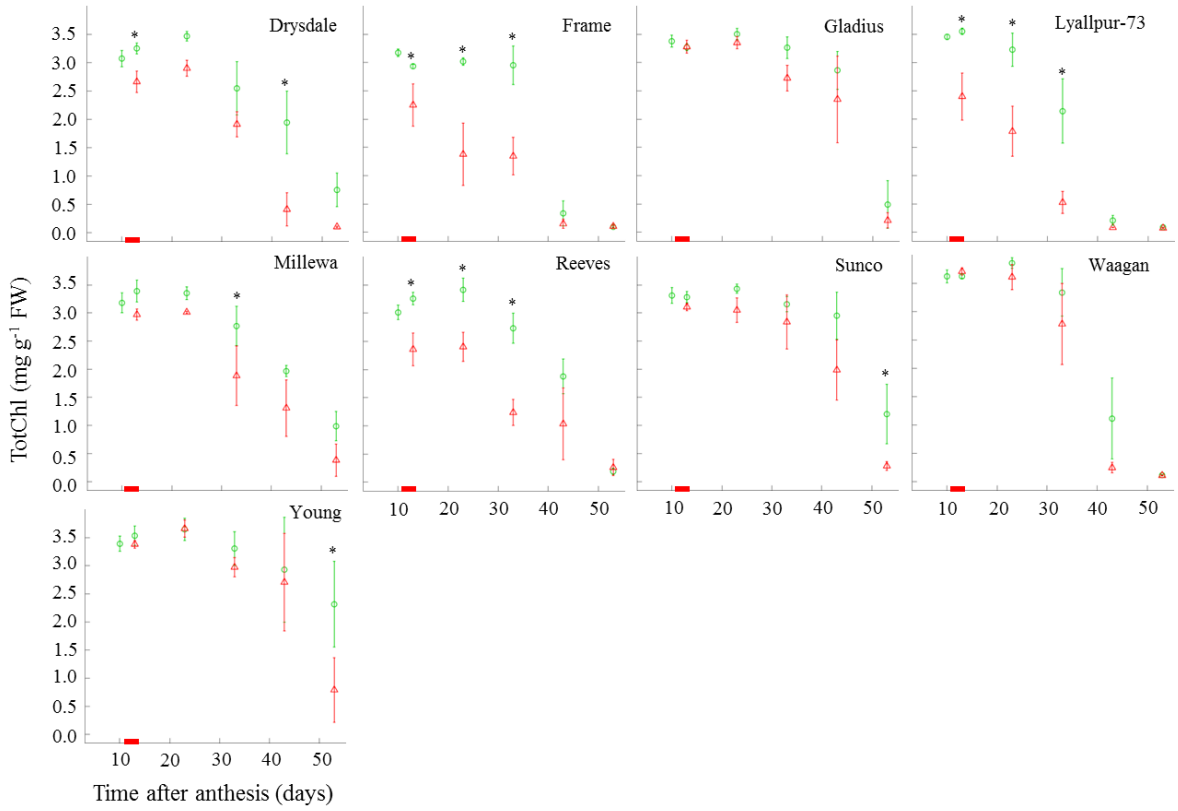


Figure 4. 7 Time courses of total chlorophyll content (TotChl, mg g^{-1} FW) of control (green circles) and heat-treated plants (red triangles) of 9 bread wheat genotypes (mean ± S.E.). Asterisks indicate a significant difference between treatments at $p < 0.05$. The red bar on the x axis represents the period of brief heat treatment.

Significant genotype and treatment effects ($p<0.001$) and an insignificant genotype-by-treatment effect ($p=0.260$ and 0.673 for chlorophyll *a* and *b*, respectively) were observed for both chlorophyll *a* and *b* content. The ranking of genotypes was the same for both treatments in both types of chlorophyll (Figure 4.8A and B). Heat responses of chlorophyll *a* and *b* were also largely similar. In other words, genotypes with higher loss in chlorophyll *a* content also showed higher loss in chlorophyll *b* content (Figure 4.8A and B). On average across all time points and all genotypes, heat reduced chlorophyll *a* and *b* by 18 and 25%, respectively. On average across all time points, chlorophyll *b* (ranged from -12%, in Gladius and Waagan, to -42% in Lyallpur-73) showed higher response in comparison to chlorophyll *a* (ranged from -7%, in Gladius, to -31% in Frame and Lyallpur-73) in all genotypes (Figure 4.8A and B).

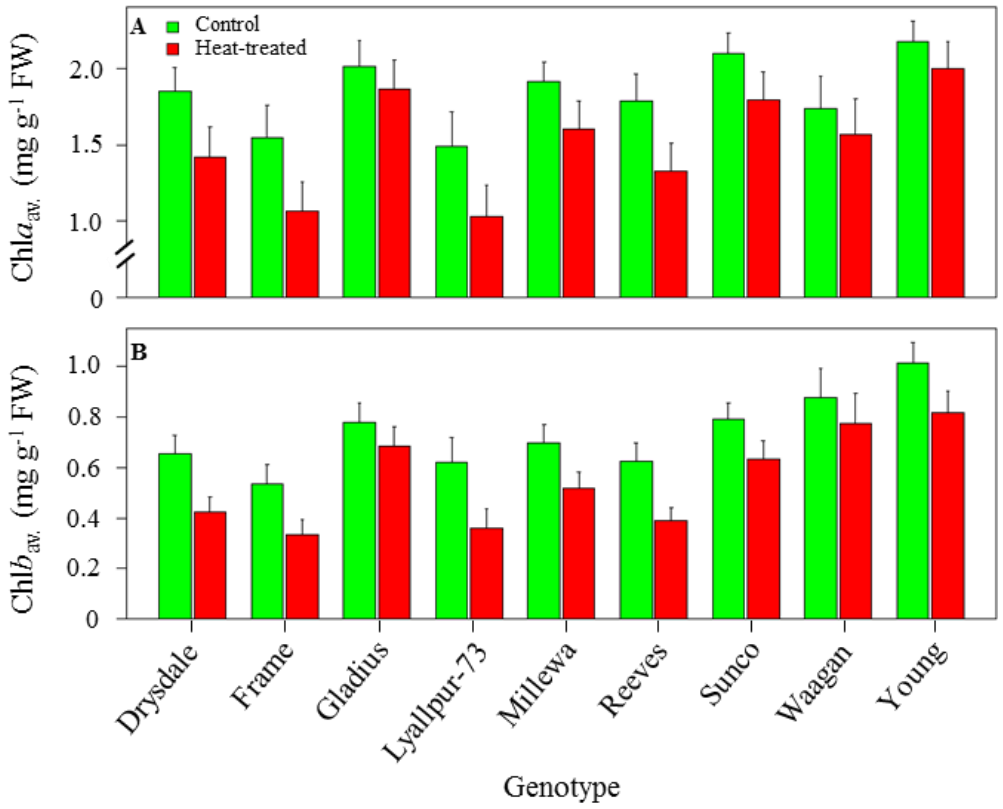


Figure 4. 8 Flag leaf chlorophyll a and b content averaged over all time points (Chl$a_{av.}$, mg g^{-1}FW, A; Chl$b_{av.}$, mg g^{-1}FW, B) in control and heat-treated plants of 9 bread wheat genotypes. Bars indicate mean + S.E.

On average across all genotypes over time, heat-induced reduction in total chlorophyll content was explained by reductions in both chlorophyll *a* and *b* (Figure 4.9); however, the pattern of heat responses of chlorophyll *a* and *b* differed over time. Heat stress had a larger negative impact on chlorophyll *b* (ranged from 23 to 38%) than chlorophyll *a* (ranged from 9 to 27%) from directly after treatment to 33 DAA, and thereafter it showed a proportionately higher adverse effect on chlorophyll *a* (37 and 67% at 43 and 53 DAA, respectively) than on chlorophyll *b* (36 and 54% at 43 and 53 DAA, respectively; Figure 4.9). Generally, this trend held true across the studied genotypes (Figures 4.10 and 4.11).

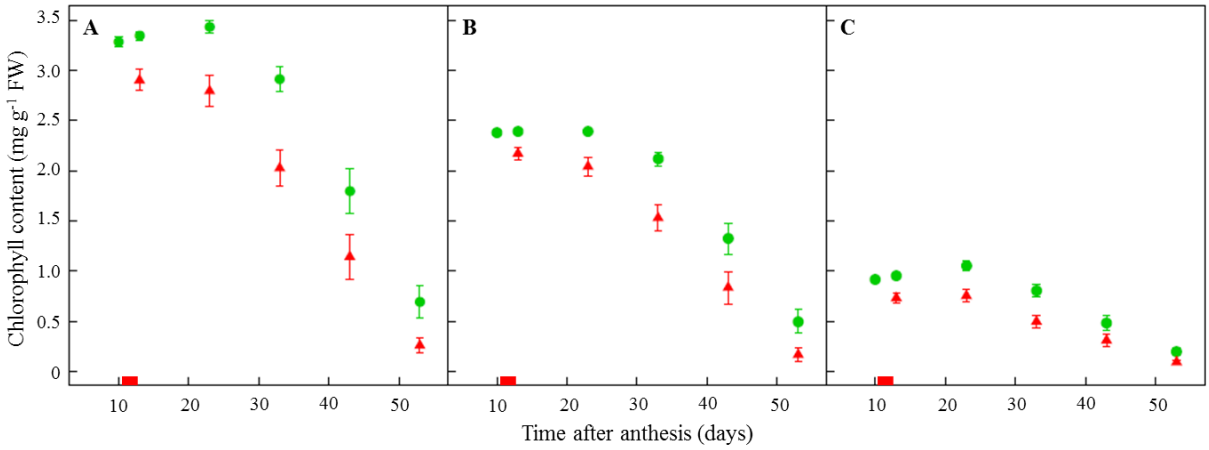


Figure 4. 9 Time courses of total chlorophyll (A), chlorophyll *a* (B) and *b* (C) content (mg g^{-1} FW) of control (green circles) and heat-treated plants (red triangles) averaged across all genotypes within each time point (mean ± S.E.). The red bar on the x axis represents the period of brief heat treatment.

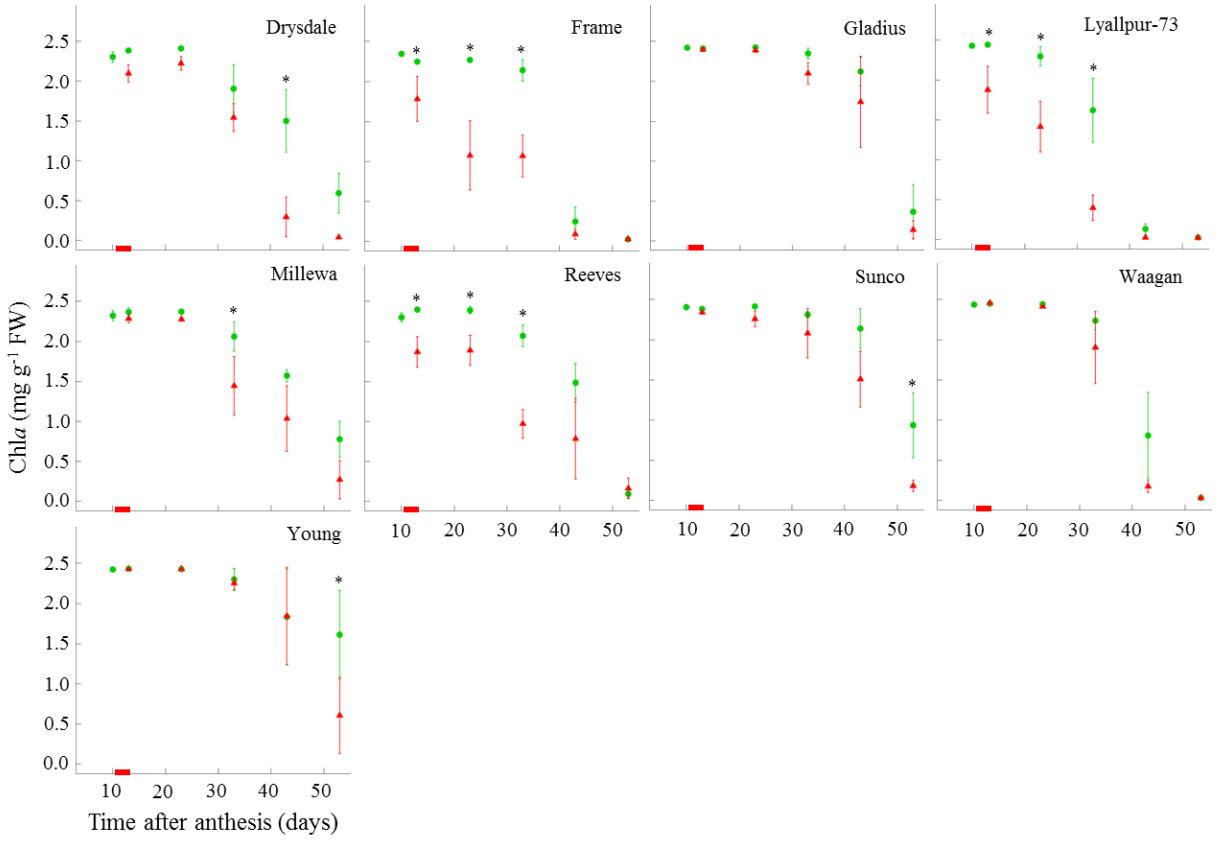


Figure 4. 10 Time courses of flag leaf chlorophyll *a* content (Chl*a*) of control (green circles) and heat-treated plants (red triangles) of 9 bread wheat genotypes (mean ± S.E.). Asterisks indicate a significant difference between treatments at $p < 0.05$. The red bar on the x axis represents the period of brief heat treatment.

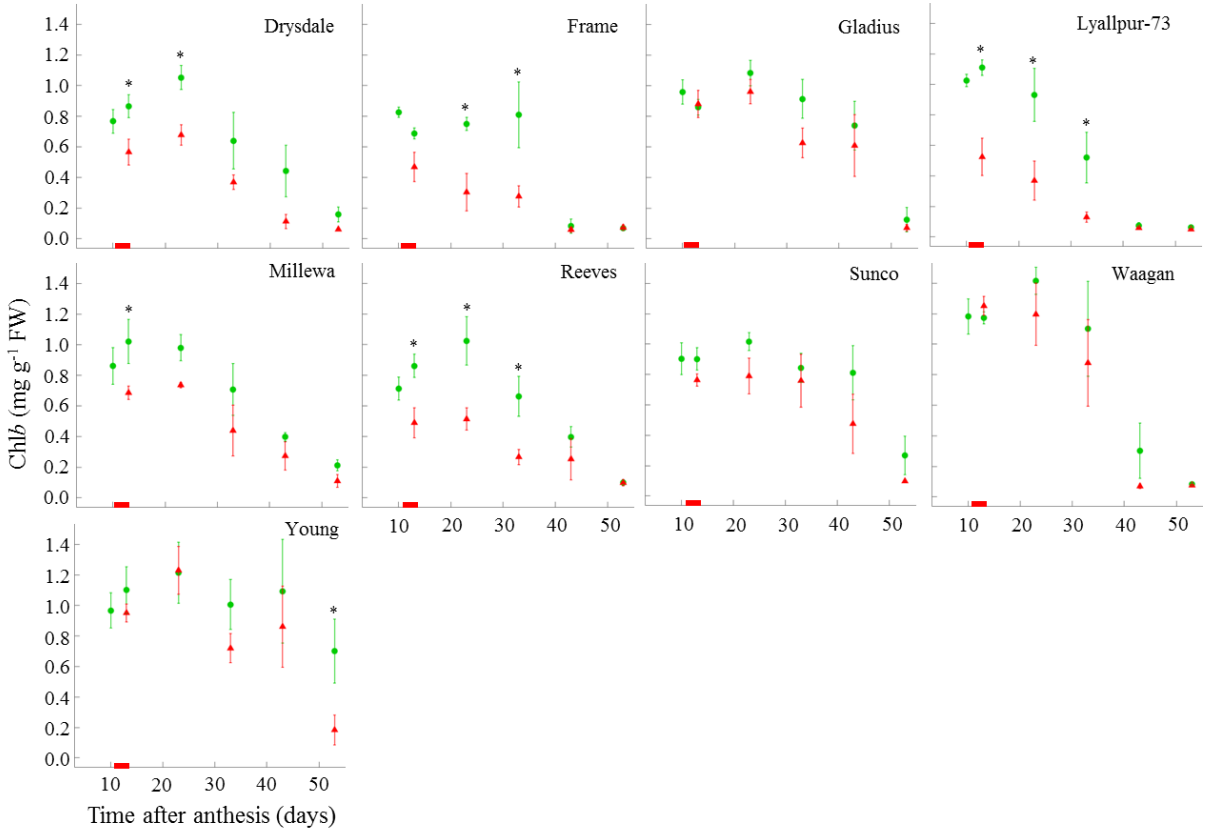


Figure 4. 11 Time courses of flag leaf chlorophyll *b* content (Chl*b*) of control (green circles) and heat-treated plants (red triangles) of 9 bread wheat genotypes (mean ± S.E.). Asterisks indicate a significant difference between treatments at $p < 0.05$. The red bar on the x axis represents the period of brief heat treatment.

4.3.6 Stem water soluble carbohydrate (WSC) content

Significant genotype and treatment effects ($p<0.001$) were observed for WSC content in all stem segments. The genotype-by-treatment effect was significant only for peduncle ($p=0.007$, 0.300 and 0.179 for peduncle, penultimate and lower internodes, respectively). On average across all genotypes, heat stress reduced WSC content averaged over all measured time points ($WSC_{cont.av.}$) in peduncle by 17% and in penultimate and lower internodes by 25%. In heat-treated peduncle, $WSC_{cont.av.}$ was 27-30% lower than in controls in Frame and Lyallpur-73, while in Gladius and Waagan it was 1-3% higher than in controls (Figure 4.12). Young and Lyallpur-73 showed the largest $WSC_{cont.av.}$ reduction in both penultimate and lower internodes in response to heat stress (31-41%), while Millewa and Drysdale showed the smallest reduction in $WSC_{cont.av.}$ in penultimate and lower internodes, respectively (10 and 6%; Figure 4.12).

On average across all genotypes and under either control or heat conditions, $WSC_{cont.av.}$ in the lower internodes (206 and 155 mg, respectively) was higher than $WSC_{cont.av.}$ in the penultimate internode (control 164 and heat 123 mg) or peduncle (control 86 and heat 71 mg).

All stem segments showed differences in $WSC_{cont.}$ (and $WSC_{conc.}$) dynamics between genotypes (Figure 4.13 and 4.2). In both control and heat-treated plants $WSC_{cont.}$ increased after 10 DAA, reached a maximum between 13 to 43 DAA depending on genotype, treatment and the stem segment, then decreased to very little by 53 DAA (Figure 4.13).

Generally, weight of the other stem components (total dry weight minus WSC) remained relatively constant over the time period of the experiment, and was not affected by heat (Appendix 4.3). Therefore, $WSC_{conc.}$ (mg WSC g^{-1} dry stem weight; Appendix 4.2) showed the same general patterns as the values of absolute $WSC_{cont.}$ (Figure 4.13) and total stem dry weight (Appendix 4.4).

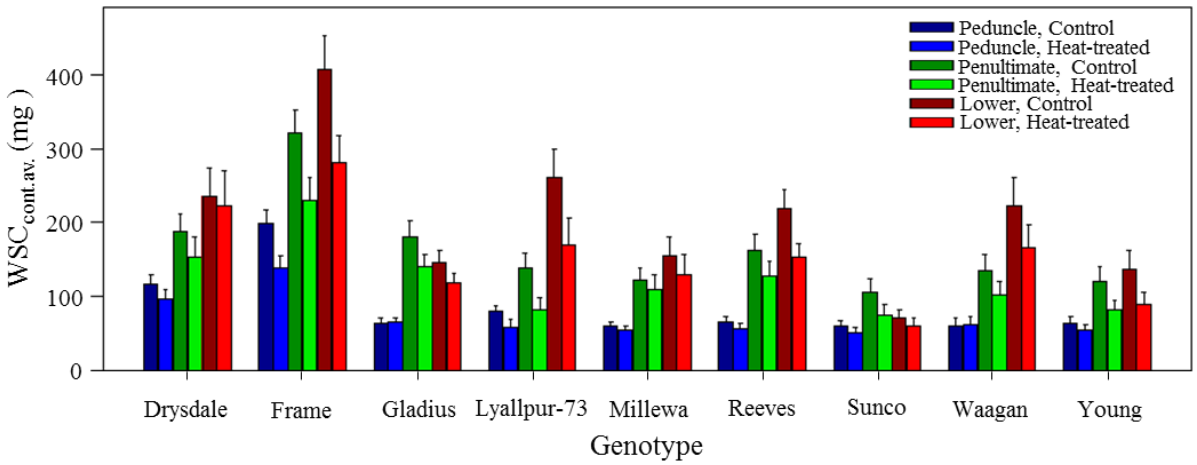


Figure 4. 12 Water soluble carbohydrate content averaged over all harvest times ($WSC_{cont.av.}$ mg; harvest times: 10 to 53 DAA), in peduncle and in penultimate and lower internodes of the main culm of 9 bread wheat varieties under control and heat conditions. Bars indicate mean + S.E.

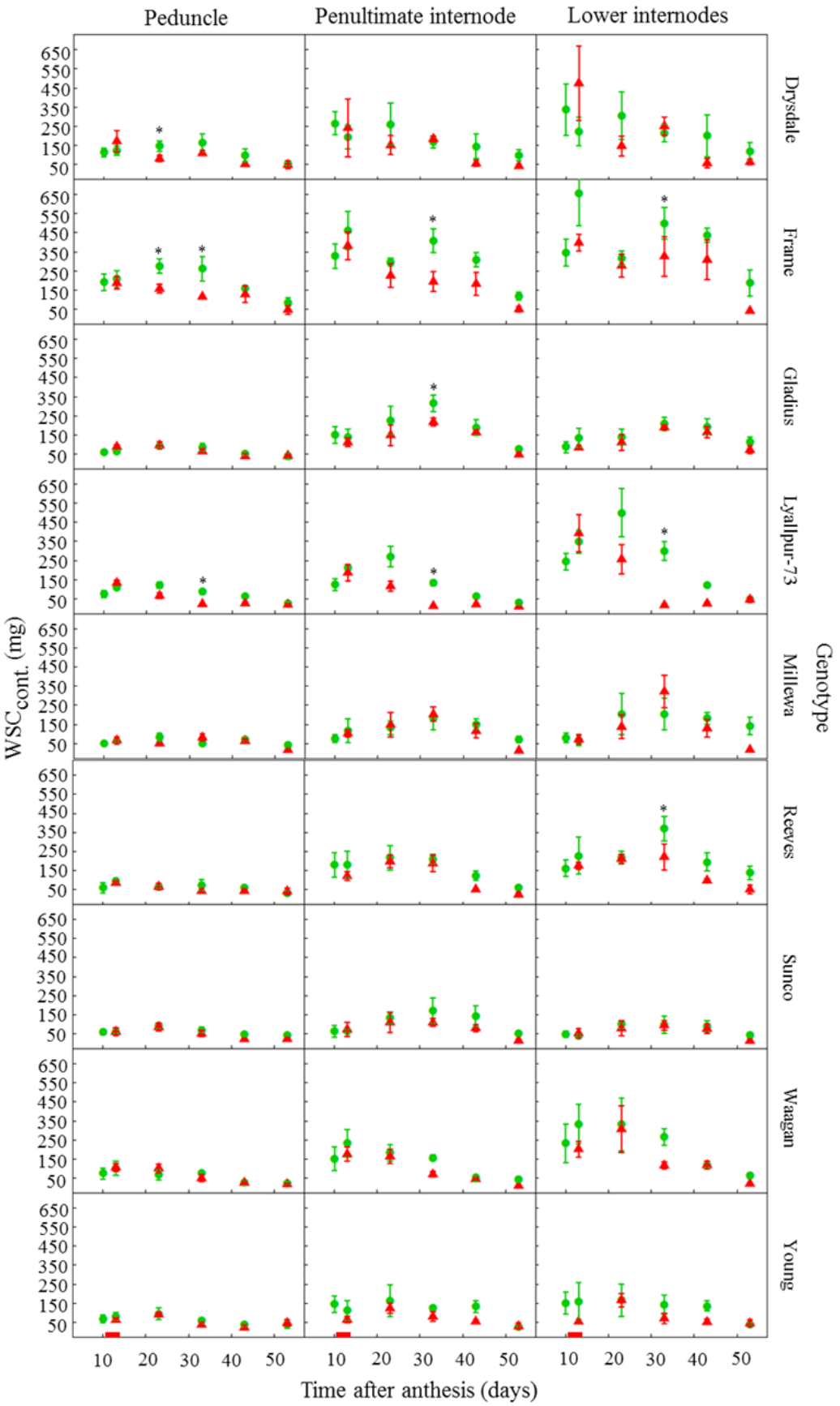


Figure 4. 13 Time courses of water soluble carbohydrate content ($WSC_{cont.}$ mg) of peduncle and penultimate and lower internodes of the main stem from control (green circles) and heat-treated plants (red triangles) of 9 bread wheat genotypes (mean ± S.E.). Asterisks indicate a significant difference between treatments at $p < 0.05$. The red bar on the x axis represents the period of brief heat treatment.

4.3.7 Maximum and minimum water soluble carbohydrate content (WSC_{max} and WSC_{min}), WSC mobilization (MWSC) and WSC mobilization efficiency (WSCME)

Significant genotypic variation ($p<0.001$) was observed for WSC_{max} in all stem segments. Heat treatment did not show a significant effect on WSC_{max} in any stem segment ($p=0.506$, 0.083 and 0.460 for peduncle, penultimate and lower internodes, respectively). Nevertheless, on average across all genotypes, heat-treated plants had smaller WSC_{max} than in controls (by 9, 20 and 11%, in the peduncle, penultimate and lower internodes, respectively) suggesting that heat may have reduced WSC_{max}. On average across all stem segments, WSC_{max} showed the greatest differences between heat-treated plants relative to controls in Frame, Reeves and Millewa (-30, -19 and +22% respectively; Figure 4.14A).

Significant genotypic variation was observed for WSC_{min} in all stem segments ($p=0.014$, 0.001 and 0.004 for peduncle, penultimate and lower internodes). Heat treatment significantly ($p<0.001$) reduced WSC_{min} in penultimate and lower internodes, by 57 and 60% respectively, while it showed no significant impact on WSC_{min} of peduncle ($p=0.085$; however heat-treated plants had 24% lower WSC_{min} than controls). The genotype-by-treatment effect was insignificant in all stem segments ($p=0.675$, 0.216 and 0.064 for peduncle, penultimate and lower internodes). Averaged across all of the stem segments, WSC_{min} showed the greatest differences between heat-treated plants relative to controls in Frame, Millewa and Sunco (-60 to -75%; Figure 4.13B), and the smallest differences relative to control in Young (+7%; Figure 4.14B).

On average, heat stress increased mobilised WSC (MWSC, calculated as the difference between maximum and minimum WSC content) in the peduncle and lower internodes, by 3 and 13%, respectively. By contrast, heat stress reduced (by an average of 6%) MWSC in the penultimate internode. Genotypes differed in their patterns of MWSC heat responses across stem segments (Figure 4.14C). In the peduncle, MWSC was greater in heat-stressed plants than in control plants in all of the genotypes except Frame and Reeves where the reverse was true (Figure 4.14C). In the penultimate internode, MWSC was greater under heat conditions than in control plants in Drysdale, Millewa and Reeves, whereas the reverse was true in other genotypes (Figure 4.14C). In the lower internodes, MWSC was larger in heat-stressed plants than in controls plants of Drysdale, Gladius, Millewa, Sunco and Waagan, whereas the reverse was true for the other genotypes (Figure 4.14C).

On average, heat stress improved WSC mobilization efficiency (WSCME, calculated as the proportion of the maximum WSC content that was mobilized), by 12, 16 and 31% in peduncle, penultimate, and lower internodes, respectively. The exceptions to this trend were

the peduncles of Gladius and Reeves and penultimate and lower internodes of Young which showed a decrease in WSCME in the heat treated plants (of 1% in Gladius, 21% in Reeves, and 4-10% in Young; Figure 4.14D). Genotypes differed in their heat response of WSCME (Figure 4.14D). On average over all stem segments, heat response of WSCME was greatest in Millewa and Sunco (+104 and + 37%, respectively) and the least in Young (+3%).

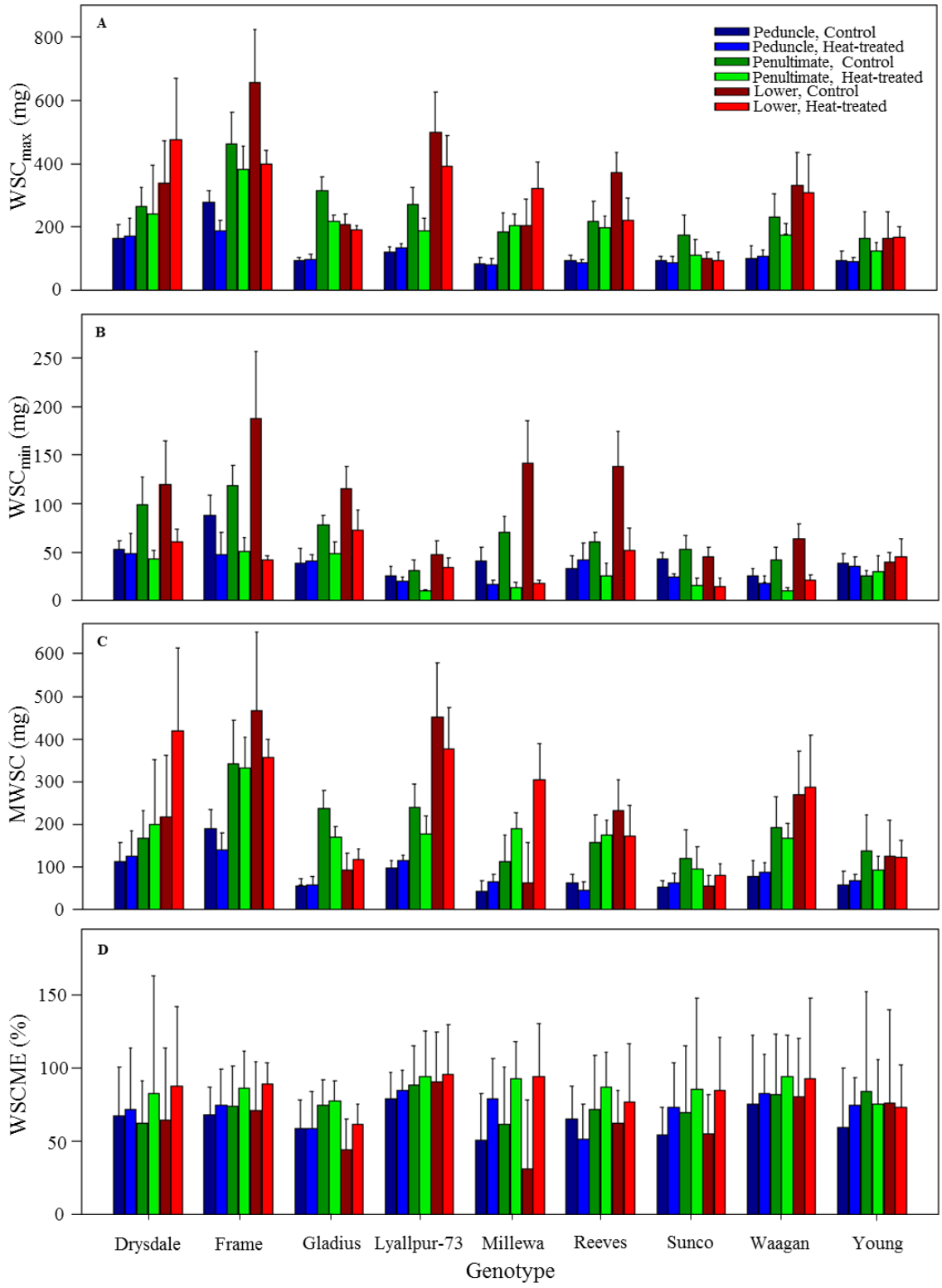


Figure 4. 14 Maximum water soluble carbohydrate content (WSC_{max}, mg; A), minimum water soluble carbohydrate content (WSC_{min}, mg; B), Mobilized WSC (MWSC, mg; C) and WSC mobilization efficiency (WSCME, %; D) of different segments of main culm (peduncle, penultimate and lower internodes) of 9 bread wheat varieties in control and heat-treated (3 days at 37/27 ºC at 10 DAA) plants. Bars indicate mean + S.E.

4.3.8 Associations between heat responses of traits

To examine the relationships between the heat responses of traits, pairwise correlation tests were performed (Table 4.2). FGW response more or less positively associated with responses of all grain growth components. FGW response showed a strong significant association with

TIP and GFD responses, while its association with MGR and SGR responses was moderate and insignificant. The associations of TIP responses with GFD responses, and of MGR responses with SGR responses, were also highly significant.

Heat responses of chlorophyll *a* and *b* (and total chlorophyll) in the flag leaf showed strong positive correlations with one another (Table 4.2), indicating that the heat responses of the two chlorophyll types tended to be similar across the genotypes. Heat responses of the flag leaf chlorophylls showed strong positive and significant associations with responses of FGW, GFD and TIP. This indicated that genotypes with the ability to retain flag leaf chlorophyll better under heat (stay-green), and by inference photosynthetic rate, tended to have a more stable grainfill duration (and TIP) and grain weight under heat.

Heat responses of WSC related traits were more or less positively correlated with one another across different stem segments (Table 4.2), suggesting there was some degree of common control of WSC accumulation and WSC remobilization between different stem segments under heat stress conditions. $WSC_{cont.av.}$ in the peduncle was the only WSC related trait whose response was significantly correlated to that of FGW. In the peduncle, DW_{av} response also showed significant correlations with responses of FGW, GFD, TIP and $WSC_{cont.av.}$, reflecting the fact that WSC content influences DW_{av}. MWSC and WSCME responses of both peduncle and lower internodes showed a moderate positive (but insignificant) association with FGW response indicating that genotypes with higher remobilization of the reserves in response to heat stress tended to be better able to maintain grain weight. Heat responses of $WSC_{max.}$ and $WSC_{min.}$ showed moderate to strong positive and negative correlations, respectively, with those of MWSC and WSCME, reflecting the fact that MWSC and WSCME parameters are calculated from $WSC_{max.}$ and $WSC_{min.}$. $WSC_{max.}$, $WSC_{cont.av.}$, MWSC and WSCME responses in lower internodes showed significant positive associations with grain growth rate responses (MGR and SGR). Hence, responses of WSCs from upper and lower stem segments tended to relate to heat responses of different aspects of grain growth (relating to grain-filling duration and rate, respectively).

In line with the aforementioned correlations, heat responses of flag leaf chlorophylls and of WSC concentration ($WSC_{cont.av.}$) and $DW_{av.}$ in the peduncle were positively and significantly associated with one another, i.e., genotypes with stay-green in the flag leaf tended to retain more WSC in the peduncle (as well as grain weight) under heat.

4.3.9 Relationships between trait potentials and heat responses of traits

Correlations between the potentials of traits (under control conditions) and the heat responses of traits were also examined (Appendix 4.5). FGW, MGR, SGR, WSC_{max} and

$DW_{av.}$ in peduncle, WSCME in penultimate and lower internodes, and $DW_{av.}$ in penultimate internodes showed significant negative correlations with the values of the same traits under control conditions (Appendix 4.5). In other words, genotypes with larger single grain weight, grain growth rate, maximum peduncle WSC, WSC mobilization efficiency, and larger stem dry weight under non-stress conditions tended to show larger reduction for these traits in response to a brief heat stress. Heat responses of chlorophyll retention related traits were positively correlated with their trait potentials, indicating that genotypes that normally had a slower senescence rates also tended to lose their chlorophyll more slowly upon heat exposure.

Table 4. 2 Genotypic correlations between response ratios of traits (Mean trait value$_{\text{Heat treatment}}$ / Mean trait value$_{\text{Control}}$). FGW, final grain weight; GFD, grain-filling duration; TIP, time to inflection point; MGR, maximum growth rate; SGR, sustained grain growth rate; TotChl$_{av.}$, total chlorophyll content averaged over all time points; Chl$a_{av.}$ and Chlb_{av}, chlorophyll a and b content averaged over all time points; WSC$_{max}$, maximum water soluble carbohydrate content; WSC$_{min}$, minimum water soluble carbohydrate content; WSC$_{cont.av.}$, water soluble carbohydrate content averaged over all harvest times; MWSC, mobilized WSC; WSCME, WSC mobilization efficiency; DW$_{av.}$, stem dry weight averaged over all harvest times.

Stem segment										Peduncle						Penultimate internode						Lower internodes					
	Trait	FGW	GFD	TIP	MGR	SGR	TotChl$_{av.}$	Chl$a_{av.}$	Chl$b_{av.}$	WSC$_{max}$	WSC$_{min}$	WSC$_{cont.av.}$	MWSC	WSCME	DW$_{av.}$	WSC$_{max}$	WSC$_{min}$	WSC$_{cont.av.}$	MWSC	WSCME	DW$_{av.}$	WSC$_{max}$	WSC$_{min}$	WSC$_{cont.av.}$	MWSC	WSCME	DW$_{av.}$
	FGW	-																									
	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	-																				
	Chl$a_{av.}$	0.94***	0.85**	0.85**	0.19	0.41	0.99***	-																			
	Chl$b_{av.}$	0.90***	0.86**	0.83**	0.09	0.31	0.98***	0.96***	-																		
Peduncle	WSC$_{max}$	0.24	0.22	0.26	0.04	0.05	0.30	0.33	0.23	-																	
	WSC$_{min}$	-0.12	-0.03	-0.10	-0.23	-0.24	0.01	0.05	-0.06	0.23	-																
	WSC$_{cont.av.}$	0.71*	0.64	0.61	0.09	0.24	0.82**	0.80**	0.81**	0.44	0.24	-															
	MWSC	0.52	0.33	0.47	0.47	0.49	0.39	0.42	0.34	0.56	-0.56	0.24	-														
	WSCME	0.48	0.26	0.41	0.54	0.56	0.30	0.31	0.27	0.05	-0.82**	0.02	0.86**	-													
	DW$_{av.}$	0.77*	0.81**	0.78*	-0.01	0.13	0.80**	0.83**	0.73*	0.55	0.32	0.79*	0.37	0.10	-												
Penultimate internode	WSC$_{max}$	-0.16	-0.41	-0.34	0.43	0.37	-0.23	-0.21	-0.30	-0.09	-0.13	0.05	0.20	0.27	-0.06	-											
	WSC$_{min}$	0.20	0.27	0.19	-0.08	0.07	0.31	0.37	0.21	-0.05	0.40	-0.03	-0.12	-0.11	0.29	-0.26	-										
	WSC$_{cont.av.}$	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	-0.08	-0.39	-0.26	0.56	0.46	-0.21	-0.20	-0.26	-0.04	-0.31	0.07	0.33	0.40	-0.1	0.95***	-0.48	0.81**	-								
	WSCME	-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***	-							
	DW$_{av.}$	0.53	0.30	0.43	0.45	0.48	0.50	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	-						
Lower internodes	WSC$_{max}$	0.36	0.01	0.17	0.70*	0.76*	0.30	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*	-					
	WSC$_{min}$	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	-				
	WSC$_{cont.av.}$	0.29	-0.09	0.10	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	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*	0.10	0.72*	-0.35	0.73*	0.83**	0.78*	0.53	0.85**	-0.43	0.53	-		
	WSCME	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*	0.00	0.70*	-0.44	0.73*	0.84**	0.82**	0.42	0.69*	-0.57	0.47	0.97***	-	
	DW$_{av.}$	0.46	0.21	0.40	0.53	0.53	0.36	0.35	0.34	0.44	0.00	0.41	0.37	0.18	0.39	0.00	-0.36	0.45	0.21	0.46	0.79*	0.54	-0.27	0.88**	0.34	0.28	-

Values are Pearson correlation coefficients, with significance levels indicated by asterisks: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

4.4 Discussion

Heat stress can adversely affect yield by influencing either source or sink related processes. Photosynthetic rate, stay-green, and assimilates available for remobilization (mainly stem reserves) are traits related to source activity/availability, while grain number, grain size, grain-filling rate and duration are mainly associated with sink capacity. This study attempted to quantify the relative importance of these traits in determining grain weight, for a range of bread wheat varieties subjected to a brief episode of severe terminal heat stress. Genotypic variation for grain weight maintenance and chlorophyll loss after a brief heat stress at early grain-filling stage (10 DAA) for the genotypes used in this study was presented in Chapter 3. Here, their analysis was extended to various grain growth parameters (i.e. SGR, MGR, TIP, and GFD), chlorophyll *a* and *b* content, and stem water soluble carbohydrate (WSC).

4.4.1 Grain number

Heat treatment had no significant effect on GNS and GNSp, reflecting the likelihood that these characters were determined prior to heat exposure (10DAA). Therefore, these traits had no significant contribution to heat responses of sink capacity in this study. No effect on grain number was reported in Chapter 3 or in other authors studies' (Bhullar and Jenner 1985; Stone and Nicolas 1995a; Tashiro and Wardlaw 1990a; Tashiro and Wardlaw 1990b; Wardlaw and Wrigley 1994) in which a similar/same short heat treatment in early grainfill was applied.

4.4.2 Grain growth and development

By contrast, the heat treatment reduced final grain size and several characteristics of individual grain growth.

Genotypes varied significantly for heat responses of both grain-filling rate and duration (Figure 4.4A, B, and D). However, the strong positive correlation between responses of FGW with response of GFD (r=0.87), but not with responses of SGR and MGR (r=0.49 and r=0.35, respectively) suggest that the GFD effects were more influential than grain-filling rate effects in determining the level of tolerance among the studied genotypes and under these conditions. This is similar to the results of a study of two wheat genotypes contrasting for heat tolerance by Stone and Nicholas (1995a) which indicated that the effect of heat stress on grain weight was mainly exerted through a reduction in GFD rather than change in grain-filling rate. By contrast, several authors reported a stronger correlation between heat response of grain-filling rate with that of grain weight and concluded that response of grain-filling rate influenced heat tolerance more than that of grain-filling duration (Hunt et al. 1991; Sofield et al. 1977; Wardlaw and Moncur 1995; Zahedi and Jenner 2003; Zahedi et al. 2003). In these studies, the

genotypes that were the most heat-tolerant showed the largest increase in grain-filling rate in response to heat stress. These conflicting results may be at least partly explained by the intensity (moderate vs. severe) and duration (brief vs. long-term) of the heat treatments used, as well as the choice of genotypes. The current study and that of Stone and Nicholas (1995) used a brief severe heat stress (37/27 °C and 40/19 °C day/night, respectively, for 3-5 days at early grain-filling), while the others used prolonged moderately high temperatures (30/25 °C day/night during the whole grain-filling period). This may suggest that different mechanisms (i.e., grain-filling rate vs. duration related) may predominate in determining maintenance of grain weight, depending on the intensity and duration of the heat stress.

Abiotic stresses during grain-filling enhance ethylene production which may cause the reduction in grain-filling period that is observed under such conditions (Beltrano et al. 1999; Hays et al. 2007). A brief heat stress (1-2 days at 38/25 °C) at 10 days after anthesis enhanced ethylene concentration in developing grains and flag leaf 6 to 12 fold in a heat sensitive variety, but did not change it in a heat tolerant variety (Hays et al. 2007). Ethylene is known to regulate growth and development and to trigger senescence and maturation in plants including wheat (Beltrano et al. 1994; Khan 2006; Pratt and Goeschl 1969; Schaller 2012). The enhanced ethylene accumulation upon heat exposure has been suggested to act as a timing signal to arrest development, trigger senescence, and shorten grain-filling duration (Hays et al. 2007). Thus, the observed adverse heat effect on grain-filling duration and variation between the genotypes for this trait (and also flag leaf senescence) may be related to differences in the levels of heat-induced ethylene production among the studied genotypes.

In contrast to the results of this study, no significant correlation was observed between GFD (as measured by spike yellowness) and grain weight response in Chapter 3, which may reflect the difficulty of measuring grainfill duration accurately by the subjective methods used in Chapter 3. Alternatively, this might have been because different sets of genotypes were used; the genotypes that were used here were biased towards some of the more contrasting types identified from Chapter 3.

Changes (reductions) in time to the inflection point (TIP) in response to the heat treatment showed the strongest positive association with FGW response ($r=0.95$; $p<0.001$) which identified this as the best of the studied traits for predicting FGW response. GFD and TIP responses were highly correlated to one another ($r=0.95$, $p<0.0001$), and are clearly both related to duration of synthetic processes. However, TIP also represents a shift between the balance between processes relating to dry matter synthesis and assimilate entry into the grain vs. senescence (Zahedi and Jenner 2003). The strong association between the responses of

FGW and TIP tends to suggest that prolonging the relative time spent in synthetic vs. senescence processes in the grain will have a greater impact on final grain weight than the duration of grain-filling *per se*. Interestingly, Zahedi and Jenner (2003) also reported FGW maintenance to be more strongly associated with TIP response than with grain-filling rate or duration responses, under conditions of long-term moderately high temperatures. Therefore, TIP might be useful indicator of heat tolerance in wheat under a range of heat stress conditions.

During the short treatment interval, the rate of dry matter accumulation in the developing grains generally increased in heat-treated plants compared with controls (Figure 4.3). In accordance to this study, Jenner (1991a) observed that a heat treatment enhanced grain-filling rate during and shortly after heat treatment. It has been shown that the rates of various developmental processes (e.g. leaf expansion or elongation) follow similar response curves over a range of temperatures (Parent et al. 2010; Parent and Tardieu 2012). The enhanced rate of dry matter accumulation during heat treatment can be at least partly explained by a general phenomenon of chemical reactions – with high temperatures, rates of chemical reactions increase because molecules (e.g., enzymes and substrates) have greater kinetic energy at higher temperatures and collide more frequently. Furthermore, when Hurkman et al. (2003) looked at mRNA expression of genes encoding some key enzymes in the starch biosynthesis pathway in the developing wheat grains they found that the peak in expression was earlier under high temperatures. It has also been shown that high temperatures increase the rate of import of photosynthate to the developing grains (Wardlaw et al. 1980). Therefore, the enhanced rate of dry matter accumulation during the treatment may be at least partly due to increased photosynthate movement into developing grains and/or enhanced conversion of sugars to starch resulting from earlier peak in expression or increased activity of starch biosynthetic enzymes.

Maximum growth rate (MGR) may reflect the maximum capacities of the synthetic activities in the grain and/or the upper limit to the import of nutrients into developing grains (Zahedi and Jenner 2003). Experiments with illuminance/shading linked the rate of dry matter accumulation in the grain with rates of photosynthate supply (Sofield et al. 1977; Wardlaw et al. 1980) suggesting grain growth rate could be affected by either current photosynthesis efficiency or amount of stem WSC mobilized. Although MGR showed some differences between control and heat-treated plants these differences were not significant (with the exception of Waagan whose MGR was significantly decreased by heat; Figure 4.4B). Therefore, on the whole, there were no significant lasting effects of heat stress on growth rate

once the stress was relieved. Potentially, effects on MGR manifested after relief from heat stress could derive from irreversible damage to the enzymes that convert sucrose to starch in the grain (Hawker and Jenner 1993), in particular soluble starch synthase which is very heat sensitive (Hawker and Jenner 1993; Jenner and Hawker 1993; Jenner 1994), from changes in mRNA expression of genes encoding enzymes for starch biosynthesis which can be brought about by heat stress (Hurkman et al. 2003), or from senescence related processes.

While there was no significant overall correlation between sustained grainfill rate (SGR) response and tolerance (smaller FGW response), genotypic differences for grain weight maintenance in this study may be at least partially explained by SGR responses to the heat treatment. Sustained grain growth rate (SGR, which is a product of both during and after treatment effects and measured during the log phase of grain growth) was reduced in the most susceptible genotypes (i.e., Frame, Lyallpur-73 and Reeves), while it was increased in most of the tolerant genotypes (i.e., Gladius, Millewa, and Sunco; Figure 4.4A). These three tolerant varieties had a relatively stable FGW, despite having considerably truncated GFD, by virtue of the fact that their SGR was increased by the treatment (Figure 4.4A and D). In Drysdale, SGR was increased by heat, yet the variety was not very tolerant, because its grain-filling period was significantly truncated (Figure 4.4A and D). Conversely, Waagan was relatively tolerant although its SGR was decreased by heat because this effect was offset by its stable grain-filling duration (Figure 4.4A and D). These results also suggest that heat responses of grain-filling rate and duration are under independent genetic control, as concluded in earlier studies (Jenner 1994; Zahedi and Jenner 2003). The lack of correlation between grain-filling duration and growth rate responses (Table 4.2) provides further support for this idea.

4.4.3 Photosynthesis and stay-green

Heat stress adversely affects photosynthetic competence by affecting both light (e.g. PSII) and dark (e.g. rubisco activase) dependent photosynthetic reactions (Haque et al. 2014). PSII has been considered as the most heat sensitive component of the electron transport chain (Ristic et al. 2007 and references cited therein). The *Fv/Fm* ratio (*Fm* is the maximum fluorescence upon exposure to a saturated flash of light and *Fv* is the difference between the fluorescence at ground state and *Fm*) is used to evaluate photosynthetic efficiency of the plants indirectly by measuring the maximum photochemical efficiency of PSII (Schreiber et al. 1995). The *Fv/Fm* ratio was considerably decreased in response to the heat treatment, and the maximum decrease in *Fv/Fm* values, occurring at 13 DAA, ranged from approximately 3.5% (in Sunco and Waagan) to 10.0% (in Lyallpur and Reeves; Figure 4.5). Generally, genotypes with better heat tolerance (smaller grain weight reduction) showed less reduction in

Fv/Fm under heat and had a larger rate of recovery in *Fv/Fm* ratio, once the heat stress was relieved (Figure 4.5). Tolerant genotypes with smaller response and faster/larger recovery of *Fv/Fm* may have greater thylakoid membrane thermo-stability, leading to less PSII inhibition than in sensitive genotypes as suggested in previous reports (Haque et al. 2014). Genotypic differences for thermo-stability of thylakoid membranes may relate to the differences in membrane lipid composition. For instance, Arabidopsis mutants deficient in the amount of digalactosyl-diacylglycerol, a component of thylakoid membranes, have been reported to be more susceptible to heat stress, showing greater damage to the photosynthetic apparatus and a more drastic decline in *Fv/Fm* ratio (Essemine et al. 2012).

The maximum *Fv/Fm* response during treatment and the *Fv/Fm* recovery response were both highly correlated with chlorophyll content responses directly after treatment ($r=0.91$ and 0.96, $p<0.01$ and $p<0.001$ respectively) and total chlorophyll content during grain-filling ($TotChl_{av.}$; $r=0.82$ and 0.91, $p<0.01$ respectively). Furthermore, there was a positive significant correlation between heat responses of *Fv/Fm* and that of chlorophyll content directly after treatment and $TotChl_{av.}$. This suggests that the chlorophyll retained in heat tolerant varieties (with the stay-green trait) was photosynthetically-competent. These findings are in accordance with earlier reports that showed a strong correlation between photosynthetic rate (measured by leaf gas exchange) or *Fv/Fm* ratio with chlorophyll content under high temperature conditions (Reynolds et al. 2000; Ristic et al. 2007; Ristic et al. 2008).

In accordance with the results in Chapter 3, chlorophyll content responses to heat during grain-filling were significantly positively associated with grain weight responses (Table 4.1) indicating that genotypes with better chlorophyll retention (stay-green), and by inference photosynthetic rate, under heat were also better able to maintain grain weight. The chlorophyll content responses during grain-filling in heat-treated plants were largely governed by the drastic loss of chlorophyll observed during the treatment period (chlorophyll content 13DAA; Figure 4.7). Regarding the average chlorophyll content averaged across all time points, chlorophyll content under control conditions was positively correlated with chlorophyll content under heat conditions ($r=0.97$, $p<0.0001$) and chlorophyll content heat responses ($r=0.85$, $p<0.01$; Appendix 4.6) indicating that genotypes with larger rates of natural senescence tended to experience larger acceleration of senescence in response to heat stress. This may suggests that heat also affected senescence by accelerating senescence processes that occur under non-stress conditions. Therefore, large total chlorophyll loss in heat sensitive genotypes seems to be the consequence of both large immediate heat damage during treatment (direct effect) and also the acceleration of natural senescence processes (after

effect). Overall these results agree with those presented in Chapter 3 and supports the notion that stay-green in flag leaves indicates/influences heat tolerance.

Chlorophyll retention response (stay-green) was highly significantly correlated with TIP and GFD. This suggests that the ability to retain chlorophyll for longer under heat (stay-green) may contribute to extended TIP and GFD. High expression of rubisco activase, soluble starch synthase and glycine decarboxylase were seen for a longer time in a stay-green durum wheat mutant in comparison with its non-stay-green parent line, which supports the idea that stay-green prolongs photosynthesis and grainfill duration (Rampino et al. 2006). Alternatively, the correlations between the flag leaf chlorophyll loss and truncation of TIP and GFD may have arisen because these processes in the upper part of the plant were synchronized under a common senescence signal, rather than being directly related by cause-effect. There was also a strong positive correlation between flag leaf chlorophyll retention and peduncle $WSC_{cont.av.}$ stability (Table 4.1), suggesting a degree of common genetic control of chlorophyll and peduncle WSC content (the same trends, albeit insignificant, were observed in the other internodes).

In general, the mechanism of heat-induced chlorophyll loss and physiological mechanisms of a stay-green phenotype are unclear (Ristic et al. 2007; Vadez et al. 2011). Previous studies suggest that heat stress impairs chlorophyll biosynthesis and hastens chlorophyll breakdown. High temperatures inhibit the activity of prophobilinogen deaminase (an enzymes involved in the chlorophyll biosynthetic pathway) and reduce protochlorophyllide (precursor of chlorophyll *a*) synthesis in wheat (Tewari and Tripathy 1998). In wheat responding to heat stress, acceleration of senescence and chlorophyll loss has been shown to be accompanied by increased activity of proteolytic enzymes (Al-Khatib and Paulsen 1984). Thylakoid membranes harbor chlorophyll so chlorophyll loss might be a consequence of heat damage to these cell structures (Ristic et al. 2007).

Abiotic stresses such as heat and drought may differentially affect synthesis/breakdown of different types of chlorophyll (change the chlorophyll *a/b* ratio) (Al-Khatib and Paulsen 1984; Almeselmani et al. 2012; Ashraf and Karim 1991; Ashraf and Mehmood 1990; Camejo et al. 2005; Wahid and Ghazanfar 2006). Here, chlorophyll *a/b* ratio generally increased due to the heat treatment from 13 to 33 DAA, which suggests a faster breakdown or decreased synthesis of chlorophyll *b* compared with chlorophyll *a* during and shortly after treatment (Appendix 4.6; Figures 4.10 and 4.11). Furthermore, the chlorophyll *a/b* ratio rose more in the sensitive genotypes than in the tolerant ones (Appendix 4.7), suggesting that chlorophyll *b* determines most of the difference in responses of total chlorophyll content between tolerant and

intolerant genotypes. Almeselmani et al. (2012) observed a similar change in chlorophyll *a/b* ratio in response to high temperatures in two wheat genotypes of contrasting heat tolerance. This could be explained by the fact that PSI is more heat stable than PSII (Georgieva 1999) and that chlorophyll *b* is less abundant in PSI than in PSII (Almeselmani et al. 2012). Perhaps the smaller increase in chlorophyll *a/b* ratio in heat tolerant genotypes derives from a more heat-stable PSII in these genotypes.

4.4.4 Water soluble carbohydrates (WSC)

WSC stored in stems has been suggested to contribute to yield formation under stresses such as heat and drought (Blum 1998; Talukder et al. 2013; Van Herwaarden et al. 1998a). In this study, a brief episode of heat stress significantly reduced the $WSC_{cont.av.}$ in various stem segments. In general, heat stress also increased WSC mobilization efficiency (Figure 4.14D). Similar effects under heat or drought stress conditions have been previously reported (Ehdaie et al. 2006; Talukder et al. 2013; Wang et al. 2012). There was also a general consistency in ranking of the genotypes for WSC concentration (average of all harvest times; data not shown) in different stem segments and in control and heat stress conditions. In other studies, wheat genotypes have been found to rank similarly for WSC concentration across various environmental conditions (Foulkes et al. 2002; Ruuska et al. 2006).

Genotypic variation was observed in the pattern of accumulation and depletion of WSC in different stem segments. Maximum WSC content was mainly reached 13 to 33 DAA depending on genotype, treatment and stem segment (Figure 4.13). Others have reported various patterns of WSC accumulation and depletion. Some observed peak WSC at anthesis (Shakiba et al. 1996), while others observed it as late as 22 DAA (Ehdaie et al. 2006; Talukder et al. 2013). These differences may relate to the different growth conditions and/or genotypes used. Averaged across all genotypes, WSC peaked in all stem segments at 23 and 13 DAA in control and heat-treated plants, respectively, then declined (Appendix 4.8), which is in agreement with patterns reported by Ehdaie et al. (2008) under well-watered or droughted field conditions. The earlier peak of WSC content in heat-treated plants may have stemmed from earlier WSC mobilization in response to the heat treatment and/or heat damage to the ability to accumulate WSC in stems.

The response of WSC content in the peduncle ($WSC_{cont.av.}$; determined by averaging over all time points) was the only WSC related trait that was significantly associated with FGW response. Heat responses of $WSC_{cont.av.}$ also showed a strong positive correlation with those of chlorophyll content which may suggest that stay-green allowed a better accumulation of reserves in peduncle under heat stress conditions. In other words, a large decline in

photosynthetic capacity, due to heat injury to the photosynthetic apparatus (shown by large decline in *Fv/Fm* ratio) and accelerated senescence (shown by chlorophyll loss) in intolerant genotypes, may have impaired the ability to accumulate stem WSC. As already mentioned, experiments with illuminance/shading have linked the rate of dry matter accumulation in the grain with rates of photosynthate supply (Sofield et al. 1977; Wardlaw et al. 1980). Grain growth rate could be affected by either current photosynthesis efficiency and/or amount of stem WSC mobilized. However, grain-filling rate did not show a significant correlation with either peduncle WSC or flag leaf senescence related traits, perhaps suggesting that neither contributed directly to FGW stability by supplying photosynthate to the grains. Instead, the correlations between loss of flag leaf chlorophyll, peduncle WSC and grain-filling duration may have arisen because these processes in the upper part of the plant were synchronized under a common senescence signal, rather than being directly related by any cause-effect relationship. It is also possible that peduncle senescence (as indicated by a reduction in WSC contained and mobilized) correlated with grain-filling duration effects because it blocked transport of photosynthates from lower sections of the plant up to the grain.

By comparison, in the lower internodes, responses of MWSC and WSCME were significantly correlated with those of grain growth rate (MGR and SGR) (Table 4.1). While the associations of these traits with FGW were moderate and insignificant, these observations nonetheless raise the possibility that they weakly contributed to FGW under heat stress through their effects on grain growth rate. In addition, WSC_{max} response (only in lower internodes) showed a significant positive correlation with that of grain-filling rate, suggesting that stability of WSC_{max} under heat was a positive trait for tolerance. Overall, these findings suggest that heat stress affected the ability to accumulate WSC in the lower internodes by damaging the WSC synthetic or photosynthetic machineries, and that this in turn affected the amount of WSC that could be remobilized into grain to effect grain growth rate. Thus, the ability to accumulate and then mobilize WSC in the lower stem internodes under heat may contribute to heat tolerance by supplying additional photosynthates to the developing grain. One reason why the lower internodes might have contributed most to grain growth rate effects was because in absolute terms, they generally contained and mobilized more WSCs than either the peduncle or penultimate internodes (Figure 4.12 and 4.14A-D).

Overall these results suggest a model for heat tolerance which is summarised in Figure 4.15.

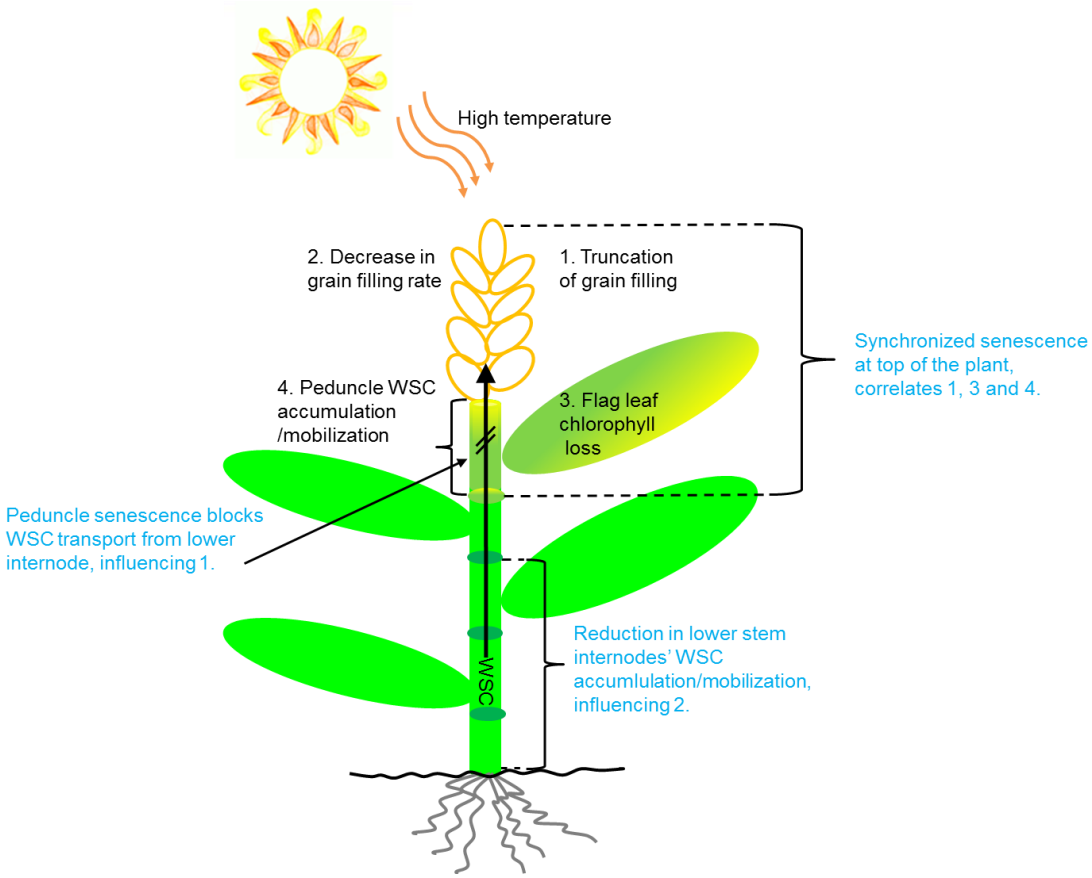


Figure 4. 15 Conceptual model of heat tolerance mechanisms in plants exposed to a 3-day heat stress at 37/27 °C day/night in a growth chamber, 10 days after anthesis. For description refer to text. WSC, water soluble carbohydrates.

4.5 Concluding remarks

Peduncle senescence (and its WSC mobilization) is associated with flag leaf senescence, and also grain-filling duration effects, WSC in lower internodes does not correlate with grain-filling duration effects, perhaps because the senescence of this part of the plant is not well synchronized with that of the top of the plant. Measuring chlorophyll content also on the lower leaves and recording senescence of different stem segments would help to confirm that in the future. Moreover, it is possible that part (or all) of the correlations observed between FGW with peduncle WSC and flag leaf chlorophyll retention were not derived by peduncle WSC and flag leaf greenness directly contributing to grain growth (via supplied photosynthates), but merely from control of these traits by common senescence-related genes active in all of these organs, possibly acting via a systemic hormone signal.

The findings suggest that stable grain-filling duration (and TIP) correlated with stay-green (current source activity) may be more influential in determining heat tolerance than stability of grain-filling rate relating to WSC content and mobilisation in the lower internodes. However, the latter mechanism may be able to confer significant tolerance in certain varieties, e.g., Millewa and Sunco. It should be emphasised that the correlations between traits found

here are based on observations of only 9 genotypes. Therefore, they need to be validated across a wider wheat germplasm set and/or by observing whether QTL for stability of grain growth parameters, stay-green, and WSC mobilisation efficiency co-segregate with those for FGW maintenance in segregating populations. It seems likely that selection for both larger grain growth rate and longer grain-filling duration under heat will be needed to achieve the best level of heat tolerance, as they are independent traits. Apart from assaying grain growth directly, stability of flag leaf stay-green and WSC in the lower internodes may provide useful selection criteria to select for these traits. Monitoring flag leaf chlorophyll loss with SPAD chlorophyll meter may be useful for estimating grain-filling duration components (including TIP) of heat tolerance.

Chapter 5: The Drysdale/Waagan molecular marker genetic map

5.1 Introduction

To maximize the chance of discovering QTL for heat tolerance, parents contrasting for the trait should be used to construct the mapping population. In Chapter 3, parents of available mapping populations were characterized for their heat tolerance at the grain-filling stage (ability to maintain grain weight under heat) under controlled conditions. A few pairs showed a significant contrast for the single grain weight response, including Drysdale × Waagan, which showed an 11% difference. Therefore, the population derived by crossing these varieties was chosen for the genetic study of heat tolerance, described in Chapter 6. The Drysdale × Waagan mapping population consisted of 184 F_1-derived doubled haploid (DH) lines, and was originally made by NSW-DPI Wagga Wagga for mapping drought tolerance derived from Waagan, using a grant from the NSW BioFirst scheme.

Both parents are elite Australian bread wheat varieties. Drysdale (Hartog*3/Quarrion) was released by GrainGene (AWB Limited, GRDC, Syngenta and CSIRO) in 2002. It was the first variety to be bred for increased water use efficiency by selecting the carbon isotope discrimination trait (Rebetzke et al. 2002; Richards 2006). It is best adapted to low/medium rainfall areas of Southern New South Wales and has also performed well in Victoria and South Australia (http://www.awb.com.au/; http://www.csiro.au/). Waagan (Janz/24IBWSN-244; 24IBWSN-244 being a CIMMYT line) was released by the NSW Department of Primary Industries in 2007. Waagan was reported to be one of the best performing varieties in NSW, particularly in the north of the state, where it ranked second only behind Suntop for yield in main-season sown (after 14 May) trials from 2008 to 2012 (Matthews et al. 2013; Matthews et al. 2015).

In addition to the contrast observed between Drysdale and Waagan for the single grain weight response to heat, the Drysdale × Waagan population has at least four other features which make it attractive for genetic studies of heat tolerance. First, as part of the NSW BioFirst funded project (Dr. Livinus Emebiri), the population plus two plants of each parent variety had been scored for a 9K iSelect SNP array (Cavanagh et al. 2013), which provided an opportunity to construct a high density molecular marker genetic map. Second, the transcriptomes of the parent varieties had been subjected to next-generation sequencing at the ACPFG, and these data are likely to help in generating additional markers, if they are required in the future, as well as in identifying candidate genes. Third, 3,000 RILs from the same cross had been made at NSW-DPI, and recombinants could be selected from among these lines to

provide additional genetic resolution for any tolerance QTL identified. Fourth, based on diagnostic molecular markers, the parent varieties were known to be closely matched for alleles at major flowering time loci (Howard Eagles and Karen Cane, personal communication; this study), suggesting that individuals from the population will have a narrow range of flowering times. The population segregated for major dwarfing loci (*Rht-B1* and *Rht-D1*) which provided the opportunity to test whether the observed association of the plant height and grain weight maintenance under heat conditions in Chapter 3 occurred by chance or due to a real genetic link between these traits. There are contradictory reports linking GA-insensitive dwarfing alleles (i.e. *Rht-B1 and Rht-D1*) to reduced abiotic stress tolerance (Alghabari et al. 2014; Blum et al. 1997; Butler et al. 2005; Law and Worland 1985) and segregation of these loci in the Drysdale × Waagan population provided the opportunity to further test the association of this particular type of height variation with heat tolerance.

Construction of molecular marker maps in wheat has previously been laborious and difficult, owing to the large and polyploid nature of the wheat genome, the low frequency of DNA sequence polymorphism (Gupta et al. 2008), and the need to screen markers individually (e.g., using restriction fragment length polymorphism markers or RFLPs, or simple sequence repeat markers or SSRs). Most recently, advances made in DNA sequencing technologies have facilitated the discovery of large numbers of single nucleotide polymorphisms (SNPs) in wheat. These represent the most abundant form of DNA sequence variation, are relatively evenly distribution throughout the genome and are amenable to high throughput array-based detection (Berkman et al. 2012). Through the efforts of the International Wheat SNP Working Group (http://wheatgenomics.plantpath.ksu.edu), SNPs were discovered and then used to construct a marker array containing almost 9,000 features (Cavanagh et al. 2013), and then a subsequent version containing 90,000 features (Wang et al. 2014), scorable using the iSelect technology. In allowing such a large number of markers to be scored in parallel, with a high degree of accuracy and at relatively little cost, arrays such as these have enhanced our capability for genetic analysis in new wheat populations. This new technology therefore provides the opportunity to conduct genetic studies of heat tolerance in a new wheat population of choice (Drysdale × Waagan), without the need for a laborious and time consuming step of generating data for individual molecular markers. Nonetheless, the volume of data generated by the 9K array presents particular data management and computational challenges, requiring use of mapping software that can handle such a volume of data (e.g. R/qtl), and an efficient data processing pipeline.

For the aforementioned reasons, the Drysdale × Waagan population was chosen for the greenhouse-based study on the genetics of heat tolerance at the grain-filling stage described in Chapter 6. The current chapter describes the construction of the Drysdale ×Waagan molecular marker genetic map using the 9K iSelect SNP data – a prerequisite for the genetic analysis. At least one of the parent varieties was found to be heterogeneous for particular chromosome regions, requiring a strategy to be developed to account for this in map construction.

5.2 Materials and Methods

5.2.1 Plant material (parents and DH population)

The Drysdale × Waagan mapping population of 184 F_1-derived doubled haploid (DH) lines was provided by Livinus Emebiri and Peter Martin, NSW-DPI. There were 13 subpopulations (H, F, K, R, B, C, G, J, D, A, M, L, and P), each derived from a separate F_1 plant. These had 31, 29, 24, 18, 13, 11, 11, 11, 9, 8, 8, 6 and 5 individuals, respectively. In each cross, Drysdale was used as the female parent and Waagan as the male. Self-seed had been harvested separately from all six female (Drysdale) plants used in crossing, although which of these plants corresponded to each F_1 was not recorded. Seed had also been collected from 10 Waagan plants. These were not necessarily the plants used as male parents, although these plants and the male parents had all been grown from the same seed packet.

5.2.2 DNA extraction

Extraction of the DNA for the SNP analysis was done at NSW-DPI using an unknown protocol. DNA for assaying parents and DH lines for phenology and height loci was extracted at the ACPFG, using a protocol similar to that described in Chapter 3.

5.2.3 Markers for flowering time and height loci

5.2.3.1 *Vrn-A1*

The primer pair BT468/BT486 described by Eagles et al. (2010) was used to assay variation in the promoter region of *Vrn-A1* gene. It gives a product of ~ 400 bp from the most common spring allele (spring allele; *Vrn-A1a*) which contains a promoter duplication, a ~ 200 bp product from the *Vrn-A1v* winter allele, and a ~ 180 bp product from the other spring allele *Vrn-A1b* (Eagles et al. 2010). PCR reactions were performed in a volume of 15 µl in a MJ Research Thermocycler (Waltham MA, USA). The reaction mixture contained 100 ng template DNA, 1× New England BioLabs ThermoPol Reaction Buffer, 0.2 mM of each dNTP, 5% dimethyl sulfoxide, 0.25 µM of each primer and 0.5U Taq DNA Polymerase (produced by Mamoru Okamoto in ACPFG). The following cycling conditions were used: an initial denaturing step of 3 min at 94 ºC, 40 cycles of 10 s at 94 ºC, 10 s at 65 ºC and 1 min at

72 ºC, and a final extension step of 10 min at 72 ºC. The PCR products were separated on 3% agarose gels. Gels contained Invitrogen SYBR® Safe stain to visualize DNA over a UV transilluminator.

5.2.3.2 *Vrn-B1*

A mixture of the three primers Intr1/B/F, Intr1/B/R3 and Intr1/B/R4 were used in one reaction to detect variation in intron-1 of the *Vrn-B1* gene (Fu et al. 2005). This primer mixture gives a product of 709 bp from the spring allele (containing a deletion) and a product of 1,149 bp from the winter allele (without the deletion) (Fu et al. 2005). The following cycling conditions were used: an initial denaturing step for 3 min at 94 ºC, 40 cycles of 10 s at 94 ºC, 10 s at 55 ºC and 2 min at 68 ºC, and a final extension step of 10 min at 68 ºC. The PCR products were separated on 1.5% agarose gels. Other details were the same as those described for the *Vrn-A1* marker.

5.2.3.3 *Vrn-D1*

A mixture of the three primers Intr1/D/F, Intr1/D//R3 and Intr1/D/R4 were used in one reaction to detect variation in intron-1 of the *Vrn-D1* gene (Fu et al. 2005). This primer mixture gives a product of 1,671 bp from the spring allele (containing a deletion) and a product of 997 bp from the winter allele (without the deletion) (Fu et al. 2005). Cycling conditions were similar to those described for *Vrn-B1* except that 60 ºC was used for annealing, and the extension time was for 2.30 min. The PCR products were separated on 1.5% agarose gels. Other details were the same as those described for the *Vrn-A1* marker.

5.2.3.4 *Ppd-B1*

At least five *Ppd-B1* alleles have been defined, namely *Ppd-B1a*, *Ppd-B1b*, *Ppd-B1c*, and *Ppd-B1d*, which have three, two, four, and one copies of the gene, respectively, and *Ppd-B1e*, which is a null allele (Díaz et al. 2012; Cane et al. 2013). According to Cane et al. (2013), Drysdale carries *Ppd-B1b* and Waagan carries *Ppd-B1c*. Across a broad set of wheat varieties they calculated that, relative to the *Ppd-B1b* allele, the average effect of the *Ppd-B1c* allele was to shorten the time to heading by 1.3 days. In the current study, a SNP in exon 3 was assayed. This SNP was found by Beales et al. (2007) to distinguish *Ppd-B1c* from other alleles (nucleotide A in *Ppd-B1c* vs. G in others). KASP primers used to assay this SNP had been developed by an international consortium (Susanne Dreisigacker, CIMMYT; Gina Brown-Guedira, USDA; Peter Sharp, University of Sydney; Keith Edwards, University of Bristol; Simon Griffiths, Dave Laurie, Cristobal Uauy, JIC; unpublished) and their sequences were obtained from CIMMYT (Susanne Dreisigacker). Other details for the *Ppd-B1* marker were the same as those described for the *Rht-B1* and *Rht-D1* markers in Chapter 3.

5.2.3.5 *Ppd-D1*

A KASP marker (sequences of primers developed by the aforementioned international consortium) was used to check for the presence of an insertion/deletion polymorphism in the *Ppd-D1* gene. The deletion of 2,089 bp, located upstream of the coding region, is diagnostic of one type of mutation that produces a photoperiod insensitive allele ('Ciano 67' allele type) (Beales et al. 2007). Other details for the *Ppd-D1* marker were the same as those described for the *Rht-B1* and *Rht-D1* markers in Chapter 3.

5.2.3.6 *Rht-B1* and *Rht-D1* (previously known as *Rht1* and *Rht2*)

Sequences of primers and other details were the same as those described for the *Rht-B1* and *Rht-D1* markers in Chapter 3.

5.2.3.7 *Rht8*-linked *gwm261* microsatellite marker

Primers for the *Rht8*-linked *gwm261* microsatellite marker (Röder et al. 1998) were used to infer which alleles were present at the *Rht8* locus. The 192 bp fragment size is most often associated with the dwarfing allele at *Rht8* (Korzun et al. 1998; Worland et al. 1998), but not always (Ellis et al. 2007). Conditions were similar to those described for *Vrn-A1* except that 55 ºC was used for the annealing step.

5.2.3.8 SNP data

Two plants of each parent variety (Drysdale, represented by DNA samples WSNP9K.091.08648 and WSNP9K.091.08660; Waagan: represented by DNA samples WSNP9K.091.08672 and WSNP9K.091.08684) and all of the DH lines were typed for the 9K SNP array. The SNP assay (9K iSelect SNP assay) was performed by Matthew Hayden (Department of Primary Industries, Victorian AgriBiosciences Centre).

5.2.4 Map construction

Initial data processing and map construction was performed using R/qtl software (Broman et al. 2003) and the WGAIM package (Taylor et al. 2012), using the steps outlined in the Results and Discussion section. Chromosomes corresponding to each linkage group (and chromosome orientation) were identified using SNP consensus map (Cavanagh et al. 2013). Plots and the map graphic were drawn using software R/qtl (Broman et al. 2003; Broman 2010) and MapChart 2.1 (Voorrips 2002), respectively.

5.3 Results and Discussion

5.3.1 Markers in flowering time and dwarfing genes

All tested single plant selections of Drysdale and Waagan carried a winter allele at *Vrn-A1* (Table1, Figure 5.1), a spring allele at *Vrn-B1* (Table1, Figure 5.2), a photoperiod insensitive

allele at *Ppd-D1* (Table 5.1, Figure 5.3), and a *gwm261* microsatellite marker allele that is associated with *Rht8* non-dwarfing alleles (Table 5.1, Figure 5.4). By contrast, polymorphism was observed between the parents for *Vrn-D1*, *Ppd-B1*, *Rht-B1*, and *Rht-D1* genes (Table 5.1, Figures 5.5, 5.6a, and 5.7a and b). The population consisted of 37 double-dwarves, 68 semi-dwarves and 39 double-talls.

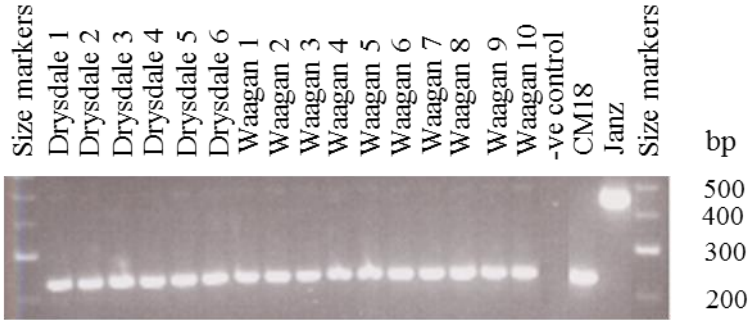


Figure 5. 1 A 3% agarose gel showing *Vrn-A1* PCR marker fragments from single plant selections of Drysdale and Waagan. Expected fragment sizes were ~400 bp (spring allele) or ~200 bp (winter allele). All of the Drysdale and Waagan selections carried the winter allele. –ve indicates the negative control (contains ultrapure water instead of template DNA), and CM18 (winter allele) and Janz (spring allele) were used as controls. The size marker lanes contain the 100 bp ladder HyperLadder II DNA size marker from Bioline.

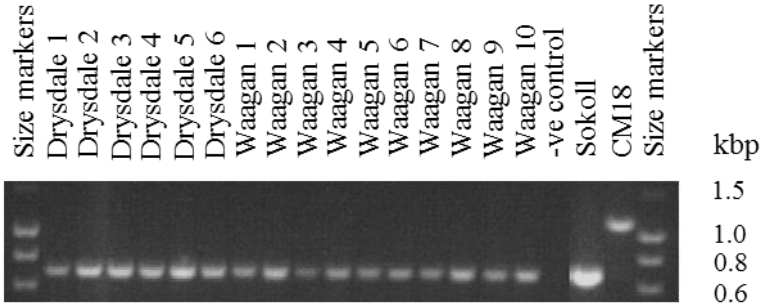


Figure 5. 2 A 1.5% agarose gel showing *Vrn-B1* PCR marker fragments from single plant selections of Drysdale and Waagan. Expected fragment sizes were 709 bp (spring allele) or 1,149 bp (winter allele). All of the Drysdale and Waagan selections showed the spring allele. –ve indicates the negative control (contains ultrapure water instead of template DNA), and Sokoll (spring allele) and CM18 (winter allele) were used as controls. The size marker lanes contain the 1kb HyperLadder I DNA size marker from Bioline.

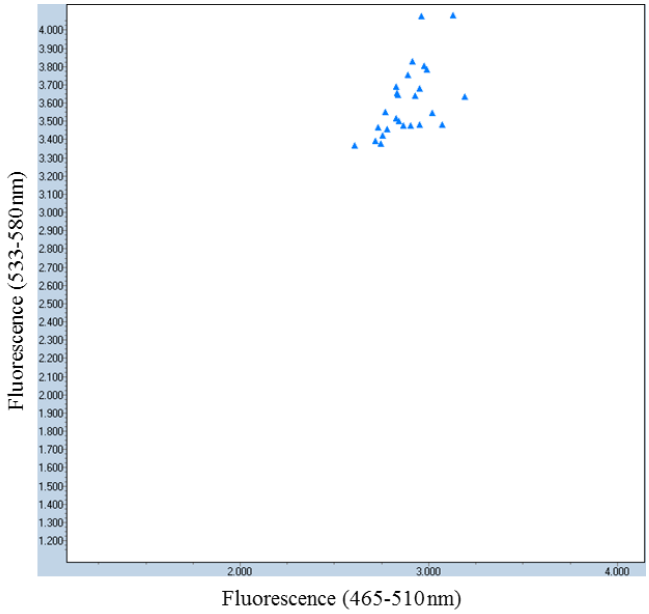


Figure 5. 3 Fluorescence data for a *Ppd-D1* KASP marker scored on Drysdale and Waagan single plant selections. Primers specific for the insensitive and sensitive alleles were labelled with FAM (fluorescence peak at wavelength 465-510 nm, blue) and VIC (fluorescence peak at wavelength 533-580 nm, green), respectively. Each dot represents a single plant and each single plant selection was tested 1-2 times (on separate plants). All tested single plant selections of Drysdale and Waagan carried an insensitive allele at *Ppd-D1*.

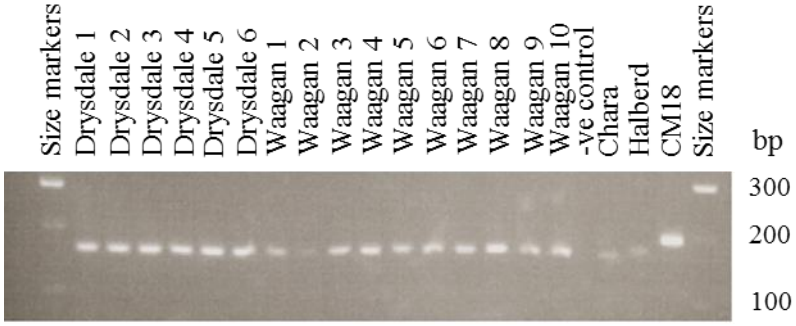


Figure 5. 4 A 3% agarose gel showing PCR marker fragments of the *gwm261* microsatellite marker, amplified from single plant selections of Drysdale and Waagan. CM18 has been reported to contain the *Rht8* dwarfing allele and to give the 192 bp *gwm261* fragment which is associated with *Rht8* dwarfing allele (Ellis et al. 2007). Chara and Halberd were also included as controls that give previously reported *gwm261* fragment sizes of 165 and 174 bp, respectively (Ellis et al. 2007). All of the Drysdale and Waagan selections showed a ~165 bp fragment, suggesting that they carried non-dwarfing *Rht8* alleles. In Chapter 5, no QTL for plant height mapped to this position on chromosome 2D, confirming lack of segregation for this gene in the DH population. The size marker lanes contain the 100 bp ladder HyperLadder II DNA size marker from Bioline.

At *Vrn-D1*, all of the Drysdale selections carried a spring allele, while the Waagan selections were heterogeneous (Table 5.1 and Figure 5.5). Waagan 1, 3, 5, 6, 7, and 9 had a winter *Vrn-D1* allele while Waagan 2, 4, 8, and 10 had a spring allele. When the whole population was scored for the *Vrn-D1* marker, all of the DH lines were seen to have a spring allele (data not shown). This indicates that only Waagan plants carrying a spring *Vrn-D1* allele contributed as parents to the population, or that such plants were used as parents but the winter *Vrn-D1* allele was not carried through to any mapping lines due to chance (e.g. in some of the smaller sub-populations).

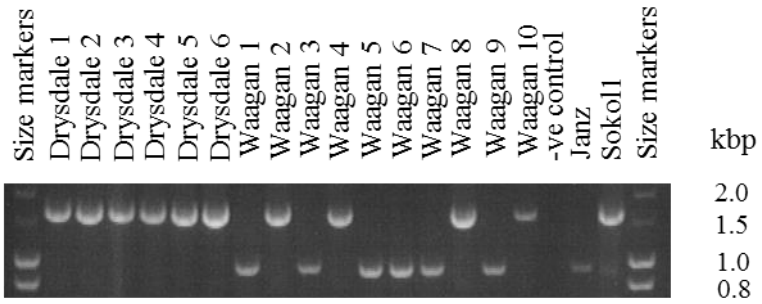


Figure 5. 5 A 1.5% agarose gel showing *Vrn-D1* PCR marker fragments from single plant selections of Drysdale and Waagan. Expected fragment sizes were 997 bp (winter allele) or 1,671 bp (spring allele). The Drysdale selections carried the spring allele while the Waagan selections were heterogeneous for winter/spring alleles at *Vrn-D1*. –ve control (contains ultrapure water instead of template DNA), and Janz (winter allele) and Sokoll (spring allele) were used as controls. The size marker lanes contain the 1kb HyperLadder I DNA size marker from Bioline.

At *Ppd-B1*, all of the Drysdale selections except Drysdale 1 carried *Ppd-B1b* allele (Table 5.1). Drysdale 1 appeared to be heterogeneous for this locus. One plant carried *Ppd-B1b* allele while the other plant carried *Ppd-B1c* allele type. All of the Waagan plants except Waagan 5 carried *Ppd-B1c* allele (Table 5.1). Waagan 5 appeared to be heterogeneous for this locus. One plant carried *Ppd-B1c* allele type while the other plant carried an unknown allele (Table 5.1, Figure 5.6). In the population, the markers for *Ppd-B1* segregated in a manner that significantly deviated from the expected 1:1 ratio ($p<0.05$) and skewed in favour of the Drysdale parent. In Chapter 5, no QTL for flowering time associated with the *Ppd-B1* marker position which may suggest there was no functional polymorphism for *Ppd-B1*segregating in this population.

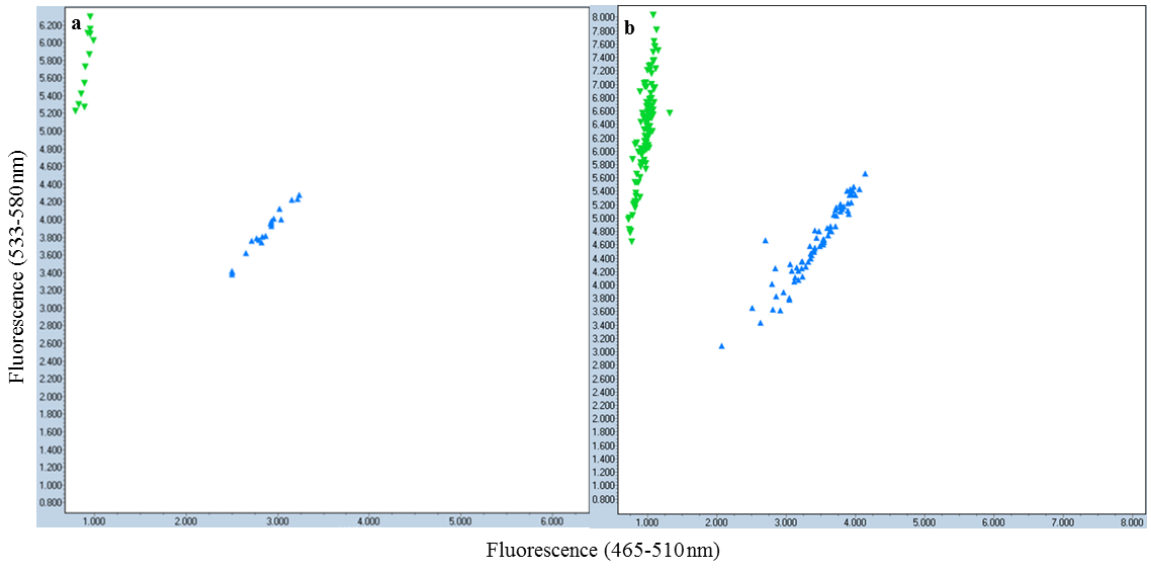


Figure 5. 6 Fluorescence data for a *Ppd-B1* KASP marker scored on Drysdale and Waagan single plant selections (a) and the DH population (b). Primers specific for the *Ppd-B1c* vs. other alleles were labelled with FAM (fluorescence peak at wavelength 465-510 nm, blue) and VIC (fluorescence peak at wavelength 533-580 nm, green), respectively. Each dot represents a single plant and each single plant selection was tested 2 times (on separate plants). All Drysdale selections, except Drysdale 1 which was heterogeneous for this locus, carried *Ppd-B1b* allele (green triangles) while all Waagan selections, except Waagan 5 which was heterogeneous for this

locus, carried a *Ppd-B1c* allele (blue triangles). Each DH line was scored only once. Lines were assigned as having the *Ppd-B1c* allele (blue; b) or *Ppd-B1b*/unknown allele (green; b).

The Drysdale selections carried a wild type allele at *Rht-B1* and a dwarfing allele at *Rht-D1*, while the reverse was the case for the Waagan selections (Table 5.1). In the population, the markers for *Rht-B1* and *Rht-D1*each segregated in a manner that was not inconsistent with an expected 1:1 ratio (p = 0.39 and p = 0.49, respectively). In Chapter 6, QTL for plant height mapped to the *Rht-B1* and *Rht-D1* marker positions, confirming that this population segregated for semi-dwarf alleles at these two loci.

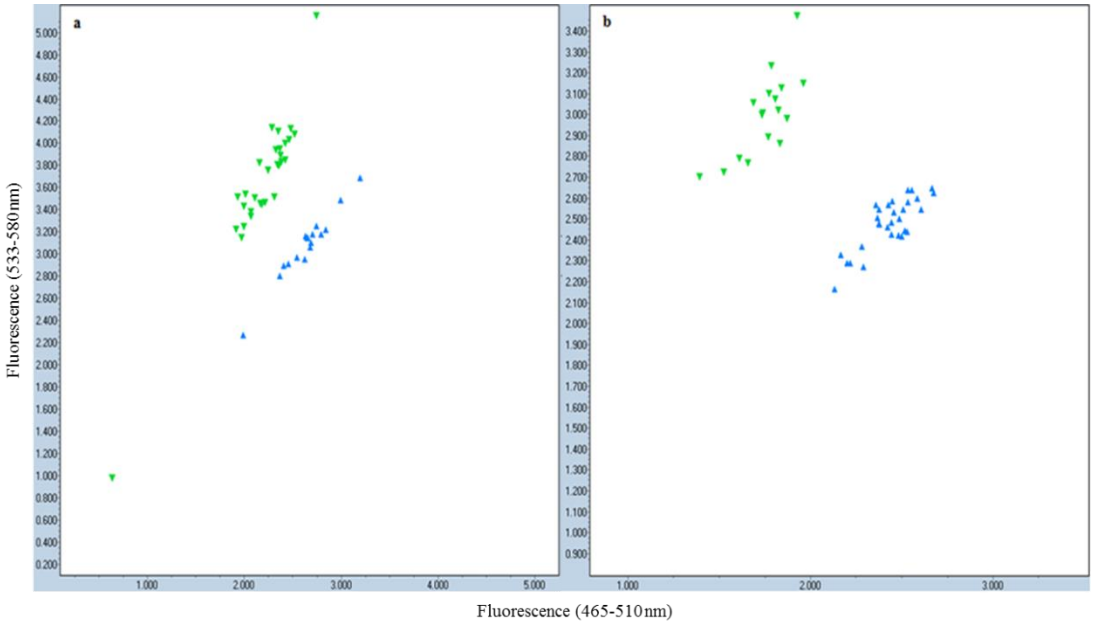


Figure 5. 7 Fluorescence data for *Rht-B1* (a) and *Rht-D1* (b) KASP markers scored on Drysdale and Waagan single plant selections. Primers specific for the wild type and mutant (dwarf) alleles were labelled with FAM (fluorescence peak at wavelength 465-510 nm, blue) and VIC (fluorescence peak at wavelength 533-580 nm, green), respectively. Each dot represents a single plant and each single plant selection was tested 2 to 3 times (on separate plants). Designations shown by blue or green were assigned based on the fluorescence intensities of the signal in the two wavelength ranges and the clustering patterns. The Drysdale selections carried the wild type allele for *Rht-B1* (blue triangles, a) and dwarfing allele for the *Rht-D1* (green triangles, b), while the reverse was true for the Waagan selections (green and blue triangles in a and b, respectively).

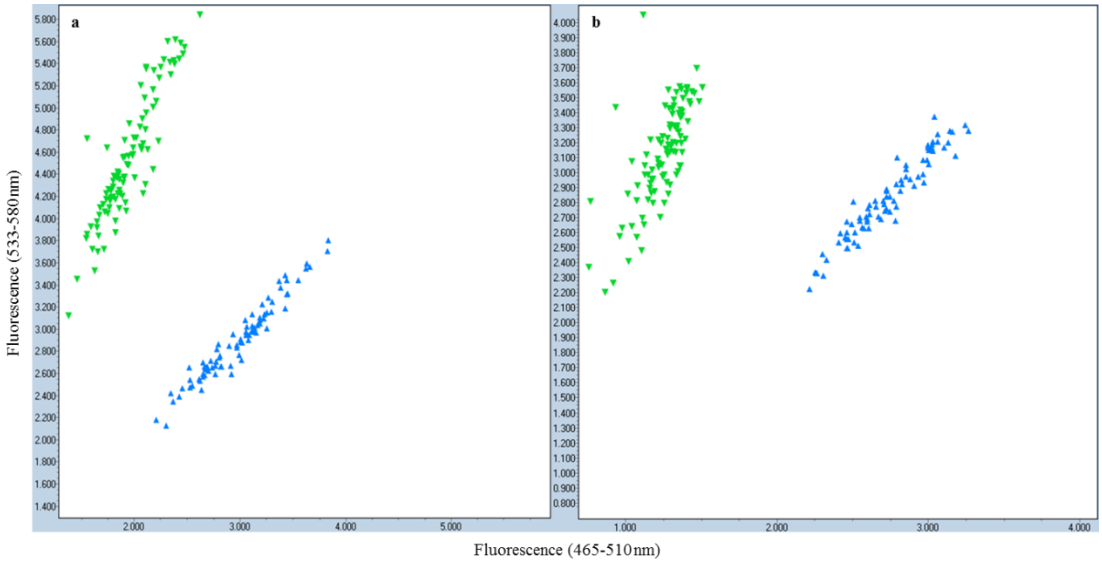


Figure 5. 8 Fluorescence data for *Rht-B1* (a) and *Rht-D1* (b) KASP markers scored on the DH lines of the mapping population. Each line was scored only once. Lines were assigned as having the wild-type allele (blue) or mutant dwarf allele (green). Other details are the same as in Figure 5. n = 183.

Table 5. 1 Alleles carried by single plant selections of Drysdale and Waagan for phenology loci, determined using diagnostic molecular markers at *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, *Ppd-B1*, *Ppd-D1*, *Rht-B1* and *Rht-D1* loci or inferred using a marker linked to *Rht8*.

Selection	*Vrn-A1*	*Vrn-B1*	*Vrn-D1*	*Ppd-B1*	*Ppd-D1*	*Rht-B1*	*Rht-D1*	*gwm261*
Drysdale 1	v	a	a	c/b	a	a	b	a
Drysdale 2	v	a	a	b	a	a	b	a
Drysdale 3	v	a	a	b	a	a	b	a
Drysdale 4	v	a	a	b	a	a	b	a
Drysdale 5	v	a	a	b	a	a	b	a
Drysdale 6	v	a	a	b	a	a	b	a
Waagan 1	v	a	v	c	a	b	a	a
Waagan 2	v	a	a	c	a	b	a	a
Waagan 3	v	a	v	c	a	b	a	a
Waagan 4	v	a	a	c	a	b	a	a
Waagan 5	v	a	v	c/unknown[Y]	a	b	a	a
Waagan 6	v	a	v	c	a	b	a	a
Waagan 7	v	a	v	c	a	b	a	a
Waagan 8	v	a	a	c	a	b	a	a
Waagan 9	v	a	v	c	a	b	a	a
Waagan 10	v	a	a	c	a	b	a	a

For *Vrn* loci, a and v represent the spring and winter alleles, respectively. For *Rht* genes, a and b represent the wild type and dwarfing alleles, respectively (or in the case of *Rht8*, the *gwm261* microsatellite allele that is most often associated with the tall or dwarfing allele, respectively). For the *Ppd-D1* gene, a represents the photoperiod insensitive allele. For *Ppd-B1* gene b and c represent *Ppd-B1b* and *Ppd-B1c*, respectively. c/b indicates that the selection was heterogeneous for the scored gene.

[Y] This line was heterogeneous for *Ppd-B1*. One selection of this line carried *Ppd-B1c* while another selection carried another allele that could not be distinguished from the results of this study.

5.3.2 Initial 9K SNPs data processing

Although it has been estimated that ~96% SNPs (8,632 markers) from the 9K array can give useful data (Cavanagh et al. 2013), in the present work only 7,759 markers had scores supplied. Some markers were omitted due to the detection of multiple-sequence variants or poor data quality.

The following were the steps used in initial processing of the data:

1. Markers that were monomorphic across all the lines (4,701 markers) were excluded.
2. Markers that had a different allele score in only 4 or fewer lines relative to the rest of the population were most likely inaccurately scored and were also excluded. The remaining 2,797 (~36%) SNPs were classified as polymorphic.
3. Following the convention, marker alleles from the maternal variety (Drysdale) were designated A, while those from the paternal variety (Waagan) were designated B. This included markers with presence/absence (AA or BB vs. Null) type scores (68 markers). The parental origin of each marker allele was determined by comparing to the scores for the two SNP-genotyped Waagan plants and two SNP-genotyped Drysdale plants.
4. All of the heterozygous (AB) marker calls were converted to missing data.
5. For 78 of the markers, the parental origins were not clear because they were monomorphic among the parental plants but polymorphic in the population, or were heterogeneous within both of the parental varieties. The markers were processed the same as the others until Step 2 of ‘Map Construction’ where their phases were assigned.
6. Duplicated lines were identified by comparing the scores for all possible pairs of DH lines. There were 33 groups (made from a total of 73 individuals), each containing two to four lines (Table 5.2). In each group, the members were identical for an unexpectedly large proportion (> 98%) of the polymorphic markers (represented by the outliers pairs circled in Figure 5.9. One line from each of the groups of identical or nearly identical lines was used for mapping molecular markers and the rest (40 lines) omitted, leaving 144 unique lines. The line with the least amount of missing data was chosen to represent each group.

Table 5. 2 Groups of highly similar DH lines. Each group contains lines which were identical for >98% of the polymorphic markers. In each group, the individual listed first is the one that was kept for map construction.

Group	DH line ID			
1	WW28436	WW28433	WW28434	WW28440
2	WW28383	WW28385	WW28390	
3	WW28424	WW28429	WW28415	
4	WW28442	WW28435	WW28437	
5	WW28448	WW28457	WW28506	
6	WW28546	WW28540	WW28542	
7	WW28368	WW28367		
8	WW28427	WW28418		
9	WW28378	WW28377		
10	WW28391	WW28394		
11	WW28444	WW28445		
12	WW28412	WW28426		
13	WW28443	WW28453		
14	WW28416	WW28430		
15	WW28363	WW28365		
16	WW28381	WW28384		
17	WW28464	WW28452		
18	WW28447	WW28458		
19	WW28514	WW28515		
20	WW28531	WW28533		
21	WW28507	WW28500		
22	WW28467	WW28460		
23	WW28483	WW28484		
24	WW28465	WW28461		
25	WW28488	WW28485		
26	WW28509	WW28494		
27	WW28472	WW28470		
28	WW28508	WW28503		
29	WW28526	WW28528		
30	WW28548	WW28545		
31	WW28473	WW28471		
32	WW28495	WW28487		
33	WW28522	WW28521		

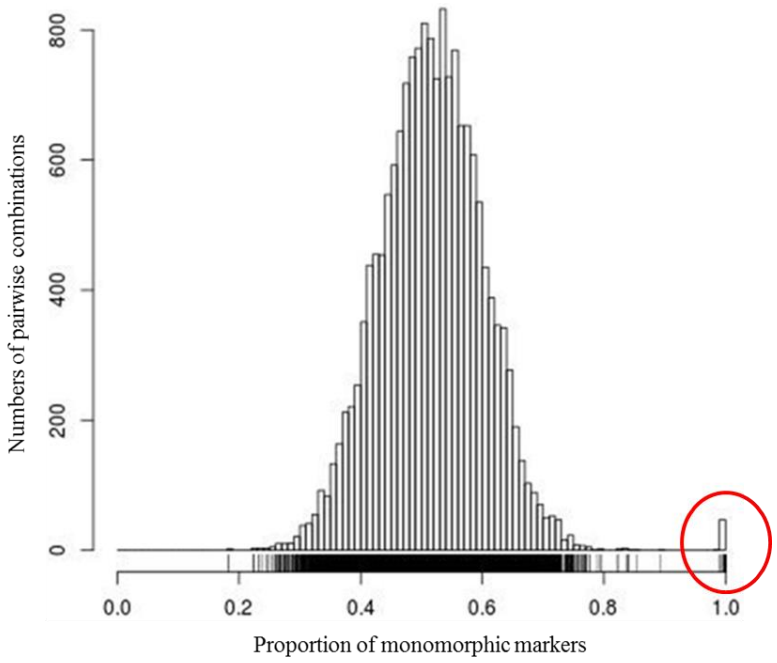


Figure 5. 9 Frequency histogram showing numbers of pairwise combinations of DH lines showing certain proportions of shared marker scores. The red circle indicates the DH line pairs that had identical allele scores for >98% of markers.

7. Three lines had a large proportion of missing data and were omitted from the analysis (ww28450, ww28454, ww28510; Figure 5.10).

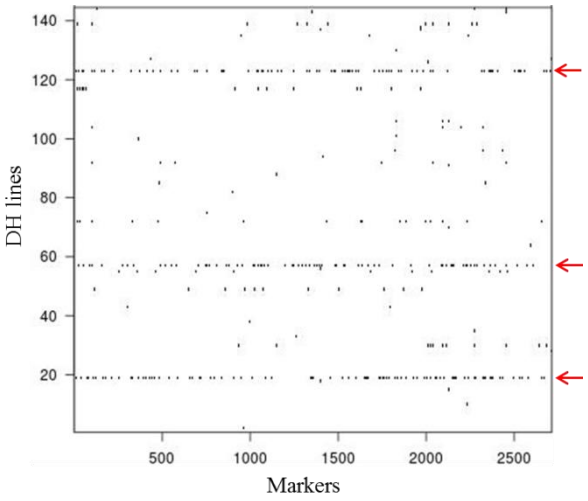


Figure 5. 10 Plot of missing scores. Black dots indicate missing scores. The three doubled-haploid (DH) lines indicated by red arrows were eliminated from the analysis.

8. Markers with large numbers of missing data points were also omitted. These were defined as markers that were genotyped for fewer than 135 DH lines (11 markers in total; Figure 5.11).

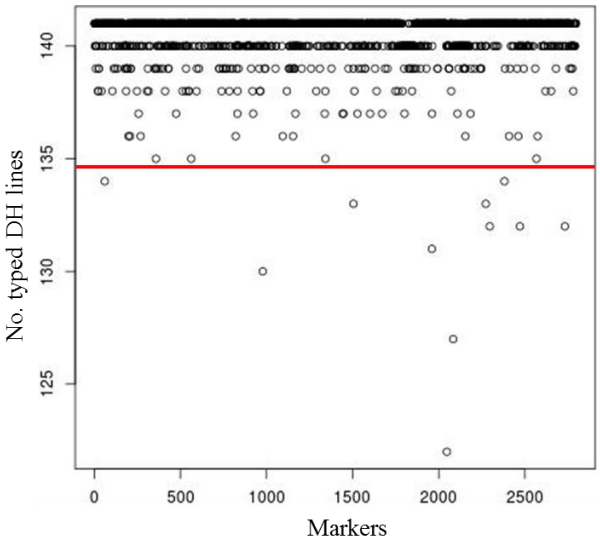


Figure 5. 11 Plot of number of typed doubled-haploid (DH) lines for each marker. Markers below the red line were omitted from the analysis. Number of DH lines = 141; 3 lines having large number of missing data were eliminated in the previous step.

5.3.3 Parent Heterogeneity

The two SNP-typed Waagan plants (represented by DNA samples WSNP9K.091.08672 and WSNP9K.091.08684) differed at only 26 (0.3%) of the total scored markers, while the two SNP-typed Drysdale plants (represented by DNA samples WSNP9K.091.08648 and

WSNP9K.091.08660) differed at 1,387 (~18%) of the scored markers. The first and second Drysdale plants differed from the Drysdale plant represented in the 9K SNP database (Cavanagh et al. 2013) at 20 and 8.3% of the markers, respectively, while there were no 9K SNP data available on this site for Waagan. This indicated a significant degree of heterogeneity in the Drysdale variety, but not in Waagan. However, given the small sample of 2 plants from Waagan, the presence of heterogeneity in Waagan cannot be ruled out. Indeed, analysis using the phenology markers showed this variety was heterogeneous at the *Vrn-D1* locus (Table 5.1). The observed heterogeneity within these varieties is not entirely surprising, since according to the Australian Plant Breeders Rights (PBR) regulations, a registered variety does not need to be 100% homozygous to be released, and many Australian wheat varieties are known to be heterogeneous at glutenin loci (Whiting 2004).

Also consistent with parental heterogeneity was the fact that some markers segregated in only a few sub-populations (70 of the 2,797 polymorphic markers segregated in only 1 to 4 sub-populations; Table 5.3). The majority of these markers clustered on specific intervals on chromosomes 1A, 2A, and 6B (positions inferred from the consensus map; Table 5.3), suggesting that these intervals were heterogeneous in one or each of the parental varieties, among the individual plants crossed to derive the population. Markers from the heterogeneous segments would be expected to lead to erroneous genetic distances and marker orders. Hence, the 70 markers segregating in only 1 to 4 sub-populations were eliminated from the analysis.

A further 199 markers segregated in only 5 to 11 of the 13 subpopulations and the majority of these markers belonged to specific intervals on chromosomes 1A, 2A, 3A, 4A, 4B, 5B, and 6B (Table 5.4). Non-segregation of these markers in two or more of the sub-populations may have been due to either parent heterogeneity or chance. Hence, these markers were kept in the analysis, but their scores in the monomorphic subpopulations were converted to missing data while their scores in the segregating subpopulations were retained.

The subsequently constructed map (see below), including the positions of markers affected by parent heterogeneity, was judged to be largely correct, based on a comparison to the consensus map (Cavanagh et al. 2013)(not shown). Hence, the aforementioned procedures largely succeeded in avoiding the potential errors of map construction caused by parent heterogeneity. The high fidelity and dense coverage provided by the 9K SNP markers allowed this issue to be recognized and dealt with to some extent; the procedure used here to avoid disruption to the map by these types of markers could be recommended in other similar situations. However, it is still possible that some errors remained, and the procedure may have led to deletion of some markers for the wrong reason; the impact of heterogeneity in the

parents cannot be traced with certainty as DNA/seeds of the parent plants of each sub-population were not collected. Doing so for future populations would be preferable, as it would make the map construction procedure simpler, and could reduce error further. Each SNP marker is only bi-allelic. Use of other marker types allowing detection of >2 alleles (e.g., SSRs) could have helped reveal the number of unique haplotypes contributed to the population by the heterogeneous parents, at any given chromosome position.

Table 5. 3 Summary of markers that segregated in only 1 to 4 subpopulations. Marker positions are according to the consensus map deposited on the website of The Australian Wheat & Barley Molecular Markers Program (www.markers.net.au).

Chromosome	Position (cM)	Number of markers	Number of segregating subpopulations
1A	37-57	17	1-2
1B	20.7	1	1
1D	27.16	1	1
2A	365-375	11	2
3A	6.46 and 30.82	2	1
3B	19.67 and 56.02	2	1-2
4A	192.35, 207.89, 238.39 and 238.58	4	3-4
4B	20.64, 126.06 and 129.48	3	2-3
6B	48-245	27	3-4
Non-designated	-	2	1-4
Total		70	

Table 5. 4 Summary of markers that segregated in only 5 to 11 subpopulations. Marker positions are according to the consensus map.

Chromosome	Position (cM)	Number of markers	Number of segregating subpopulations
1A	1.19,16-19, and 174-190	24	9-11
1B	132.02	1	11
2A	305-375	19	5-11
2B	368.17 and 369.6	2	5-11
2D	64.89, 133.82 and 159.87	5	9-11
3A	43.48, 57.57 and 208-227	12	7-9
3B	88.96, 101.23 and 239.53	5	7-8
4A	5.72, 6.29, 15.87, 115.03 and 211-221	10	8-11
4B	5-81	23	5-11
5A	187.62	1	9
5B	180-186	9	11
6A	1.11 and 121.06	2	6
6B	48-57 and 133-243	83	5-10
7A	192.87 and 268.03	2	7-10
Non-designated	-	1	9
Total		199	

5.3.4 Markers with segregation distortion and genotype frequencies

Out of 2,716 remaining markers, 6 were highly significantly distorted ($p<1e^{-4}$) and 2 were suspected of having genotyping errors, so they were eliminated from the analysis. According to the consensus map, these markers occurred on chromosomes 1B, 2A, 2B, 6A and 7A and those on the same chromosomes were not closely linked. The most likely explanation for the distorted segregation of these markers are inaccurate scoring of these markers or detection of multiple loci by individual markers. For the remaining markers AA or BB scores occurred at frequencies of between 0.2 and 0.8 (Figure 5.12). On average, maternal Drysdale alleles (A) occurred at a frequency of 50.8%, while paternal Waagan alleles (B) occurred at a frequency of 49.2%.

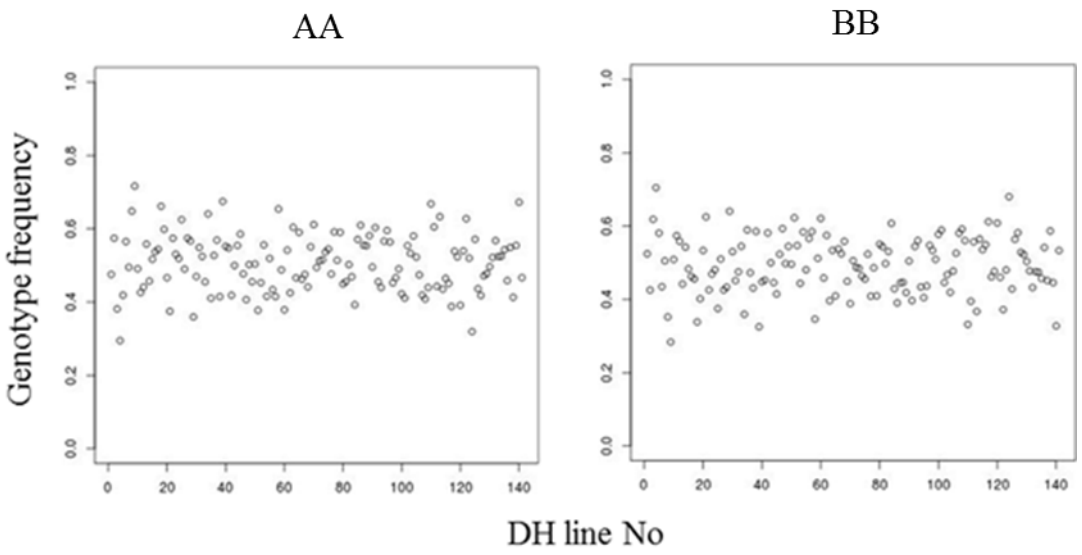


Figure 5. 12 Genotype frequencies by individual. The frequency of BB is simply 1 minus the frequency of AA. DH, doubled-haploid.

5.3.5 Map construction

After processing the data in the aforementioned ways, the following steps were taken to construct the map:

1. Markers with identical scores (including missing scores in the same line) were identified using the "findDupMarkers" function in R/qtl with setting "exact.only = TRUE". This identified 923 non-redundant groups. One marker from each of these groups was used for map construction.

2. Markers were grouped into linkage groups using a minimum LOD (logarithm of odds) of 5 for linkage, and a maximum recombination frequency of 0.4. Some pairs of linkage groups were found to be associated with one another by a high LOD score but a recombination frequency greater than 0.5, indicating that the markers from these linkage groups were scored in opposite phase. Within each such pair, the linkage group in the wrong

phase was identified as the one composed mostly of markers whose phase was originally uncertain based on the parental genotypes (see point 8 of section Initial 9K SNPs data processing). The phase of the markers from those linkage groups (total 26 markers) was corrected and the linkage groups re-formed, making 39 linkage groups. A heatplot showing LOD scores for linkage and recombination fractions for pairs of markers (Figure 5.13) indicated that the allele phases at that point were largely correct.

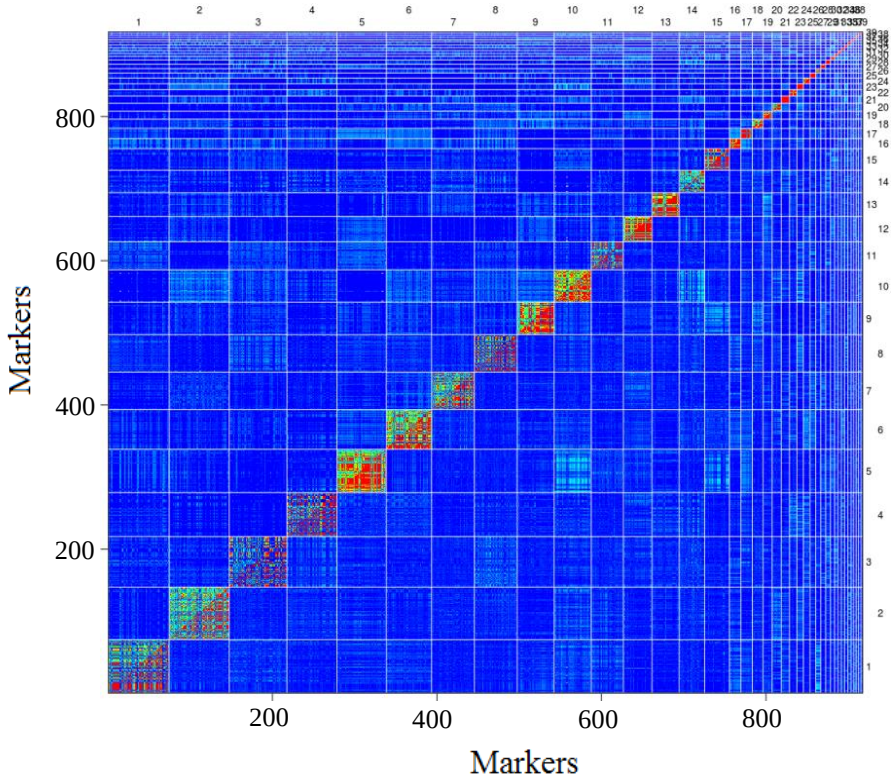


Figure 5. 13 Heatplot indicating recombination fractions (upper-left half of figure) and LOD scores for linkage (lower-right half of figure) for all pairs of markers. Markers are arranged in numbered linkage groups from the largest to the smallest linkage group. Markers are located randomly within the linkage groups, as they have not been ordered at this stage. LOD score increases and recombination fraction decreases with progression through the colour series blue-green-yellow-red. The lack of green, yellow or red signals in the lower-right half of the figure (lower-right triangle) shows that the allele phase of the markers is largely correct.

3. Markers were ordered within linkage groups using the "orderMarkers" and "Ripple" functions in the R/qtl software. The former establishes a rough order and the latter explores alternate orders to improve the marker order. According to Figure 5.14, markers seemed to be largely in the right order and in the right phase.

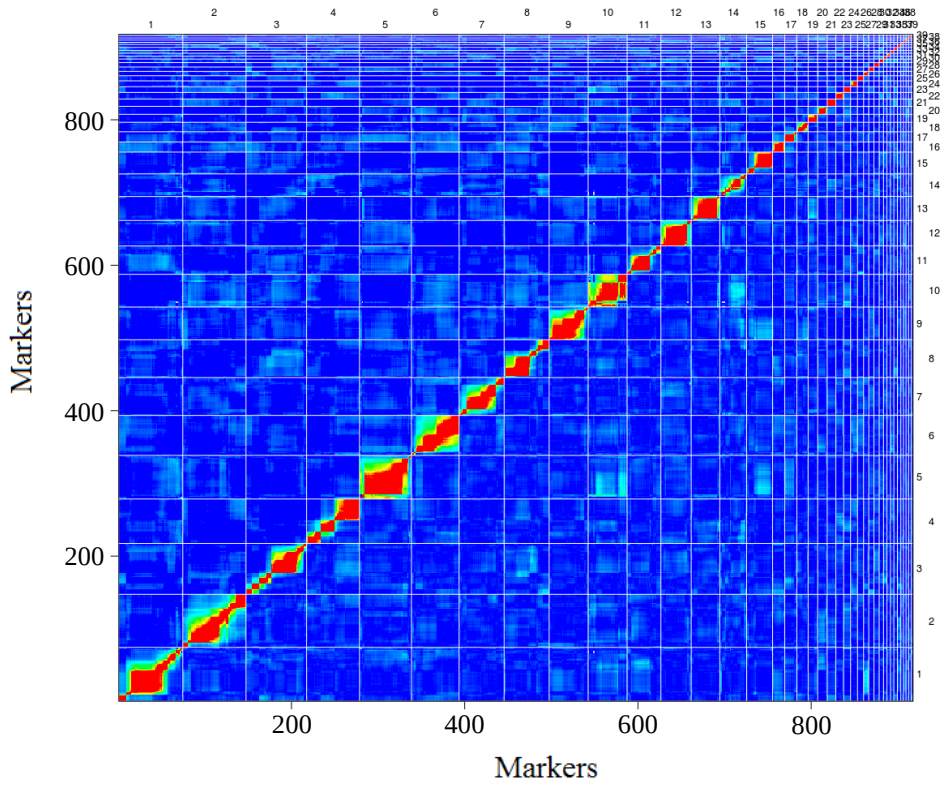


Figure 5. 14 Figure 5.14 Heatplot indicating the recombination fractions (upper-left half of figure) and LOD scores for linkage (lower-right half of figure) for all pairs of markers after the markers had been ordered. LOD score increases and recombination fraction decreases with progression through the colour series blue-green-yellow-red. Alignment of the red signals along the diagonal indicates that the marker orders are largely correct.

4. The "calc.errorlod" function in R/qtl was applied to identify 'singleton' scores resulting from highly improbable double crossovers (error LOD > 4). These scores have a high chance of being errors and were therefore converted to missing data. Figure 5.15 illustrates an example of three such scores on linkage group 1, which were subsequently eliminated.

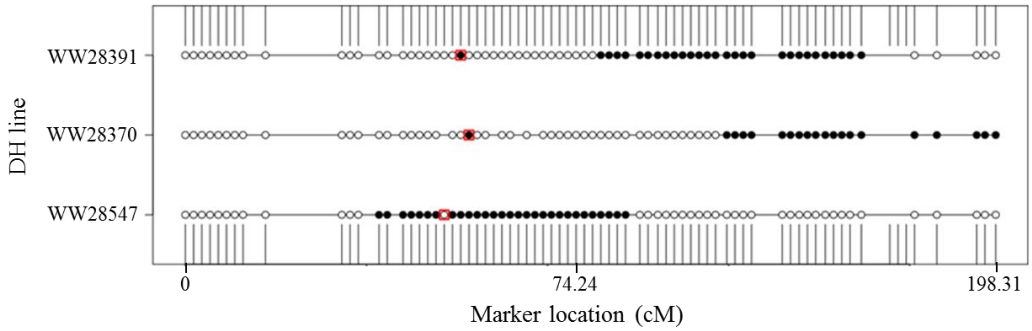


Figure 5. 15 Marker scores for 3 doubled-haploid (DH) lines on linkage group 1 with potential erroneous marker scores flagged by red squares. White and black circles correspond to AA and BB marker scores, respectively. The absence of a circle at a marker position indicates a missing score.

5. There were two DH lines showing more than 40 crossovers (WW28380 and WW28535), while the remaining individuals showed only 10-35 crossovers (Figure 5.16).

The former were considered likely to contain a large number of false scores (e.g. due to poor DNA quality) and were therefore omitted.

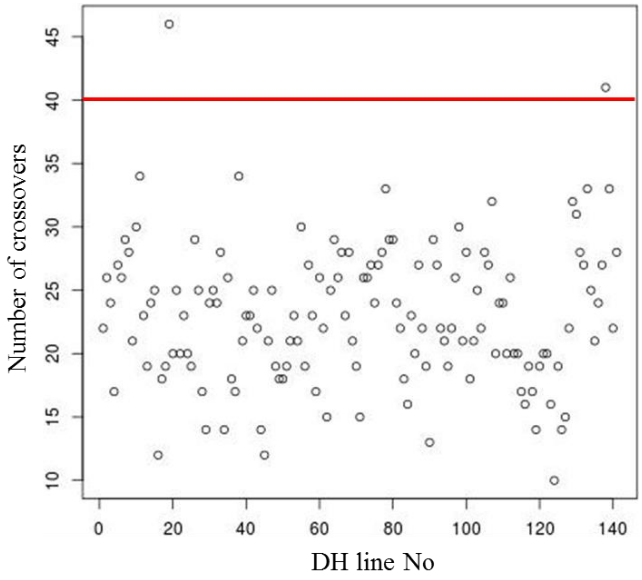


Figure 5. 16 Number of observed crossovers in each doubled-haploid (DH) line. DH lines with more than 40 crossovers (above the red line) were omitted.

6. After eliminating unlikely double crossovers and the two DH lines showing large numbers of crossovers, the markers were reordered. This helped to improve the map position of a few of the markers, as judged by comparison to the consensus map.

7. Linkage groups were oriented by aligning markers to the consensus map. The linkage groups were oriented so the short arm was at the top and began at the zero cM position. The marker orders were also compared with those of the consensus maps. In most cases the marker orders corresponded with those in the consensus map (not shown).

8. Recombination fractions were converted to cM distances using the Kosambi mapping function.

9. Many markers mapped to the same position, indicating that they differed only for the pattern of missing scores. The "findDupMarkers" function was therefore applied again, this time choosing the "exact.only = FALSE" option, which finds the markers that have no differences in their available marker scores. This reduced the number of non-redundant markers from 923 to 548. To utilize all available scores, consensus scores were determined for each group of co-localizing markers using the "fix.map" function of the WGAIM package (Taylor et al. 2012; Taylor 2013). These scores were then used to form the new map of 548 non-redundant loci (Figure 5.17).

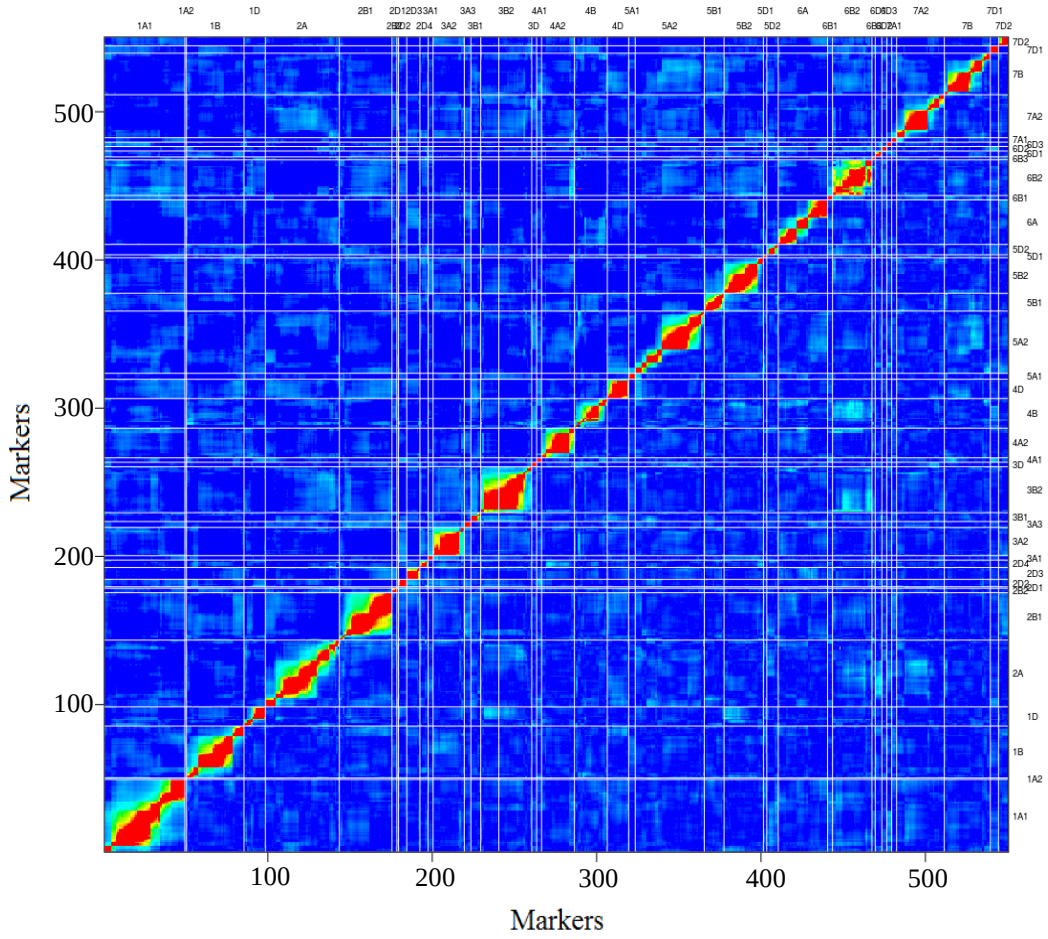


Figure 5. 17 Heatplot indicating the recombination fractions (upper-left half of figure) and LOD scores (lower-right half of figure) for all pairs of genetically non-redundant markers. Markers are arranged in order and by chromosome or chromosome fragment, from chromosome 1A (left) to 7D (right). LOD score increases and recombination fraction decreases with progression through the colour series blue-green-yellow-red. The irregular pattern on chromosome 6B probably reflects the large amount of parent heterogeneity (Table 4) and resulting missing data on this chromosome.

5.3.6 Mapping of markers for *Ppd-B1*, *Rht-B1* and *Rht-D1*

After SNP map construction, the *Ppd-B1*, *Rht-B1* and *Rht-D1* marker data were added to the SNP data. When the linkage groups were reformed, using a minimum LOD for linkage of 5 and a maximum recombination frequency of 0.4, the *Ppd-B1*, *Rht-B1* and *Rht-D1* markers located to the expected chromosomes (2B, 4B and 4D respectively; they added to the number of non-redundant loci). The markers were then reordered just on chromosomes 2B, 4B and 4D, and recombination fractions converted to cM, to establish the locations of *Ppd-B1*, *Rht-B1* and *Rht-D1* on the maps (Figure 5.18).

5.3.7 The map

The map has a total length of 2,447.4 cM and an average spacing of 4.4 cM between neighbouring markers, and all 21 chromosomes were represented (Table 5.5). Markers were well distributed across all chromosomes except chromosome 3D which only had 3 loci covering 3 cM. Chromosomes 1A, 2B, 2D, 3A, 3B, 4A, 5A, 5B, 5D, 6B, 6D, 7A, and 7D were fragmented into multiple linkage groups, with chromosome 2D represented by the most

linkage groups (four). Large gaps (30.8 to 37.8 cM) within linkage groups were also found on chromosomes 2A, 2B, 3B, 5A, 6A, and 7B (Table 5.5). Chromosome 1A had the most mapped loci (77 unique markers) and the chromosome 5A map was the longest (224.4 cM). The A and B genomes were represented by 252 and 221 unique loci, and total lengths of 998.0 and 1085.2 cM, respectively. The D genome was represented by 78 unique loci and a total length of 364 cM (Table 5.6). The average lengths of intervals between markers on the A, B, and D genomes were 4.0, 4.9 and 4.7 cM, respectively (Table 5.6). Marker distribution among the homoeologous groups varied from 10 to 18% of the unique mapped loci. Homoeologous chromosome groups 2 and 5 had the largest number of unique loci and greatest coverage (100 markers and 425.0 cM, and 91 markers and 445.0 cM, respectively). Homoeologous chromosome group 4 had the fewest unique loci and shortest total length (56 markers; 280.2 cM; Table 5.6).

Linkage groups were most fragmented on the D genome. In general, this is the genome with the most sequence conservation among wheat genotypes, and its low level of polymorphism makes it hardest to map (Langridge et al. 2001). The results obtained here are in accordance with the earlier published maps in which the D genome was only partially covered (Akbari et al. 2006; Cadalen et al. 1997; Chalmers et al. 2001; Paillard et al. 2003; Semagn et al. 2006).

Large gaps (30.8 to 37.8 cM) were also observed in the map on the A and B genomes. These gaps reflect lack of polymorphism between the parents in these regions; based on the consensus map, markers in those intervals were available on the 9K SNP array. The absence of polymorphism in those regions might be because those segments in the parents were identical due to descent from a common ancestor.

Many molecular marker linkage maps have been published in wheat (http://wheat.pw.usda.gov/ggpages/map_summary.html). The Drysdale × Waagan map covers 2,447.4 cM which is similar to the consensus maps of Somers et al. (2004) (2,569 cM), and Semagn et al. (2006) (2,595.5 cM). However, it is significantly shorter than other maps, of 2,937 to 4,110 cM (Akbari et al. 2006; Chalmers et al. 2001; Paillard et al. 2003; Quarrie et al. 2005), and the ITMI population map of 5,256 cM (reviewed by Langridge et al. 2001), which has been regarded as the most comprehensive map in wheat in terms of genome coverage. However, a longer map does not necessarily mean a better quality map, because a longer map may arise from more scoring errors.

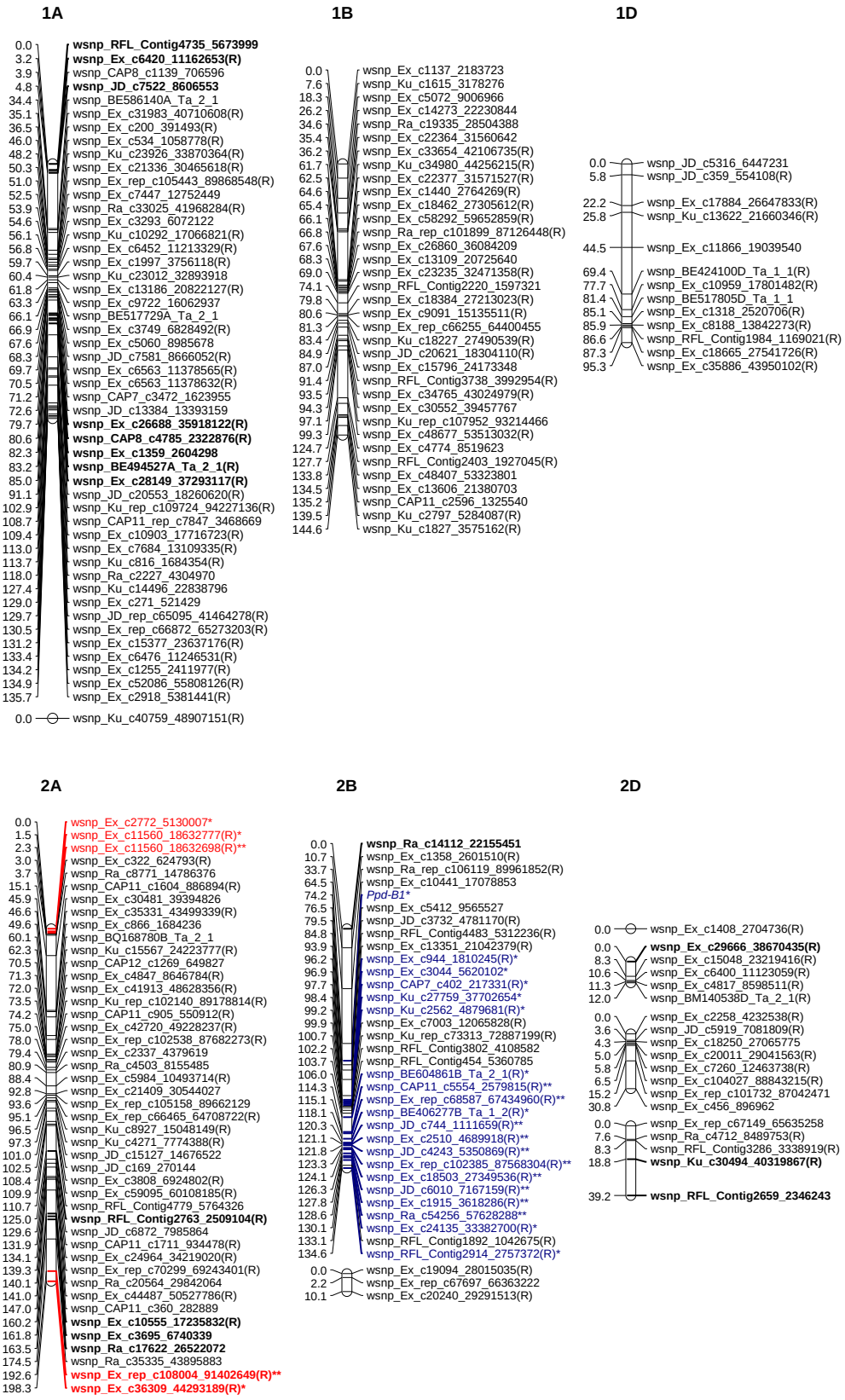


Figure 5. 18 The genetic linkage map made from 139 Drysdale/Waagan DH lines and 550 genetically non-redundant markers. An R in parenthesis after the marker name indicates that the marker is the representative of a group of genetically redundant markers. Linkage groups were ordered and oriented along each chromosome by aligning to the wheat consensus SNP map, so that the end of the short arm was at the top. The numbers to the left of each linkage group indicate cM distances from the top of each linkage group. Markers in Bold were segregating in only 5 to 11 subpopulations and may therefore identify chromosome segments that were heterogeneous within a parent variety. Markers that showed a significant (*, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively) segregation distortion are in blue or red to indicate an excess of Drysdale or Waagan alleles, respectively.

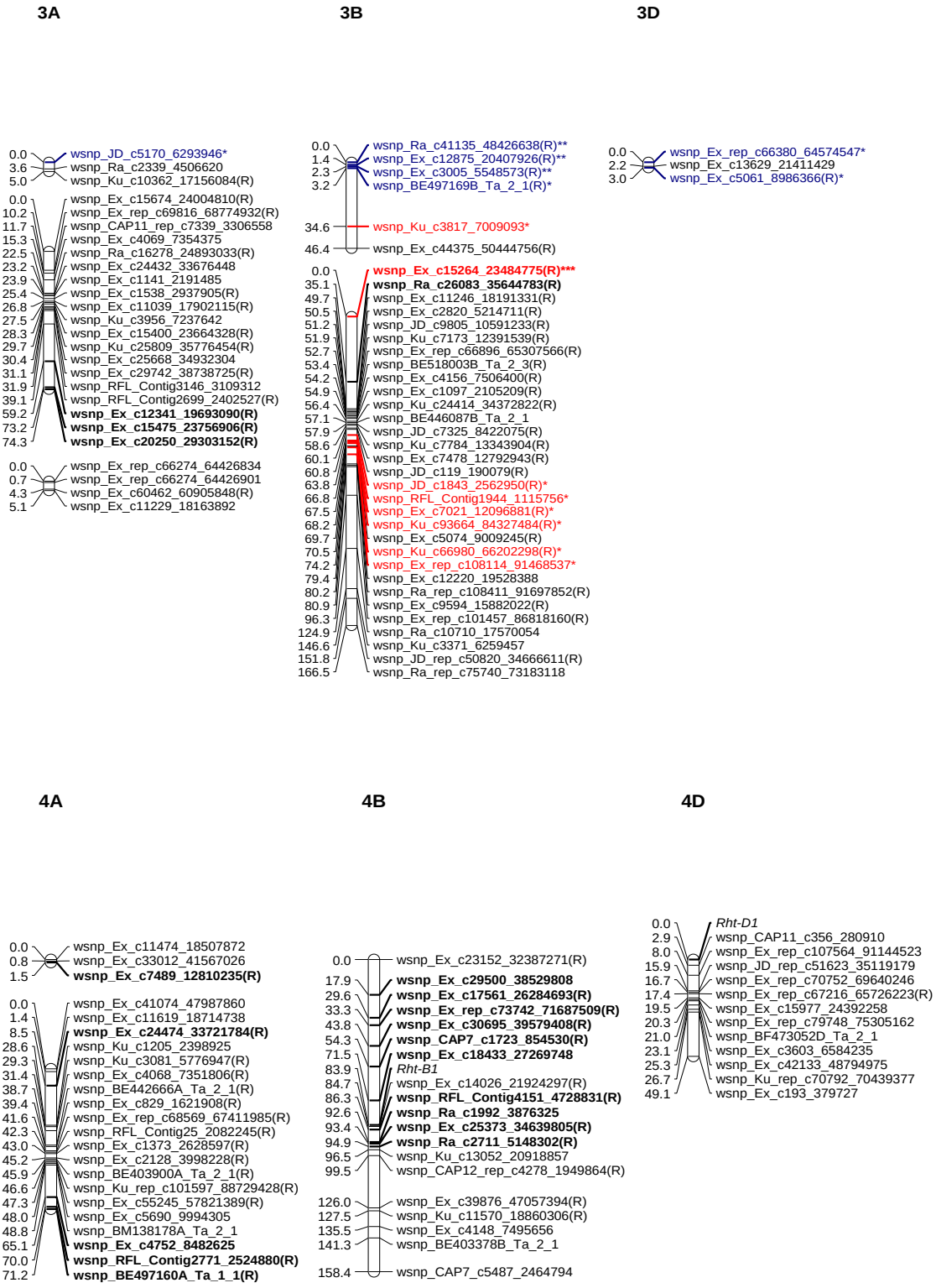


Figure 5.18 Continued

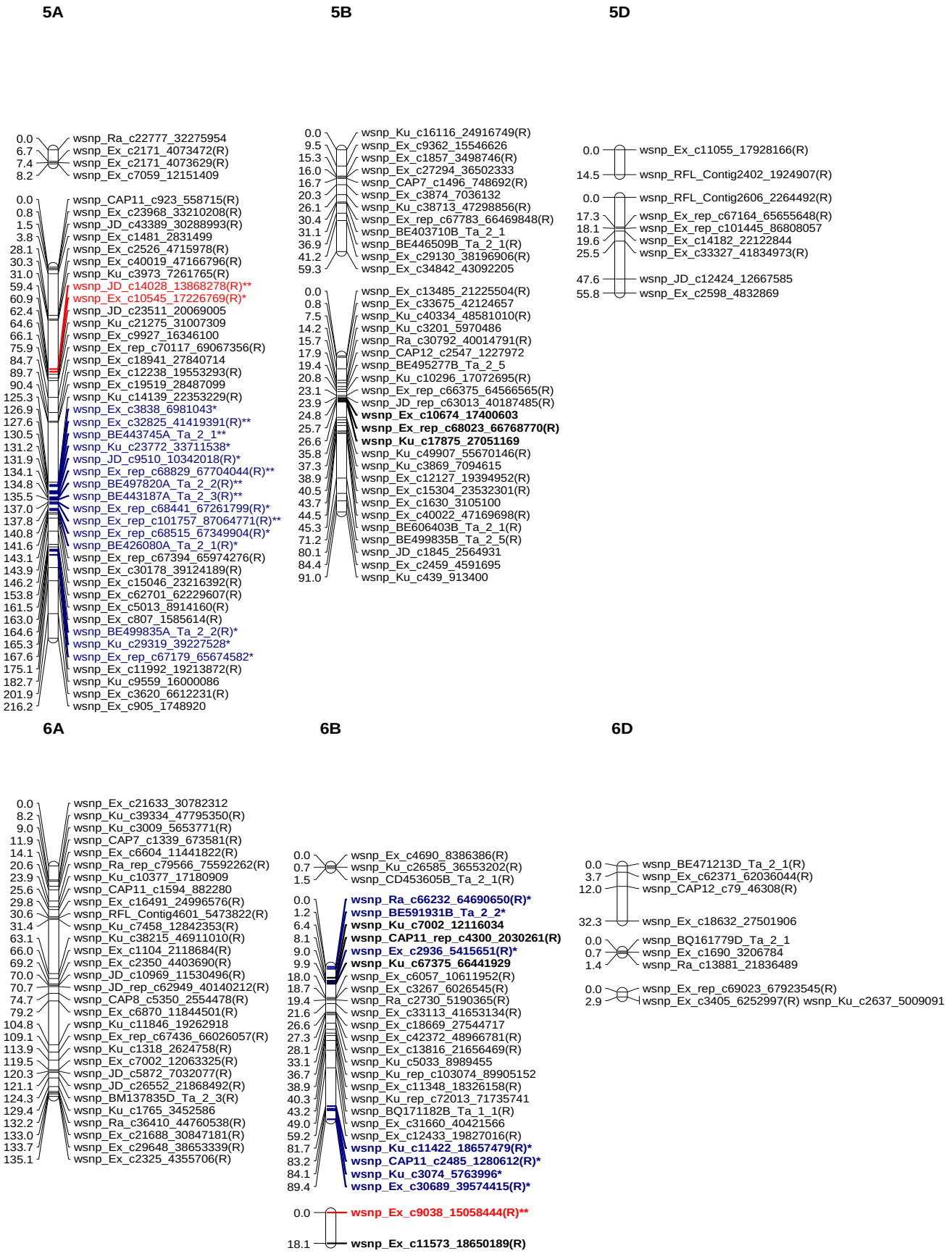


Figure 5.18 Continued

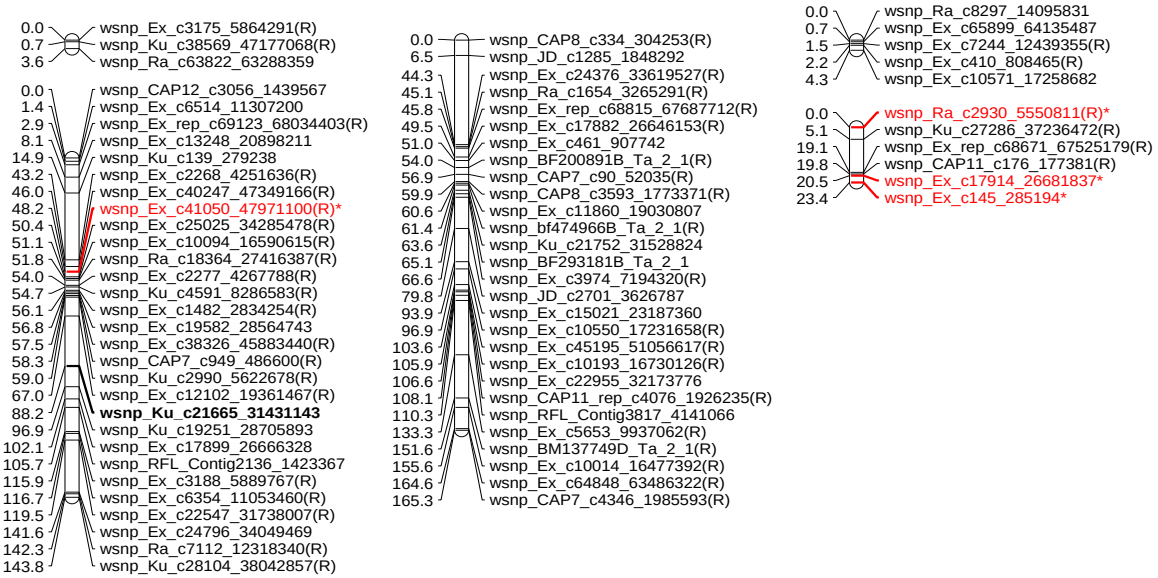


Figure 5.18 Continued

Table 5. 5 Summary of the map by chromosome

Chromosome	Number of non-redundant loci	Number of all loci	Length (cM)	Marker density* (cM/non-redundant locus)	Maximum spacing
1A	50	182	135.70	2.71	29.60
1B	35	127	144.60	4.13	25.60
1D	13	75	95.30	7.33	25.00
2A	45	267	198.30	4.41	30.80
2B	36	178	144.70	4.01	30.80
2D	19	56	82.00	4.32	20.40
3A	26	147	84.40	3.25	20.00
3B	37	183	212.90	5.75	35.10
3D	3	12	3.00	1.00	2.20
4A	23	129	72.70	3.16	20.00
4B	20	55	158.40	7.92	26.60
4D	13	18	49.10	3.78	22.40
5A	46	195	224.40	4.88	34.90
5B	36	160	150.30	4.18	25.90
5D	9	26	70.30	7.81	22.00
6A	30	230	135.10	4.50	31.70
6B	29	283	109.00	3.76	22.50
6D	10	27	36.60	3.66	20.30
7A	32	159	147.40	4.61	28.20
7B	28	163	165.30	5.90	37.80
7D	11	39	27.70	2.52	14.00
Overall	551	2711	2447.40	4.44	37.80

*Not including the gap between linkage groups, when chromosomes were mapped as multiple linkage groups

Table 5. 6 Summary of the map by genome and homoeologous chromosome groups

Genome/ Chromosome group	Number of non-redundant loci	Number of all loci	Length (cM)	Marker density* (cM/non-redundant locus)
A genome	252	1309	998.00	3.96
B genome	221	1149	1085.20	4.91
D genome	78	253	364.00	4.67
Group 1	98	384	375.60	3.83
Group 2	100	501	425.00	4.25
Group 3	66	342	300.30	4.55
Group 4	56	202	280.20	5.00
Group 5	91	381	445.00	4.89
Group 6	69	540	280.70	4.07
Group 7	71	361	340.40	4.79
Overall	551	2711	2447.40	4.44

*Not including the gap between linkage groups, when chromosomes were mapped as multiple linkage groups

5.3.8 Segregation distortion

Skewed segregation has been reported in many previous studies (Akbari et al. 2006; Cadalen et al. 1997; Cavanagh et al. 2013; Chalmers et al. 2001; Francki et al. 2009; Kammholz et al. 2001; Paillard et al. 2003; Peleg et al. 2008; Semagn et al. 2006). Significant segregation distortion was observed for 12.7% of loci (70 out of 551; Figure 5.19) in this study. Loci showing significant segregation distortion in favour of Drysdale alleles (50 loci) were more frequent than those in favour of Waagan alleles (20 loci). The majority of significantly distorted loci were clustered to intervals on chromosomes 2B, 3B, 5A and 6B (Figures 5.18 and 5.19). Chromosomes 2A, 3A, 3D, 7A and 7D had few distorted markers.

Segregation distortion can occur due to several reasons such as parent heterogeneity, selection associated with the doubled haploid production procedure, outcrossing, genotyping errors, introgressed alien chromatin segments, gametophytic competition, and also random chance (Francki et al. 2009; Kammholz et al. 2001; Paillard et al. 2003; Peleg et al. 2008; Singh et al. 2007; Xu et al. 1997). Parental heterogeneity and genotyping errors have been accounted for here to some degree, as outlined in the previous sections. A combination of the aforementioned factors may lead to segregation distortion in the Drysdale × Waagan population. Here, the largest level of segregation distortion was observed on chromosome 2B. Segregation distortion on chromosome 2B has been reported in various studies (Cadalen et al. 1997; Cavanagh et al. 2013; Campbell et al. 1999; Chalmers et al. 2001; Kammholz et al. 2001; Paillard et al. 2003) and has been attributed to the introgression of *Sr36* locus from *Triticum timopheevii* (Cavanagh et al. 2013; Huang et al. 2012). Loci affecting gametophyte competition and gamete vigour which result in segregation distortion have been reported on homoeologous group 5 chromosomes (5A, 5B, and 5D) in wheat and its relatives (Faris et al. 1998; Peng et al. 2000; Kumar et al. 2007). In the present study chromosome 5A showed the

second largest level of segregation distortion. Other possible causes for the observed segregation distortion in the Drysdale × Waagan mapping population could be the heterogeneity within one or both parents, and selection associated with doubled haploid production in the wheat × maize method. In general, it has been suggested that the segregation distortion may have a negligible impact on marker order and map length (Hackett and Broadfoot 2003), and thus may not be an issue in this work.

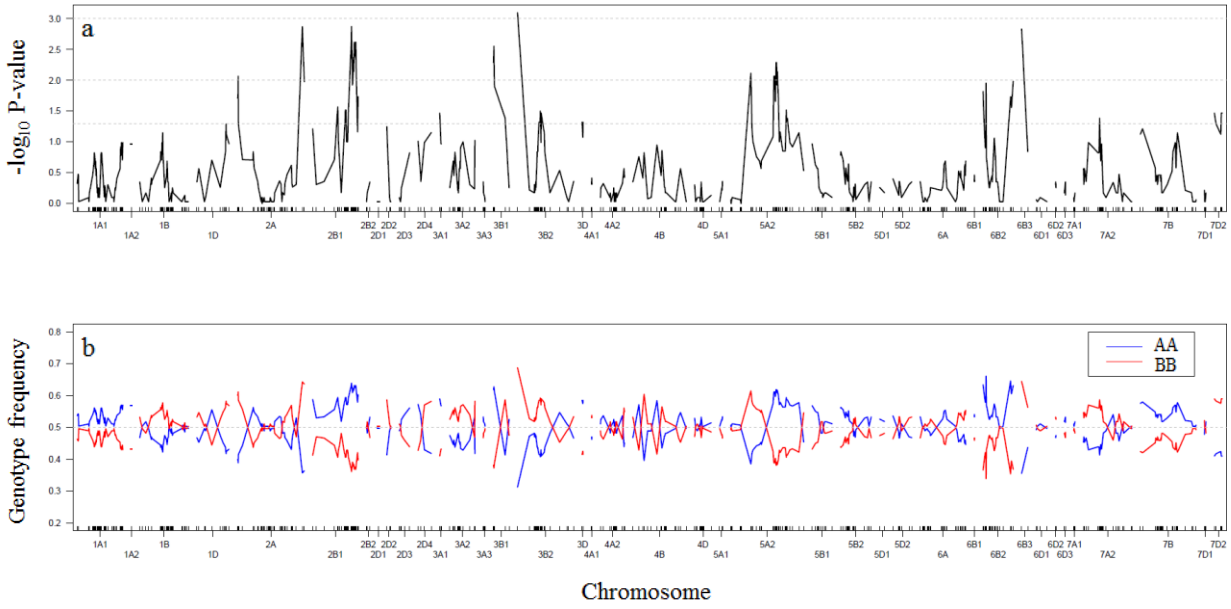


Figure 5. 19 Summary of segregation distortion across the Drysdale/Waagan molecular marker genetic map. a) – $\log_{10}$ P-values from test of 1:1 segregation at each marker. Dashed horizontal lines represent significance at levels $p < 0.05$, $p < 0.01$, and $p < 0.001$ from the bottom to the top, respectively. b) Genotype frequency at each marker. Blue and red lines indicate AA and BB genotypes frequencies, respectively.

5.4 Concluding remarks

A 9K SNP array was used to construct a molecular marker genetic map for a new mapping population derived from crosses between two Australian bread wheat varieties (Drysdale/Waagan). The constructed map is believed to be highly accurate, based on the evidence outlined, and provides a valuable resource for wheat genetic studies. The map will be used to map QTL for heat tolerance and related heat responses (Chapter 6) and could also be used for mapping other agronomically important traits (e.g. drought tolerance and disease resistance), and to support map-based cloning of the gene(s) controlling the target trait(s) in the future.

Chapter 6: QTL mapping of heat tolerance in wheat (*Triticum aestivum* L.) under a brief episode of heat stress at grain-filling

6.1 Introduction

Terminal heat stress, which mostly refers to rising temperature during grain-filling, is one of the major constraints for wheat production and affects 40% of wheat growing regions worldwide (Reynolds et al. 2001). Moreover, the proportion of wheat growing environments that are heat-stressed is likely to increase with the current trend of climate change (Ortiz et al. 2008). Therefore, breeding for heat tolerance is one of the main priorities of wheat breeding programs, to help cope with climate change and the growing global demand for grain (Kumar et al. 2013).

Wheat has the optimum temperature of 15 ºC for achieving maximum grain mass and 3-4% yield reduction has been estimated to result from each 1°C above this optimum (Chowdhury and Wardlaw 1978; McDonald et al. 1983; Wardlaw et al. 1989a). Yield loss resulting from high temperatures after anthesis is mainly due to grain weight reduction rather than changes in grain number (Tashiro and Wardlaw 1990a; Wardlaw et al. 1989a; Wardlaw et al. 1989b). A range of physiological and biochemical processes are adversely affected by high temperatures in wheat including development and senescence, respiration, photosynthesis, and starch deposition in developing grains (Al-Khatib and Paulsen 1984; Almeselmani et al. 2012; Bhullar and Jenner 1985; Jenner 1994; Zahedi and Jenner 2003). Variation in a range of physiological and structural traits, such as cell membrane thermal stability (Blum and Ebercon 1981; Fokar et al. 1998), stay-green (Kumari et al. 2007; Lopes and Reynolds 2012; Reynolds et al. 2001), stomatal conductance (Reynolds et al. 1994), photosynthetic rate (Al-Khatib and Paulsen 1989; Al-Khatib and Paulsen 1990; Reynolds et al. 2000), chlorophyll fluorescence (Peck and McDonald 2010; Sharma et al. 2012), canopy temperature (Amani et al. 1996; Kumari et al. 2007; Reynolds et al. 1994; Reynolds et al. 1998), epicuticular wax (Mason et al. 2010), stem carbohydrate reserves (Blum et al. 1994; Blum 1998; Talukder et al. 2013; Yang et al. 2002a) and grain-filling rate and duration (Stone and Nicolas 1995a; Talukder et al. 2013; Zahedi and Jenner 2003), are believed to contribute to the variation among wheat genotypes for heat tolerance. A few of these traits such as canopy temperature depression, yield and its components have been used to screen for heat tolerance in wheat (Reynolds et al. 2001; Reynolds et al. 1994). However, evaluation of these traits is expensive, time consuming and laborious in large-scale breeding programs. The unpredictable nature of heat stress (timing, magnitude, and duration), its frequent co-occurrence with other stresses (e.g. drought), and growth-stage-specific effects make

screening for heat tolerance challenging. This may explain the limited progress of breeding programs for heat tolerance (Kumar et al. 2013) despite the reported variation for heat tolerance related traits among wheat genotypes (Reynolds et al. 1994; Stone and Nicolas 1995b; Wardlaw et al. 1989a).

QTL mapping is a useful method for understanding the genetic control of complex traits (e.g. heat and drought tolerance) (Mason et al. 2013). Mapping loci affecting variation in heat tolerance could potentially lead to the development of markers for breeding heat tolerance and eventual isolation of heat tolerance genes. The markers could be used to select for heat tolerance in breeding programs, avoiding the aforementioned difficulties in selecting for heat tolerance directly in the field. However, the genetic basis of heat tolerance in wheat is still poorly understood (Kumar et al. 2013). Chromosomal regions have been reported for better performance under heat conditions in wheat based on various agronomical and physiological traits including yield and its components (Mason et al. 2013; Mohammadi et al. 2008b; Paliwal et al. 2012; Tiwari et al. 2013), grain-filling duration (Mason et al. 2010; Mohammadi et al. 2008a; Yang et al. 2002b), senescence related traits (Kumar et al. 2010; Vijayalakshmi et al. 2010), and canopy temperature depression (Mason et al. 2011; Paliwal et al. 2012; Tiwari et al. 2013). Also, some efforts have been made to map loci affecting heat susceptibility indices, an estimate of genotypic performance under stress relative to non-stress conditions, adjusted for stress intensity in the particular experiment (Fischer and Maurer 1978), for yield and its components, grain-filling duration, and temperature depression (Mason et al. 2010; Mason et al. 2011; Mason et al. 2013; Mohammadi et al. 2008b; Paliwal et al. 2012; Tiwari et al. 2013). These studies suggest that genes on almost all of the wheat chromosomes can contribute to heat tolerance, although most of these QTL have been located to the B genome (Kumar et al. 2013). While the aforementioned studies represent a significant advance, so far there is no marker linked to genetic effect on heat tolerance that has been proven yield benefit under field conditions, and a heat tolerance gene has not been isolated from wheat (Cossani and Reynolds 2012).

Genetic mapping of variation for heat responses can shed light on the genetic and physiological basis of heat tolerance and make progress towards identifying marker(s) linked to heat tolerance and the cloning of heat tolerance genes. This can help wheat breeding programs and improve our understanding of heat tolerance mechanisms. To help address these needs in the current study, a doubled-haploid wheat mapping population derived from two Australian elite bread wheat varieties was evaluated for tolerance to a brief episode of heat stress imposed in a growth chamber to identify QTL for heat susceptibility indices, as

well as absolute values, for grain number and size, grain-filling duration, flag leaf chlorophyll retention related traits, and plant architectural traits.

6.2 Materials and methods

The doubled haploid population and its parents were assayed for heat tolerance under controlled conditions in two experiments in 2012. Seeds were sown on 16th and 17th of March and 21st and 22nd of July in the first and second trial, respectively.

6.2.1 Plant material

An F_1-derived doubled haploid population (DH) of 144 lines, made by crossing the two Australian wheat varieties, Drysdale (Hartog*3/Quarrion; female; relatively heat susceptible) and Waagan (Janz/24IBWSN-244; 24IBWSN-244 is a CIMMYT line; male; relatively heat tolerant), and their parents (6 and 10 single plant selections of Drysdale and Waagan, respectively) were used in the present study.

6.2.2 Plant growth, heat stress conditions and phenotype data collection

Plant growth and heat stress conditions were similar to those described in Chapter 3. Briefly, plants were initially grown under control conditions in a naturally lit greenhouse (The Australian Plant Accelerator, University of Adelaide, Waite Campus, Adelaide). They were pruned back to the single main culm by removing tillers as they appeared, similar to previous heat tolerance studies, to allow better light penetration into the plant canopy (Tashiro and Wardlaw 1990b; Wardlaw et al. 1989b). Measured greenhouse conditions were approximately 23/19 ºC, 14/10 h day/night (Table 6.1). Plants were well watered and fertilized fortnightly with a commercial plant fertilizer (Thrive all-purpose soluble fertilizer) from one month after sowing to maturity to avoid drought and nutrition stress. For each plant, anthesis date was recorded. Each plant destined for heat treatment was transferred to a growth chamber (BDW120, Conviron) at 10 days after its anthesis date (days after anthesis, DAA), to be exposed to a brief heat stress (37/27ºC day/night temperature for 3 days), before being returned to the greenhouse. Pots were placed in trays of water to ~2-cm depth while in the chamber to minimize drought stress. Each plant was evaluated for several traits during growth and at maturity, as summarized in Table 6.2.

Table 6. 1 Measured temperatures (ºC) across the growing periods in greenhouses in Experiments 1 and 2. Anthesis and maturity occurred May-June and July-August in the first trial and in September-October and November-December in the second trial, respectively.

Trial	Month	Average day temperature	Average night temperature	Average minimum temperature	Average maximum temperature	Minimum temperature	Maximum temperature	Days > 30ºC
Experiment 1								
	March	24.1	19.9	18.5	28.8	17.7	33.5	2
	April	23.4	19.3	18.1	26.6	16.5	29.8	0
	May	22.3	18.8	17.7	25.0	16.3	26.8	0
	June	22.2	18.8	17.7	24.7	16.7	25.4	0
	July	22.3	18.7	17.7	25.1	17.5	29.5	0
	August	22.2	18.6	17.5	25.0	17.3	25.9	0
Experiment 2								
	July	22.3	18.6	17.6	26.1	17.5	29.7	0
	August	22.2	18.6	17.5	25.0	17.2	25.8	0
	September	22.8	18.8	17.6	26.0	17.0	29.9	0
	October	23.2	18.3	16.9	28.6	14.7	33.2	9
	November	24.7	19.2	17.7	29.4	14.9	33.0	11
	December	23.3	20.9	19.5	26.6	17.1	31.0	2

Table 6. 2 Traits evaluated in the Drysdale × Waagan DH population and its parents.

Trait	Abbreviation	Measurement method
Days to anthesis	DTA	Days from sowing to the day that exerted anthers first became visible
Grain weight spike^{-1}(g)	GWS	Total grain weight of spike at maturity, measured after grain weight stabilized at room temperature for ~4 weeks
Grain number spike^{-1}	GNS	Total grain number of spike at maturity
Single grain weight (mg)	SGW	GWS/GNS
Grain-filling duration (days)	GFD	Days from anthesis to 95% senescence of spike, visually scored
Days to maturity (days)	DTM	Days from sowing to 95% senescence of spike, visually scored
Chlorophyll contentat10, 13 and 27 DAA* (SPAD units)	ChlC10DAA ChlC13DAA ChlC27DAA	Relative chlorophyll content of the flag leaf measured using a portable SPAD chlorophyll meter (SPAD-502; Minolta Co. Ltd., Japan) at 10, 13 and 27 DAA, corresponding to directly before and after treatment and 14 days after treatment (Figure 6.1)
Area under SPAD curve	AUSC¥	AUSC provides an indication of absolute chlorophyll content in heat-treated and control plants from 10 to 27 DAA (Figure 6.1)
Chlorophyll loss rate 10 to 13DAA (SPAD units day^{-1})	ChlR13	Linear rate of chlorophyll loss between SPAD 10 and 13 DAA points, representing the loss during the treatment time interval; Figure 6.1
Chlorophyll loss rate 10 to 27 DAA (SPAD units day^{-1})	ChlR27	Chlorophyll loss rate determined by a linear regression of the three SPAD measurements; incorporates losses during and after the treatment time interval; Figure 6.1
Flag leaf senescence (days)	FLSe	Days from anthesis to 95% senescence of the flag leaf, visually scored
Shoot weight (g)	ShW	Above-ground biomass (stem + leaves; excluding spike) at maturity, measured after oven drying at 85ºC for 3days
Plant height (cm)	PH	Plant height from the soil surface to tip of the spike, excluding awns, at maturity
Harvest index (%)	HI	(GWS / (GWS + ShW)) × 100
Flag leaf length (cm)	FL	The length of the flag leaf from base of the blade to the leaf tip, measured at 10 DAA
Flag leaf width (cm)	FW	The length of the widest section of the flag leaf blade, measured at 10 DAA
Heat susceptibility index	HSI¥	Calculated for yield components and heat tolerance related traits

*Days after anthesis

¥ $AUSC = \sum_{i=1}^{i-1}[\left(\frac{X_i+X_{(i+1)}}{2}\right) \times \left(t_{(i+1)} - t_i\right)$, where X_i is the relative chlorophyll content (SPAD units) on the i^{th} date, t_i is the date on which the chlorophyll content was measured, and n is the number of dates on which chlorophyll content was recorded.

¥ $HSI = (1 - Y_{Heat}/Y_{Control})/(1 - X_{Heat}/X_{Control})$ (Fischer and Maurer, 1978), where Y_{Heat} and $Y_{Control}$ are the means for each genotype under heat-treatment and control environments, and X_{Heat} and $X_{Control}$ are means of all lines under heat-treatment and control conditions, respectively. The denominator indicates stress intensity.

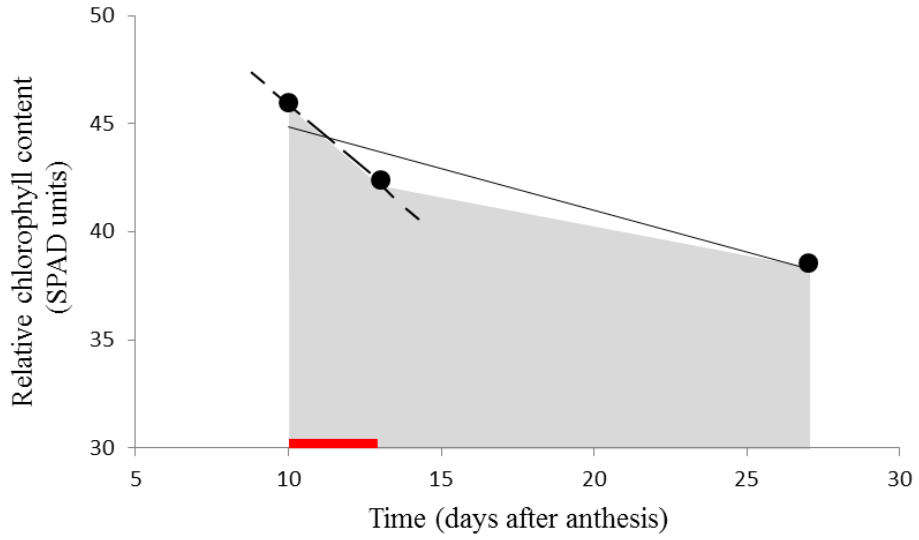


Figure 6. 1 Schematic of relative chlorophyll (SPAD) readings taken from the flag leaf of one hypothetical plant over time using a SPAD chlorophyll meter, defining chlorophyll loss/retention parameters. The red bar represents the period of heat treatment, and the black circles indicate the SPAD readings taken 10, 13 and 27 DAA. The slopes of the black dashed and solid lines represent chlorophyll loss rates between 10 and 13 DAA, and between 10 and 27 DAA (linear regression of the three points), respectively. The grey shaded area represents the area under the SPAD progress curve (AUSC), which is an estimate of absolute chlorophyll content considering all 3 measurements together.

6.2.3 Molecular marker analysis

The details of molecular marker analysis were presented in Chapter 5. In brief, the DH lines were typed for the 9K iSelect array developed by the International Wheat SNP Working Group (Cavanagh et al. 2013). They were also typed for SNP polymorphisms within the *Ppd-B1* photoperiod response gene and the *Rht-B1* and *Rht-D1* semi-dwarfing genes, using competitive allele-specific PCR (KASP) assays (LGC Genomics, London, UK; www.lgcgenomics.com).

6.2.4 Construction of the molecular marker map

A genetic linkage map was generated from 548 genetically non-redundant SNP markers plus markers for the *Ppd-B1*, *Rht-B1* and *Rht-D1* genes, using R/qtl software. Linkage groups were formed using a logarithm of odds (LOD) threshold of 5 and a maximum recombination frequency threshold of 0.4. Then, markers were ordered within linkage groups. Recombination fractions were converted to cM distances using the Kosambi mapping function (Kosambi 1943). Linkage groups were assigned to chromosomes and oriented according to the SNP consensus wheat genetic map (Cavanagh et al. 2013) (for more details see Chapter 5). The map graphic was drawn using MapChart 2.1 (Voorrips 2002).

6.2.5 Experimental design and statistical analysis

The experiments were designed as a randomized block split-plot with genotype (parents and DH lines) and temperature treatments (control vs. heat) as main plots and subplots respectively. Thus, for any given genotype, the control plant and the plant assigned to the heat treatment were neighbours within each main plot replicate. Two replicates of the DH population were intended in each experiment, but some lines were subsequently found to be highly genetically similar and assumed to be identical (see Chapter 5) providing up to 8 replicates of some lines. Parent varieties were replicated 6 to 8 times.

Each trait within an experiment was analysed separately using a linear mixed model that accounted for genetic and non-genetic sources of variation. For the vector of trait observations, $\boldsymbol{y} = (\boldsymbol{y_1}, \ldots, \boldsymbol{y_n})$ the linear mixed model was defined as:

$$\boldsymbol{y} = \boldsymbol{X\tau} + \boldsymbol{Zu} + \boldsymbol{Z}_g\boldsymbol{g} + \boldsymbol{e}$$

where $\boldsymbol{\tau}$, is a vector of fixed effects and $\boldsymbol{u}$ and $\boldsymbol{g}$ are vectors of non-genetic and genetic random effects, respectively. $\boldsymbol{X}$, $\boldsymbol{Z}$ and $\boldsymbol{Z}_g$ are design matrices which associate the trait observation with the appropriate combination of fixed and random effects. The genetic effects were assumed to have distribution $\boldsymbol{g} \sim N\,(\boldsymbol{0}, \boldsymbol{\Sigma}_g \otimes \boldsymbol{I}_g)$ where $\boldsymbol{\Sigma}_g$ is a 2×2 matrix with diagonal elements $(\delta^2_{g_c}, \delta^2_{g_h})$ representing the genetic variance for the control and heat treatments and $\boldsymbol{I}_g$ is the identity matrix. The residual error was assumed to be distributed as $e \sim N\,(\boldsymbol{0}, \delta^2\boldsymbol{R}(\rho_r, \rho_c))$ where δ^2 is the residual variance and $\boldsymbol{R}(\rho_r, \rho_c)$ is a correlation matrix containing a separable AR1 × AR1 autoregressive process with parameters ρ_r and ρ_c representing the correlation along the rows and columns of the experimental layout. For each of the traits within each experiment a generalized heritability (H^2), which is an estimate of the broad-sense heritability, developed by Cullis et al. (2006) and Oakey et al. (2006) was calculated for each treatment using:

$$H^2 = 1 - \frac{E}{2\delta^2_g}$$

where E is the average pairwise prediction error variance of the best linear unbiased predictors (BLUPs) and δ^2_g is the genetic variance for the treatment. All models were fitted using the flexible linear mixed modelling software ASReml-R (Butler et al. 2009) available in the R statistical computing environment (R Development Core Team 2014).

BLUPs derived from the linear mixed model were used for QTL analysis. QTL analysis was performed separately for traits under either control or heat conditions, and for trait HSIs, for each experiment, using GenStat 16 (http://www.vsni.co.uk/genstat). Initially, linkage

analysis was performed using simple interval mapping, then the selected candidate QTL were used as co-factors for composite interval mapping (CIM), setting the minimum co-factor proximity to 30 cM. For CIM, a 10 cM maximum step size and a genome wide significance level of $\alpha = 0.05$ was chosen. Map diagrams were drawn using MapChart 2.1 (Voorrips 2002).

Pairwise Pearson correlation tests were done to study relationships between heat susceptibility indices (HSIs) of traits with their potentials (mean value of control plants) and between HSIs of different traits. Pearson correlation tests were performed using Psych package (Revelle 2011).

6.2.6 Comparing physical location of 3BS QTLs from this and previous studies

Locations of QTL for heat tolerance related traits mapped to 3BS in this and previous studies were compared by determining locations of markers on the wheat 3B reference sequence, by using BLAST searches at the URGI website (http://wheat-urgi.versailles.inra.fr/). Map diagram was drawn using MapChart 2.1 (Voorrips 2002).

6.3 Results

6.3.1 Phenotypic analysis

6.3.1.1 Parental lines

Means ± standard errors (SEs) of traits for the Drysdale × Waagan DH population and the parent varieties are presented in Table 6.3. Heat treatment did not have any significant overall effect on GWS, GNS, and SGW in either parent in Experiment 1, while it significantly reduced GWS and SGW of both parents in Experiment 2. Percent change of yield components due to heat ranged from 0.6 to 2.7% in Experiment 1 and from 2.0 to 11.0% in Experiment 2. Compared to earlier experiments (Chapters 3 and 4) where the parents showed a difference of 11% for SGW response, they differed less in the two current experiments (4.5 and 0.6% contrast in Experiments 1 and 2, respectively) – a contrast which may have been derived from within-genotype variation or differences in growth conditions. GFD and DTM of both parents decreased in response to the heat treatment (ranged from 1.9 to 6.0% in Experiment 1 and from 3.5 to 8.3% in Experiment 2), with Drysdale responding more than Waagan. Heat treatment impacted flag leaf chlorophyll retention related traits in Drysdale (by 4.9 to 945.5% in Experiment 1 and 3.3 to 572.7% in Experiment 2) more than in Waagan (1.1 to 600.0% in Experiment 1 and 1.2 to 91.7% in Experiment 2). In both experiments and for all chlorophyll retention related traits, the heat effects were significant in Drysdale (except ChlR27 in

Experiment 2) but non-significant in Waagan. There was no significant difference between control and heat-treated plants for ShW and HI in Experiment 1, while heat stress significantly reduced ShW in Waagan and HI in both parents in Experiment 2. On average, Drysdale flowered 12-15 days earlier and was 8-9 cm taller than Waagan.

6.3.1.2 DH lines

In the DHs, overall mean values for almost all of the measured traits (except GNS in both experiments, HI in Experiment 1 and ShW in Experiment 2) were significantly affected by the brief heat treatment (Table 6.3). For all traits, the range of HSIs and absolute trait values was larger in DHs than in the parents (Table 6.3). Heat treatment reduced the overall mean values across DH lines for GWS by 4.6%, and for SGW by 4.1% in Experiment 1, and for both traits by ~ 11.0% in Experiment 2. Heat stress reduced GFD by 8.6 and 7.3%, and DTM by 4.4 and 3.1%, in Experiment 1 and 2, respectively, indicating that the brief heat treatment accelerated development by ~ 5 days in both experiments. As expected, there was no significant difference for chlorophyll content before heat exposure (ChlC10DAA) between the two treatments. Heat stress showed a very similar impact on chlorophyll content measured directly after treatment (ChlC13DAA) between two experiments, reducing the mean value in the DH lines by ~ 4.0%. During the heat treatment period, chlorophyll loss rate was greater in heat-treated plants relative to the control plants (ChlR13 trait; by 636.4% in Experiment 1 and by 725.0% in Experiment 2). Mean chlorophyll content of DH lines measured two weeks after heat treatment (ChlC27DAA) was 5.1 and 7.6% lower in heat-treated plants than in unheated control plants in Experiment 1 and 2, respectively. The area under the SPAD progress curve (AUSC) was reduced by heat by 3.4% in Experiment 1 and by 4.9% in Experiment 2. Chlorophyll loss rate from 10 to 27 DAA (ChlR27) was higher in the heat treated plants, by 5.0% in Experiment 1 and by 62.5% in Experiment 2, which suggests there was a difference between experiments for chlorophyll gain/loss after treatment. The time from anthesis to the date that flag leaf became ~95% senesced (FLSe; visually scored) was reduced by heat stress in both experiments (12.5 and ~ 4.0% in Experiment 1 and 2, respectively). Shoot dry weight at maturity (ShW) and harvest index (HI) were both reduced by the heat treatment, by 2.9 and 0.9% in Experiment 1, and by 1.5 and ~4.0% in Experiment 2, respectively. The overall mean values for days from sowing to anthesis, plant height, flag leaf length and width of the DH lines was very similar for heat-treated and unheated control plants in both experiments.

Table 6. 3 Means ± S.E. for traits measured in the two experiments of the Drysdale × Waagan population and its parents. DTA, days from sowing to anthesis; GWS, grain weight spike^{-1} (g); GNS, grain number spike^{-1}; SGW, single grain weight (mg); GFD, grain-filling duration (days from anthesis to 95% senescence of spike); DTM, days to maturity (days from sowing to 95% senescence of spike); ChlC10DAA, chlorophyll content 10 days after anthesis (corresponding to the measurement before treatment in heat-treated plants; SPAD units); ChlC13DAA, chlorophyll content 13 days after anthesis (corresponding to first measurement after treatment in heat-treated plants; SPAD units); AUSC, area under SPAD curve; ChlR13, linear rate of chlorophyll loss between SPAD at 10 and 13 DAA (SPAD units day^{-1}), representing the loss during the treatment time interval; ChlR27, linear rate of chlorophyll loss considering all of the three SPAD measurements (10, 13 and 27 DAA; SPAD units day^{-1}) which incorporates losses during and after the treatment time interval; FLSe, days from anthesis to 95% flag leaf senescence; ShW, shoot dry weight (g); PH, plant height (cm); HI, harvest index (%); FL, flag leaf length (cm) and FW, flag leaf width (cm).

Experiment/Traits	Drysdale		Waagan		DH mean		DH Range (Minimum, Maximum)		
	Control	Heat	Control	Heat	Control	Heat	Control	Heat	HSI[a]
Experiment 1									
DTA	56.98 ± 0.55	57.29 ± 0.54^{ns}	68.74 ± 1.03	69.27 ± 1.03^{ns}	58.70 ± 0.25	58.74 ± 0.25^{ns}	49.00, 73.00	49.00, 72.00	-
GWS	1.72 ± 0.05	1.73 ± 0.05^{ns}	1.55 ± 0.04	1.58 ± 0.04^{ns}	1.53 ± 0.02	1.46 ± 0.02**	0.54, 2.72	0.47, 2.83	1.00
GNS	40.21 ± 1.11	41.29 ± 1.13^{ns}	44.66 ± 1.00	44.40 ± 1.05^{ns}	41.55 ± 0.47	41.36 ± 0.47^{ns}	23.00, 73.00	14.00, 68.00	0.37
SGW	43.02 ± 0.65	42.13 ± 0.72^{ns}	34.92 ± 0.51	35.79 ± 0.43^{ns}	36.83 ± 0.34	35.33 ± 0.35***	17.66, 54.40	17.48, 52.22	0.97
GFD	62.07 ± 0.48	58.36 ± 0.49***	56.41 ± 0.29	53.11 ± 0.33***	60.03 ± 0.17	54.83 ± 0.19***	47.00, 68.00	44.00, 64.00	1.00
DTM	119.02 ± 0.67	115.64 ± 0.69**	124.80 ± 1.00	122.39 ± 0.91*	118.71 ± 0.31	113.53 ± 0.30***	103.00, 136.00	97.00, 130.00	0.99
ChlC10DAA	49.34 ± 0.24	49.54 ± 0.23^{ns}	47.45 ± 0.22	47.27 ± 0.23^{ns}	47.65 ± 0.16	47.71 ± 0.15^{ns}	35.90, 54.80	39.10, 55.00	-
ChlC13DAA	49.67 ± 0.25	46.75 ± 0.35***	47.74 ± 0.24	47.16 ± 0.26^{ns}	47.99 ± 0.15	45.95 ± 0.22***	36.10, 55.30	31.60, 54.10	1.01
ChlC27DAA	49.42 ± 0.25	47.01 ± 0.39***	47.77 ± 0.23	48.29 ± 0.23^{ns}	48.73 ± 0.19	46.24 ± 0.28***	8.20, 55.30	11.90, 54.40	1.01
AUSC	842.16 ± 4.10	800.81 ± 5.74***	811.33 ± 3.86	809.80 ± 4.07^{ns}	814.17 ± 2.62	785.85 ± 3.87***	526.15, 939.35	445.70, 920.85	1.01
ChlR13	0.11 ± 0.03	-0.93 ± 0.09***	0.10 ± 0.02	-0.04 ± 0.03^{ns}	0.11 ± 0.01	-0.59 ± 0.04***	-0.57, 1.13	-5.30, 0.83	1.00
ChlR27	0.00 ± 0.01	-0.10 ± 0.02***	0.01 ± 0.01	0.07 ± 0.01^{ns}	0.00 ± 0.01	-0.05 ± 0.01***	-2.44, 0.18	-1.76, 0.19	1.58
FLSe	68.90 ± 1.08	63.33 ± 0.94***	63.61 ± 0.70	62.09 ± 0.77^{ns}	65.74 ± 0.54	57.51 ± 0.51***	31.00, 99.00	31.00, 91.00	0.96
ShW	1.48 ± 0.04	1.51 ± 0.03^{ns}	1.45 ± 0.03	1.51 ± 0.03^{ns}	1.39 ± 0.02	1.35 ± 0.02*	0.52, 2.84	0.59, 2.99	1.04
PH	85.47 ± 1.09	86.98 ± 0.65^{ns}	77.39 ± 0.56	77.38 ± 0.65^{ns}	78.86 ± 1.00	78.81 ± 1.00^{ns}	41.70, 124.50	40.00, 122.70	-
HI	47.04 ± 0.56	46.77 ± 0.47^{ns}	45.16 ± 0.46	44.82 ± 0.46^{ns}	45.8 ± 0.16	45.39 ± 0.18^{ns}	35.37, 56.61	33.85, 58.33	1.00
FL	24.18 ± 0.48	24.73 ± 0.35^{ns}	24.04 ± 0.48	23.97 ± 0.48^{ns}	23.37 ± 0.18	23.37 ± 0.17^{ns}	11.90, 35.30	15.70, 34.50	-
FW	1.56 ± 0.03	1.58 ± 0.02^{ns}	1.55 ± 0.02	1.54 ± 0.01^{ns}	1.54 ± 0.01	1.53 ± 0.01^{ns}	1.05, 2.00	0.90, 2.20	-
Experiment 2									
DTA	68.24 ± 0.24	68.34 ± 0.25^{ns}	83.90 ± 0.98	82.11 ± 0.90^{ns}	70.82 ± 0.25	70.85 ± 0.25^{ns}	57.00, 95.00	59.00, 84.00	-
GWS	3.61 ± 0.08	3.21 ± 0.08***	3.85 ± 0.07	3.44 ± 0.07***	3.02 ± 0.05	2.69 ± 0.04***	0.99, 6.34	0.97, 5.28	0.97
GNS	64.74 ± 1.54	62.93 ± 1.74^{ns}	77.54 ± 1.45	75.97 ± 1.71^{ns}	60.35 ± 0.82	60.72 ± 0.81^{ns}	24.00, 107.00	22.00, 112.00	-2.20
SGW	55.90 ± 0.43	51.31 ± 0.42***	49.81 ± 0.33	45.43 ± 0.31***	49.75 ± 0.26	44.22 ± 0.27***	32.70, 62.91	28.33, 56.88	1.00
GFD	57.19 ± 0.35	52.44 ± 0.34***	47.35 ± 0.48	44.43 ± 0.35***	53.14 ± 0.19	49.26 ± 0.19***	44.00, 78.00	28.00, 60.00	1.00
DTM	125.43 ± 0.39	120.78 ± 0.37***	131.17 ± 0.84	126.54 ± 0.72***	123.95 ± 0.27	120.13 ± 0.25***	109.00, 145.00	107.00, 132.00	0.99
ChlC10DAA	48.43 ± 0.28	48.20 ± 0.28^{ns}	48.36 ± 0.22	48.65 ± 0.24^{ns}	47.79 ± 0.16	47.64 ± 0.17^{ns}	33.20, 55.90	32.10, 55.20	-
ChlC13DAA	48.76 ± 0.27	46.63 ± 0.35***	48.01 ± 0.24	48.60 ± 0.30^{ns}	47.55 ± 0.17	45.66 ± 0.29***	32.40, 55.80	16.70, 54.30	1.02
ChlC27DAA	44.77 ± 0.56	43.28 ± 0.57*	44.00 ± 0.98	45.71 ± 0.57^{ns}	44.07 ± 0.26	40.69 ± 0.44***	27.80, 55.60	4.80, 53.10	1.02
AUSC	800.46 ± 5.77	771.64 ± 6.77**	775.66 ± 8.27	804.43 ± 6.15^{ns}	782.84 ± 3.23	743.61 ± 5.49***	552.00, 947.35	291.20, 914.45	1.02
ChlR13	0.11 ± 0.03	-0.52 ± 0.10***	-0.12 ± 0.03	-0.01 ± 0.05^{ns}	-0.08 ± 0.01	-0.66 ± 0.06***	-1.27, 0.67	-9.80, 1.03	1.00
ChlR27	-0.24 ± 0.03	-0.27 ± 0.03^{ns}	-0.38 ± 0.06	-0.20 ± 0.03^{ns}	-0.24 ± 0.01	-0.39 ± 0.02***	-1.50, 0.15	-2.47, 0.15	0.93
FLSe	58.00 ± 0.53	54.29 ± 1.05**	43.04 ± 0.98	44.28 ± 0.75^{ns}	54.92 ± 0.38	52.79 ± 0.50***	32.00, 78.00	9.00, 87.00	1.00
ShW	2.14 ± 0.03	2.09 ± 0.03^{ns}	2.37 ± 0.05	2.20 ± 0.04*	1.94 ± 0.03	1.91 ± 0.03^{ns}	0.75, 4.64	0.72, 4.75	0.78
PH	89.53 ± 0.60	89.6 ± 0.73^{ns}	81.11 ± 0.37	81.17 ± 0.45^{ns}	81.72 ± 1.02	82.11 ± 1.02^{ns}	47.00, 132.10	47.80, 138.30	-
HI	54.59 ± 0.45	52.49 ± 0.56***	53.60 ± 0.27	52.58 ± 0.30**	53.05 ± 0.25	50.93 ± 0.23***	32.09, 65.85	31.55, 61.52	0.98
FL	31.53 ± 0.36	31.79 ± 0.45^{ns}	25.02 ± 0.43	25.95 ± 0.50^{ns}	29.00 ± 0.17	29.12 ± 0.18^{ns}	17.30, 39.00	19.70, 39.80	-
FW	1.83 ± 0.02	1.86 ± 0.02^{ns}	1.89 ± 0.02	1.86 ± 0.02^{ns}	1.82 ± 0.01	1.82 ± 0.01^{ns}	1.20, 2.30	1.20, 2.30	-

[a]Average HIS calculated across all DH lines

^{ns}Non-significant difference between control and heat-treated plants

*, **, and *** indicate significant difference between control and heat-treated plants at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

6.3.2 Heritability

Heritability (H^2) for each trait/treatment/experiment is presented in Table 6.4. DTA showed heritabilities ranging from 0.59 to 0.68. Estimated heritability for yield components (GWS, GNS and SGW) were high under both control and heat stress conditions, ranging from 0.64 to 0.85 (Table 6.4). GWS and SGW had higher heritability than GNS. Moreover, SGW showed a slightly higher heritability under heat stress conditions than under control conditions. GFD and DTM appeared to have high heritability under both conditions. However, GFD showed higher heritability under heat stress conditions, whereas the reverse held true for DTM. Heritability for flag leaf chlorophyll retention, chlorophyll loss rate and FLSe were high under heat stress conditions (0.71-0.87), while they ranged from 0.00 to 0.77 under control conditions. The large difference in heritability of chlorophyll loss rate from 10 to 13 DAA (ChlR13) between control and heat-treated plants (0.00 vs. 0.87 in control and heat-treated plants, respectively) reflects lack of detectable difference between lines for change in chlorophyll content from 10 to 13 DAA and a large difference between lines for chlorophyll retention during heat treatment (10 to 13 DAA) in heat-treated plants. PH had the highest heritability among the measured traits (0.96), followed by ShW (0.86-0.91) which is highly associated with PH. HI showed a low heritability in first experiment (0.30-0.44) while it had a high heritability in the second (0.76-0.78). FL showed a moderate heritability (0.45-0.50), while FW appeared to have high heritability (0.60-0.71).

Table 6. 4 Heritability (H^2) of the traits for each treatment/experiment. DTA, days from sowing to anthesis; GWS, grain weight spike^{-1}; GNS, grain number spike^{-1}; SGW, single grain weight; GFD, grain-filling duration; DTM, days to maturity; ChlC10DAA, chlorophyll content 10 days after anthesis; ChlC13DAA, chlorophyll content 13 days after anthesis; AUSC, area under SPAD curve; ChlR13, linear rate of chlorophyll loss between SPAD at 10 and 13 DAA; ChlR27, linear rate of chlorophyll loss considering all of the three SPAD measurements (10, 13 and 27 DAA); FLSe, days from anthesis to 95% flag leaf senescence; ShW, shoot dry weight; PH, plant height; HI, harvest index; FL, flag leaf length and FW, flag leaf width.

Trait	H^2			
Treatment	Control		Heat	
Experiment	Experiment 1	Experiment 2	Experiment 1	Experiment 2
DTA	0.61	0.66	0.59	0.68
GWS	0.83	0.85	0.85	0.80
GNS	0.64	0.76	0.64	0.74
SGW	0.79	0.80	0.81	0.82
GFD	0.65	0.72	0.71	0.79
DTM	0.60	0.67	0.55	0.63
ChlC10DAA	0.76	0.72	0.75	0.73
ChlC13DAA	0.65	0.61	0.84	0.84
ChlC27DAA	0.65	0.39	0.87	0.82
AUSC	0.71	0.50	0.87	0.82
ChlR13	0.00	0.00	0.87	0.87
ChlR27	0.25	0.33	0.84	0.81
FLSe	0.77	0.60	0.71	0.74
ShW	0.86	0.91	0.88	0.91
PH	0.96	0.96	0.96	0.96
HI	0.30	0.78	0.44	0.76
FL	0.49	0.50	0.45	0.50
FW	0.60	0.71	0.60	0.68

6.3.3 Correlations

Summaries of correlations between HSIs for different traits, and between HSIs and trait potentials (trait value under control conditions), are given in Tables 6.5 and 6.6, respectively. Low to strong correlations were observed among HSIs of the different traits (Tables 6.5). HSIs of main-spike yield components were moderately to highly correlated. HSI of GWS showed a strong positive correlation with HSIs of GNS and SGW in both experiments, while HSI of SGW and GNS showed a moderately significant negative correlation in Experiment 1, which tends to suggest a trade-off between heat responses of grain size and number. HSI of GWS and SGW showed positive correlations with other traits in both Experiments. These correlations were particularly strong and significant for most of the flag leaf chlorophyll retention related traits, i.e. genotypes able to better maintain SGW and GWS under heat conditions (tolerant) also tended to maintain flag leaf chlorophyll content and to have slower chlorophyll loss rate in response to heat stress. This suggested a functional relationship between stay-green and heat tolerance. The HSI of ShW also showed positive and significant associations with the HSIs of yield components and most of the flag leaf chlorophyll retention related traits, suggesting that under heat, the ability to maintain grain mass and chlorophyll was also functionally related to the ability to maintain shoot mass.

Correlations were observed between HSIs and potentials (trait in control plants) for some traits. Positive associations indicate a greater response of the lines with higher potential for the trait, while the negative associations suggest a weaker response for plants with higher trait potential values. There was no significant correlation between potentials of PH, FL, and FW with HSIs of yield components (GWS, GNS and SGW) in either experiment (Table 6.6). DTA showed low to moderate correlation with HSIs of GFD, DTM, and FLSe in Experiment 1, and with HSIs of all yield components, as well as with HSIs of ChlC27DAA, and ChlR27, in Experiment 2. Late flowering genotypes tended to have a greater response (more susceptibility) for the developmental (GFD and DTM) and some stay-green related traits (ChlR27 and FLSe). A positive correlation was observed between HSIs of GNS, SGW, DTM, FLSe and HI and the potentials of these same traits, in one or both experiments. By contrast, negative correlations were found between HSIs of ChlC13DAA, ChlC27DAA, AUSC, ChlR13 and ChlR27 and their potentials in both experiments, although the magnitude of the correlations differed between experiments (Table 6.6). i.e., genotypes with higher absolute value for yield components (GNS and SGW), DTM, FLSe, and HI also tended to show higher response to the heat treatment, while for most stay-green related traits, genotypes with slower natural senescence (higher chlorophyll content *per se* and slower chlorophyll loss rate) also showed lower response upon heat exposure.

Table 6. 5 Genotypic correlations between heat susceptibility indices (HSIs) of traits in Experiment 1 (below diagonal) and Experiment 2 (above diagonal). GWS, grain weight spike^{-1}; GNS, grain number spike^{-1}; SGW, single grain weight; GFD, grain-filling duration; DTM, days to maturity; ChlC13DAA, chlorophyll content 13 days after anthesis; AUSC, area under SPAD curve; ChlR13, linear rate of chlorophyll loss between SPAD at 10 and 13 DAA; ChlR27, linear rate of chlorophyll loss considering all of the three SPAD measurements (10, 13 and 27 DAA); FLSe, days from anthesis to 95% flag leaf senescence; ShW, shoot dry weight; HI, harvest index.

Trait	GWS	GNS	SGW	GFD	DTM	ChlC13 DAA	ChlC27 DAA	AUSC	ChlR13	ChlR27	FlSe	ShW	HI
GWS	-	0.83***	0.47***	0.25**	0.29***	0.32***	0.42***	0.36***	0.38**	0.43***	0.00	0.56***	0.70***
GNS	0.62***	-	-0.04	0.11	0.22**	0.08	0.12	0.06	0.13	0.22**	-0.07	0.50***	0.54***
SGW	0.56***	-0.26**	-	0.22**	0.12	0.52***	0.63***	0.59***	0.55***	0.42***	0.13	0.28***	0.40***
GFD	0.24**	-0.11	0.43***	-	0.43***	0.18*	0.46***	0.34***	0.24**	0.43***	0.54***	-0.08	0.35***
DTM	0.25**	-0.04	0.35***	0.67***	-	0.12	0.21*	0.16*	0.15	0.21*	0.11	0.20*	0.25**
ChlC13DAA	0.40***	0.14	0.35***	0.15	0.14	-	0.72***	0.89***	0.85***	0.39***	0.08	0.23**	0.23**
ChlC27DAA	0.43***	0.04	0.49***	0.38***	0.23**	0.77***	-	0.93***	0.70***	0.68***	0.48***	0.14	0.37***
AUSC	0.44***	0.11	0.46***	0.27**	0.17*	0.91***	0.93***	-	0.78***	0.56***	0.32***	0.16	0.29***
ChlR13	0.33***	0.02	0.41***	0.19*	0.14	0.86***	0.66***	0.77***	-	0.56***	0.12	0.24**	0.27***
ChlR27	0.14	0.08	0.07	-0.06	-0.01	0.15	0.18*	0.19*	0.13	-	0.35***	0.14	0.3***
FlSe	0.22**	-0.06	0.36***	0.65***	0.38***	0.15	0.37***	0.27***	0.22**	-0.01	-	-0.21**	0.13
ShW	0.64***	0.39***	0.42***	0.27***	0.16	0.44***	0.48***	0.48***	0.37***	0.02	0.26**	-	-0.04
HI	0.51***	0.30***	0.24**	-0.01	0.13	0.07	0.08	0.09	0.02	0.12	0.07	-0.21*	-

Values are Pearson correlation coefficients, with significance levels indicated by asterisks: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Table 6. 6 Correlations between trait potentials (mean value in control plants; for those that were measured before treatment including ChlC10 DAA, FL and FW also just the value in control plants was used for the correlation analysis) and heat susceptibility indices (HSIs) in the two experiments. DTA, days from sowing to anthesis; GWS, grain weight spike^{-1}; GNS, grain number spike^{-1}; SGW, single grain weight; GFD, grain-filling duration; DTM, days to maturity; ChlC10DAA, chlorophyll content 10 days after anthesis; ChlC13DAA, chlorophyll content 13 days after anthesis; AUSC, area under SPAD curve; ChlR13, linear rate of chlorophyll loss between SPAD at 10 and 13 DAA; ChlR27, linear rate of chlorophyll loss considering all of the three SPAD measurements (10, 13 and 27 DAA); FLSe, days from anthesis to 95% flag leaf senescence; ShW, shoot dry weight; PH, plant height; HI, harvest index; FL, flag leaf length and FW, flag leaf width.

		Trait potentials																
	Experiment/Trait	DTA	GWS	GNS	SGW	GFD	DTM	ChlC10 DAA	ChlC13 DAA	ChlC27 DAA	AUSC	ChlR13	ChlR27	FLSe	ShW	PH	HI	FL	FW
HSIs	**Experiment 1**																		
	GWS	0.09	0.00	-0.01	0.02	-0.01	0.14	-0.06	-0.10	-0.06	-0.07	-0.18*	-0.06	0.02	-0.07	-0.05	0.32***	0.06	0.08
	GNS	0.00	0.20*	0.33***	-0.06	-0.08	-0.03	0.12	0.09	0.11	0.11	-0.09	0.02	-0.01	0.14	0.07	0.15	0.11	0.08
	SGW	0.13	-0.14	-0.29***	0.13	0.11	0.23**	-0.14	-0.17*	-0.14	-0.15	-0.15	-0.08	0.06	-0.18*	-0.08	0.23**	0.02	0.02
	GFD	0.19*	-0.26**	-0.20*	-0.20*	0.31***	0.34***	-0.13	-0.18*	-0.12	-0.16	-0.25**	-0.07	0.35***	-0.34***	-0.31***	0.17*	0.12	0.28***
	DTM	0.17*	-0.17*	-0.14	-0.12	0.33***	0.53***	-0.17*	-0.20*	-0.13	-0.17*	-0.10	0.05	0.26**	-0.20*	-0.18*	0.08	0.04	0.23**
	ChlC13DAA	-0.11	-0.13	-0.14	0.01	0.05	0.02	-0.32***	-0.35***	-0.33***	-0.35***	-0.08	0.02	0.03	-0.11	0.01	0.00	0.04	-0.05
	ChlC27DAA	-0.07	-0.24**	-0.23**	-0.08	0.13	0.06	-0.24**	-0.28***	-0.15	-0.22**	-0.07	0.04	0.12	-0.26**	-0.11	0.11	0.02	-0.07
	AUSC	-0.13	-0.18	-0.17*	-0.05	0.09	0.03	-0.25**	-0.30***	-0.21	-0.25**	-0.06	0.05	0.06	-0.19*	-0.05	0.07	0.00	-0.05
	ChlR13	-0.04	-0.08	-0.15	0.08	0.05	0.10	-0.37***	-0.42***	-0.39**	-0.42***	-0.17*	0.00	0.02	-0.07	0.08	0.01	0.09	-0.11
	ChlR27	-0.07	-0.01	-0.05	0.06	-0.03	-0.02	0.07	0.05	0.03	0.05	-0.10	-0.10	-0.07	-0.01	0.15	0.01	-0.11	-0.19*
	FlSe	0.27**	-0.25**	-0.13	-0.27**	0.30***	0.35***	-0.16*	-0.22**	-0.15	-0.20*	-0.25**	0.01	0.60***	-0.32***	-0.31***	0.22**	0.22**	0.33***
	ShW	0.10	-0.07	0.00	-0.11	-0.04	0.06	-0.05	-0.09	-0.06	-0.07	-0.15	0.01	0.03	-0.05	-0.11	0.00	-0.03	0.06
	HI	0.07	0.03	-0.07	0.12	0.11	0.16	-0.05	-0.05	-0.04	-0.04	-0.06	-0.06	0.09	0.04	0.07	0.14	0.10	0.08
	Experiment 2																		
	GWS	0.31***	0.46***	0.49***	0.15	-0.04	0.26**	-0.13	-0.18*	-0.38***	-0.30***	-0.21*	-0.47***	-0.25**	0.23**	0.13	0.32***	-0.04	0.12
	GNS	0.21**	0.33***	0.43***	-0.04	-0.05	0.17*	-0.05	-0.07	-0.21**	-0.16	-0.09	-0.20*	-0.13	0.14	0.06	0.25**	-0.04	0.08
	SGW	0.23**	0.14	0.10	0.19*	0.03	0.20*	-0.26**	-0.31***	-0.41***	-0.40***	-0.26**	-0.57***	-0.16	0.07	0.03	0.19*	-0.01	0.03
	GFD	0.15	0.33***	0.33***	0.14	-0.04	0.29***	0.03	-0.02	-0.24**	-0.15	-0.21*	-0.53***	-0.22**	0.34***	0.30***	0.02	-0.05	-0.01
	DTM	0.17*	0.17*	0.18*	0.05	0.05	0.49***	-0.12	-0.12	-0.19*	-0.18*	-0.07	-0.24**	-0.05	0.16	0.13	0.04	-0.04	-0.02
	ChlC13DAA	-0.10	0.01	-0.03	0.13	0.11	-0.06	-0.32***	-0.32***	-0.30***	-0.34***	-0.11	-0.49***	0.05	-0.01	0.13	0.04	0.13	-0.19*
	ChlC27DAA	0.18*	0.27***	0.23**	0.23**	-0.08	0.16	-0.25**	-0.31***	-0.37***	-0.38***	-0.30***	-0.86***	-0.19*	0.20*	0.22**	0.10	0.12	-0.07
	AUSC	0.06	0.17*	0.13	0.19*	0.00	0.07	-0.29***	-0.33***	-0.30***	-0.34***	-0.21*	-0.71***	-0.08	0.11	0.18	0.07	0.14	-0.11
	ChlR13	0.05	0.12	0.10	0.14	0.03	0.08	-0.36***	-0.38***	-0.42***	-0.44***	-0.24**	-0.60***	-0.07	0.05	0.15	0.11	0.11	-0.09
	ChlR27	0.42***	0.49***	0.48***	0.29***	-0.24**	0.33***	-0.08	-0.19*	-0.54***	-0.4***	-0.43***	-0.84***	-0.41***	0.36***	0.31***	0.19*	0.07	0.03
	FlSe	0.05	0.19*	0.18*	0.06	-0.08	0.1	0.16	0.12	0.02	0.08	-0.14	-0.49***	0.02	0.15	0.1	0.03	0.05	0.14
	ShW	0.13	0.10	0.11	0.07	-0.02	0.08	-0.11	-0.12	-0.21*	-0.18*	-0.06	-0.15	-0.13	0.11	0.05	-0.02	-0.15	-0.02
	HI	0.11	0.30***	0.32***	0.07	0.15	0.23**	-0.17*	-0.21*	-0.27**	-0.26***	-0.18*	-0.36***	-0.02	0.05	0.00	0.47***	-0.03	0.18*

Values are Pearson correlation coefficients, and significance level indicated by $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

6.3.4 The molecular marker map

The molecular marker genetic map made from a set of 551 genetically non-redundant markers was described in Chapter 5.

6.3.5 HSI QTL (heat responses of the traits)

Ten QTL were detected for heat susceptibility indices (HSIs), on 7 of the 21 wheat chromosomes, namely 1A, 3B, 4A, 4B, 5A, 6B, and 7B, with individual QTL explaining between ~6 to 40% of the phenotypic variance (Table 6.7; Figure 6.2). Both parents contributed favourable alleles for heat tolerance. In some cases, HSI QTL co-located with QTL for absolute trait values under control and/or heat conditions. QTL for absolute trait values are summarized in Table 6.8 and illustrated in Figure 2.

The QTL region on the short arm of chromosome 3B was the only HSI QTL region detected in both experiments and appeared to have pleiotropic effects (influencing the HSI of several traits). In both experiments, a QTL was detected in this interval (0-11 cM with QTL peak occurring at the 0-3.15 cM interval in most of the cases) for HSI of each of the traits: GWS, SGW, indicators of chlorophyll content (ChlC13DAA, ChlC27DAA, and AUSC), and for chlorophyll loss rate during the treatment (ChlR13). In all cases, Waagan contributed the heat tolerance allele (smaller HSI). The QTLs explained ~15 to 22% of the phenotypic variance for GWS, ~11 to 20% of the variance for SGW and ~13 to 40% of the variance for the flag leaf chlorophyll retention related traits. QTL were also detected in this interval for HSIs of GFD, FLSe, ShW in Experiment 1, and for chlorophyll loss rate between 10 to 27 DAA (ChlR27), and for HI, in Experiment 2. The QTLs explained between 10 and ~ 22% of the variance for these traits. Waagan also contributed the alleles for stability of these traits under heat stress (smaller HSI). These response QTLs on 3BS co-localized with QTLs for trait values under either control or heat conditions, for GWS, SGW, chlorophyll content (ChlC10DAA, ChlC13DAA, ChlC27DAA and AUSC), chlorophyll loss rate (ChlR13 and ChlR27), GFD, FLSe, ShW and HI, with Waagan contributing the allele for the larger values of traits, except for HI, for which Drysdale contributed the positive allele.

Several HSI QTLs were detected in just one experiment. Chromosomes 4A and 4B had the highest number of HSI QTLs after chromosome 3BS. Two QTL regions were associated with HSIs on chromosome 4A. The first controlled HSI for ChlC27DAA and AUSC in Experiment 1, with the Waagan allele conferring tolerance. It was in the *wsnp_Ex_c11474_18507872/ wsnp_Ex_c33012_41567026* marker interval and explained ~6 to 7% of the phenotypic variance. This QTL co-localized with a QTL for the absolute trait value under control conditions for GFD (QGfd.aww-4A1) which had Drysdale contributing longer GFD. The

second region on 4A had a QTL for HSI of GFD, closely associated with marker *wsnp_RFL_Contig25_2082245(R),* in Experiment 2. This locus had Drysdale contributing the allele for stable GFD and explained ~8% of the phenotypic variance. This region co-localized with QTLs for absolute trait values under heat conditions for GFD (QGfd.aww-4A2) and for PH under both control and heat conditions (QPh.aww-4A), with Drysdale and Waagan alleles contributing longer GFD and greater PH, respectively.

On chromosome 4B, HSI QTL were detected for GFD (QHgfd.aww-4B), FLSe (QHflse.aww-4B; Experiment 1), and ChlR27 (QHchlr27.aww-4B; Experiment 2), and these explained ~8 to 18% of the phenotypic variances for the respective traits. For each QTL, the Drysdale allele was associated with heat tolerance. QHgfd.aww-4B and QHflse.aww-4B were in the same marker interval, *wsnp_CAP12_rep_c4278_1949864(R)/wsnp_Ex_c39876_47057394*, and co-localized with a series of overlapping QTLs for absolute values of several traits (GFD, DTM, GNS, FW, and FL) across a large proportion of the chromosome. The QTL for HSI of ChlR27 (QHchlr27.aww-4B) co-localized with QTLs for flowering time (QDta.aww-4B), and absolute trait values of GWS and FLSe (both expressed under both control and heat conditions).

On chromosome 5A, HSI QTL were detected for DTM (QHdtm.aww-5A; Experiment 1) and GFD (QHgfd.aww-5A; Experiment 2), and these explained 10 to 12% of the phenotypic variances. Favourable alleles (trait stability under heat) were contributed by Waagan and Drysdale, respectively. QHgfd.aww-5A co-localized with QTL for control plant SGW, GFD, HI, and FW, with the Waagan allele conditioning longer GFD and wider FW, and the Drysdale allele conditioning larger SGW and HI.

On chromosome 6B, HSI QTL were detected in the marker interval *wsnp_Ex_c9038_15058444/wsnp_Ex_c11573_18650189(R)* for SGW and ChlR13 in Experiment 2. These explained ~12 and 9% of phenotypic variance, respectively, and the Drysdale allele was associated with stability of both traits under heat stress (smaller HSI). This interval also contained QTL for absolute values in heat-treated plants for AUSC and ChlR13, with the Drysdale allele being favourable for both traits.

HSI QTL were detected for ChlR27 (QHchlr27.aww-1A; Experiment 2) and FLSe (QHflse.aww-7B; Experiment 1) on chromosome 1A and 7B, respectively. These explained ~7.5 to 11% of the phenotypic variances and in both cases the Waagan allele contributed to heat tolerance. The QTL for HSI of ChlR27 on chromosome 1A, at the *wsnp_Ku_c40759_48907151(R)* locus, co-localized with QTL for absolute trait values of

GWS, GNS, ChlC10DAA, and ChlR27. At this locus, Drysdale contributed larger GWS and GNS in Experiment 1 and larger ChlC10DAA in Experiment 2 in control plants, and Waagan contributed slower ChlR27 in control and heat-treated plants in Experiment 2. The QTL on 7B, QHflse.aww-7B, co-localized with QTL for absolute trait values for flowering time (QDta.aww-7B), FLSe, GFD, DTM and FL, with Drysdale contributing the larger trait values.

Table 6. 7 Summary of heat susceptibility index (HSI) QTLs detected in the Drysdale × Waagan DH population. Linkage group, position of each QTL, experiment (Exp) that the QTL was detected, closest marker(s), LOD score, percentage of explained variation (R^2), additive effect, and high value allele (Drysdale, D; Waagan, W) are presented. Red highlights indicate QTLs detected for response of grain weight (GWS and SGW), and QTLs for responses of other traits that co-localized with them. Hgws, HSI of grain weight spike^{-1}; Hgns, HSI of grain number spike^{-1}; Hsgw, HSI of single grain weight; Hgfd, HSI of grain-filling duration; Hdtm, HSI of days from sowing to maturity; Hchlc13, HSI of chlorophyll content 13 days after anthesis; Hchlc27, HSI of chlorophyll content 27 days after anthesis; Hausc, HSI of area under SPAD curve; Hchlr13, HSI of linear rate of chlorophyll loss between SPAD 10 and 13 DAA points; Hchlr27, HSI of linear rate of chlorophyll loss considering all of the three SPAD measurements (10, 13 and 27 DAA); Hflse, HSI of days from anthesis to 95% flag leaf senescence; Hshw, HSI of shoot dry weight; Hhi, HSI of harvest index.

Trait/QTL	Linkage group	Position	Exp[a]	Marker(s)	LOD	R^2	Additive[b]	Allele[c]
GWS								
QHgws.aww-3B	3B1	0.00	E2	*wsnp_Ra_c41135_48426638(R)*	5.91	14.97	0.377	D
	3B1	3.15	E1	*wsnp_BE497169B_Ta_2_1(R)*	8.79	21.64	1.160	D
SGW								
QHsgw.aww-3B	3B1	1.44	E2	*wsnp_Ex_c12875_20407926(R)*	4.73	10.82	0.160	D
	3B1	3.15	E1	*wsnp_BE497169B_Ta_2_1(R)*	8.08	20.11	0.921	D
QHsgw.aww-6B	6B3	9.06	E2	*wsnp_Ex_c9038_15058444/ wsnp_Ex_c11573_18650189(R)*	3.78	12.07	0.169	W
GFD								
QHgfd.aww-3B	3B1	3.15	E1	*wsnp_BE497169B_Ta_2_1(R)*	4.36	10.00	0.118	D
QHgfd.aww-4A	4A2	42.27	E2	*wsnp_RFL_Contig25_2082245(R)*	3.78	8.39	0.120	W
QHgfd.aww-4B	4B	99.47	E1	*wsnp_CAP12_rep_c4278_1949864(R)*	5.54	12.39	0.132	W
QHgfd.aww-5A	5A2	134.08	E2	*wsnp_Ex_rep_c68829_67704044*	4.78	11.58	0.140	D
DTM								
QHdtm.aww-5A	5A2	216.16	E1	*wsnp_Ex_c905_1748920*	4.05	10.29	0.161	W
ChlC13DAA								
QHchlc13.aww-3B	3B1	0.00	E2	*wsnp_Ra_c41135_48426638(R)*	9.97	23.89	0.806	D
	3B1	1.44	E1	*wsnp_Ex_c12875_20407926(R)*	16.58	35.74	0.670	D
ChlC27DAA								
QHchlc27.aww-3B	3B1	0.00	E2	*wsnp_Ra_c41135_48426638(R)*	5.26	13.37	0.616	D
	3B1	1.44	E1	*wsnp_Ex_c12875_20407926(R)*	21.25	38.60	1.282	D
QHchlc27.aww-4A	4A1	0.00	E1	*wsnp_Ex_c11474_18507872*	4.65	7.39	0.561	D
AUSC								
QHausc.aww-3B	3B1	0.00	E 2	*wsnp_Ra_c41135_48426638(R)*	7.34	18.32	0.669	D
	3B1	1.44	E1	*wsnp_Ex_c12875_20407926(R)*	20.47	38.29	0.913	D
QHausc.aww-4A	4A1	0.00	E1	*wsnp_Ex_c11474_18507872*	3.83	6.09	0.364	D
ChlR13								
QHchlr13.aww-3B	3B1	0.00	E1	*wsnp_Ra_c41135_48426638(R)*	16.27	39.68	0.554	D
	3B1		E2		13.21	27.21	0.795	D
QHchlr13.aww-6B	6B3	18.11	E2	*wsnp_Ex_c11573_18650189(R)*	4.79	8.94	0.456	W
ChlR27								
QHchlr27.aww-1A	1A2	0.00	E2	*wsnp_Ku_c40759_48907151(R)*	5.9	11.00	0.302	D
QHchlr27.aww-3B	3B1	0.00	E2	*wsnp_Ra_c41135_48426638(R)*	9.33	19.35	0.400	D
QHchlr27.aww-4B	4B	135.47	E2	*wsnp_Ex_c4148_7495656*	4.65	8.44	0.264	W
FLSe								
QHflse.aww-3B	3B1	1.44	E1	*wsnp_Ex_c12875_20407926(R)*	6.70	14.42	0.271	D
QHflse.aww-4B	4B	117.19	E1	*wsnp_Ex_c39876_47057394*	6.53	17.68	0.300	W
QHflse.aww-7B	7B	45.80	E1	*wsnp_Ex_rep_c68815_67687712(R)*	3.92	7.47	0.195	D
ShW								
QHshw.aww-3B	3B1	3.15	E1	*wsnp_BE497169B_Ta_2_1(R)*	9.31	22.75	1.620	D
HI								
QHhi.aww-3B	3B1	0.00	E2	*wsnp_Ra_c41135_48426638(R)*	4.06	10.30	0.291	D

[a]E1 and E2 indicate Experiment 1 and 2, respectively.
[b]Indicate the additive effect
[c]D and W indicate Drysdale or Waagan allele increased the value of HSI (contributed to higher susceptibility)

6.3.6 QTL for absolute trait values

In addition to HSIs (heat responses of the traits), QTL were also detected for performance *per se* (absolute trait values) of the traits under control and heat conditions. QTL were detected on 16 of the 21 wheat chromosomes for the absolute trait values under control and/or heat-treated plants, with individual QTL explaining ~1 to 56% of the phenotypic variances in the four experiments/treatments and with some chromosomal regions being pleiotropic, i.e., affecting several traits (Table 6.8; Figure 6.2). Both parents contributed favorable alleles for the studied traits. In the D genome, 2D was the only chromosome to show any QTL.

6.3.6.1 DTA and PH

There was no major flowering time effect segregating in the DH population. Three minor QTL were detected for flowering time, on chromosome 2B, 4B, and 7B (Table 6.8; Figure 6.2). The QTL on chromosome 4B showed the highest LOD score and was the only one detected in both experiments, but it determined differences in time to anthesis of only 1.5-1.6 d. It explained 36-37% of the phenotypic variance, with the Waagan allele delaying flowering. The QTL on chromosomes 2B and 7B were expressed just in one experiment each and in both cases the Drysdale allele delayed anthesis (by only 0.7-0.9 d). They explained 7-14% of the phenotypic variance for that trait. A SNP in the flowering time gene *Ppd-B1* was mapped in the population. However, it was located ~10 cM from the weak flowering time QTL identified on chromosome 2B and the assayed SNP is not documented to alter gene function.

Several QTL were detected for PH (Table 6.8; Figure 6.2). QTL on chromosomes 4B and 4D corresponded to *Rht-B1* and *Rht-D1* loci, consistent with the fact that the parents differed for the known functional SNPs in these genes (Chapter 5). Together, these two loci explained 88.5-93.5% (added across loci) of the total phenotypic variance in all four experiments/treatments. QTLs on chromosome 3A and 4A were also detected in all four experiments/treatments, but explained only 1-2% of the phenotypic variance. QTL on chromosomes 1A and 2A were each just detected in one experiment/treatment, and explained 1-2% of the phenotypic variance. *Rht-B1* and *Rht-D1*had the strongest additive effects on PH (11 and 14 cm, respectively), followed by the QTL on chromosome 4A (~2.5-3 cm).

6.3.6.2 Yield components (GNS, GWS and SGW)

QTL for yield related traits including GNS, GWS, and SGW were detected on 11 chromosomes with some chromosome regions affecting several traits (Table 6.8). Two QTL were detected on chromosome 1A for yield components expressed under control conditions.

At the first locus, at marker *wsnp_Ex_c200_391493(R)*, the Waagan allele was associated with larger SGW (Experiment 1), and at the second locus, at marker *wsnp_Ku_c40759_48907151(R)*, the Drysdale allele was associated with both larger GWS and GNS (Experiment 2). A heat specific QTL for SGW was detected on chromosome 1B in Experiment 2 explaining 6.75% of phenotypic variance. At this locus the Waagan allele increased SGW by an average of 0.97 mg. QTL were detected in the chromosome 2D interval (*wsnp_Ku_c30494_40319867(R)/wsnp_RFL_Contig2659_2346243*) for GWS and SGW, in Experiment 1 and 2, respectively. The QTL was expressed under both control and heat conditions but had a stronger additive effect under heat conditions. The QTL explained ~6-8% of phenotypic variances, depending on the experiment/treatment, and in all cases Drysdale contributed the favorable allele. Two heat specific QTL were detected on chromosome 3B for grain weight (either SGW or both SGW and GWS) with Waagan contributing the favorable allele, in both experiments. On group-4 chromosomes, QTL were detected for all three yield components on chromosomes 4B and 4D. These QTL were co-located at/near loci controlling PH (*Rht-B1* and *Rht-D1* loci) and DTA (at the *wsnp_Ex_c4148_7495656* marker). At *Rht-B1*, the tall (Drysdale) allele was associated with larger GWS and SGW. At or near to *wsnp_Ex_c4148_7495656* the Waagan allele for late flowering was associated with both larger GWS and GNS, with the effect being dependent on the experiment and treatment (larger effect in Experiment 2 and under control conditions). At *Rht-D1*, the tall (Waagan) allele was associated with both larger GWS and SGW. On group-5 chromosomes, QTL were detected on chromosome 5A and 5B for SGW under control and under both treatments, respectively. Both QTL on group-5 were detected in Experiment 2 with Drysdale contributing favourable alleles. On group-6, QTL were detected on chromosomes 6A and 6B. A QTL on chromosome 6A was detected for SGW under control conditions in both experiments and under heat conditions in Experiment 2, with the Drysdale allele increasing SGW. This QTL had a stronger effect and explained more phenotypic variance under control conditions. The 6B QTL interval appeared to be pleiotropic, affecting GWS (in Experiment 2) and GNS (in Experiment 1) in heat-stressed plants with the Drysdale allele increasing values of both traits.

6.3.6.3 GFD and DTM

QTL were detected for GFD and DTM on 9 chromosomes (1A, 2A, 3B, 4A, 4B, 4D, 5A, 6A and 7B) with some regions affecting both traits.

Individual QTL explained from ~4 to 21% of the phenotypic variances for GFD. QTL on chromosomes 3B, 4B, 4D and 7B had the largest additive effects on GFD. The QTL on chromosomes 4B and 4D were detected at/near loci affecting PH (*Rht-B1*and *Rht-D1* loci) or

DTA (at marker *wsnp_Ex_c4148_7495656*). At *Rht-B1*, the dwarfing (Waagan) allele was associated with longer GFD. At or near to *wsnp_Ex_c4148_7495656* and at *Rht-D1*, the Drysdale alleles for early flowering and dwarfing were both associated with longer GFD. The QTL on chromosome 7B was detected in both Experiments (at least for heat conditions) while the remaining GFD QTL were detected in one experiment/treatment only.

Individual QTL explained ~6 to 30% of the phenotypic variance for DTM, with QTL on chromosomes 4B and 7B having the largest additive effects. At/near *wsnp_Ex_c4148_7495656* on chromosome 4B, the Waagan allele for late flowering was associated with greater DTM and that was consistent between both experiments/treatments. QTL on chromosome 7B at *wsnp_CAP8_c334_304253* were detected in both experiments under heat, and under control conditions in Experiment 2, and another QTL on the same chromosome was detected at *wsnp_Ex_c24376_33619527* for control conditions in Experiment 1. In each case the Drysdale allele increased DTM. QTL were detected for DTM under heat conditions in Experiment 2 at *Rht-B1* and *Rht-D1* loci. At both loci the dwarfing alleles were associated with increased DTM.

6.3.6.4 Flag leaf chlorophyll retention related traits (ChlC, ChlR, and FLSe)

QTL for flag leaf chlorophyll retention related traits were detected on 10 chromosomes (1A, 2A, 2B, 2D, 3B, 4B, 4D, 5A, 6B and 7B) (Table 8) with individual QTL explaining ~4 to 54% of the phenotypic variance.

The QTL interval on 3BS was the main one for chlorophyll content effects measured at different time points (10, 13, and 27 DAA) and for AUSC, and these were detected in all four treatments/experiments. The QTLs showed much larger effects in heat-treated plants than in controls for ChlC13DAA, ChlC27DAA and AUSC. The QTL on 3BS was also the dominant QTL interval for chlorophyll loss rate under heat-stress conditions: ChlR13 in both experiments and for ChlR27 in Experiment 1. The QTL was also detected for FLSe in Experiment 1. The QTL explained up to 23 and 54% of phenotypic variance in control and heat-treated plants, respectively. At this locus the Waagan allele was always associated with larger chlorophyll content, slower chlorophyll loss rate, and longer time from anthesis to 95% flag leaf senescence (FLSe).

Other QTLs were expressed just in one treatment/experiment combination, with the exception of QChlc27.aww-2A, QAusc.aww-2A, and QFlse.aww-4B1 which were expressed in control plants in both experiments and QFlse.aww-4D which was expressed in control plants in both experiments and in heat-treated plants in Experiment 2. QFlse.aww-4B1 and QFlse.aww-4D coincided with *Rht-B1* and *Rht-D1* and in both cases the dwarfing alleles were

associated with longer time from anthesis to 95% flag leaf senescence (FLSe, visually scored). Other notable QTL were QAusc.aww-5A, QAusc.aww-6B, and QChlr10-13.aww-6B that were heat specific, and QChlc13.aww-2D, QChlc27.aww-2D, QAusc.aww-2D, QChlc13.aww-5A, QChlr27.aww-1A and QFlse.aww-4B2 (at/near QTL affecting flowering time, with the Drysdale allele associated with earlier flowering and increased FLSe) that were large-effect QTLs expressed in both heat-treated and control plants but having larger effect in heat-treated plants. Except QChlr27.aww-1A, for which the Waagan allele conditioned the slower chlorophyll loss rate, favorable alleles were contributed by Drysdale (larger chlorophyll content, slower chlorophyll loss rate, and longer FLSe). These QTLs explained 5 to 28% of phenotypic variance depending on trait/treatment/experiment.

6.3.6.5 ShW and HI

QTL affecting ShW and HI were distributed on 9 (1A, 2D, 3A, 3B, 4A, 4B, 4D, 6B and 7B) and 6 (3B, 4B, 4D, 5A, 7A, and 7B) chromosomes, respectively.

Not unexpectedly, *Rht-B1* and *Rht-D1* loci had the largest effect on ShW (explaining 19 to 49% of the phenotypic variance), in all experiments/treatments, with the tall alleles being associated with larger ShW. At *Rht-B1* the additive effect was stronger under heat conditions, while the reverse held true at *Rht-D1* in both experiments. At a QTL at *wsnp_Ex_c4148_7495656*, the Waagan allele (also conditioning late flowering) was associated with larger ShW. This locus explained 5 to 10% of the phenotypic variance and was expressed in both experiments/treatments. QTLs were also detected on chromosome 2D, explaining 3 to 6% of phenotypic variance, with Drysdale contributing favourable alleles. QTL on chromosomes 1A, 3A, 3B, 4A and 6B were detected just under heat conditions, in one experiment each. Except QTLs on chromosome 3B (QShw.aww-3B1 and QShw.aww-3B2) which accounted for ~3 to 14% of the phenotypic variance for ShW, other loci detected for the same trait explained less than 2% of the phenotypic variance. These loci, except QShw.aww-3A, were not associated with PH effects. Favourable alleles were contributed by Drysdale at loci on chromosomes 3A and 6B and by Waagan at other loci. A minor QTL was also detected on chromosome 7B in Experiment 1 under both control and heat conditions, accounting for ~3% of the variation.

None of the detected QTLs for HI were consistent between experiments/treatments, and most of them were detected in Experiment 2. QTL at/near *Rht-B1* and *Rht-D1* were detected in Experiment 2, and those QTL accounted for the greatest phenotypic variance (11-25%), LOD scores and additive effects. The additive effect was larger in controls than heat-stress conditions at *Rht-B1*, while the reverse was true at *Rht-D1*. At both loci the dwarfing alleles

were associated with larger HI. Further QTL were detected on chromosomes 3B, 5A and 7A in control conditions in Experiment 2, and on 5A and 7B in heat conditions in Experiment 1. No HI QTL was detected in control conditions in Experiment 1. The Drysdale alleles at loci on chromosomes 3B and 5A increased HI, while the Waagan alleles at loci on group-7 chromosomes were associated with larger HI.

6.3.6.6 FL and FW

Five and six QTL were detected for flag leaf length (FL) and width (FW), respectively (Table 8). QTL were located on chromosomes 2A, 2B, 3A, 4A, 4B, 5A, 5B and 7B. A QTL on chromosome 2B was detected for FL in both experiments and explained 13.5-14% of the phenotypic variance. For FW, QTL were detected on chromosomes 4B and 5B in both experiments and together accounted for 28-42% of the variance. Other QTL were detected in one experiment. Waagan and Drysdale both contributed favorable alleles for each trait, depending on the locus. However, favorable alleles were mainly contributed by Drysdale for FL and by Waagan for FW.

Table 6. 8 Summary of QTLs detected in the Drysdale × Waagan DH population for absolute trait values, in control (C) or heat-treated (H) plants. Linkage group, position of each QTL, experiment (Exp) that the QTL was detected, closest marker(s), their LOD score, percentage of explained variation (R^2), additive effect, and high value allele (Drysdale, D; Waagan, W) are presented. For DTA, ChlC10DAA, FL, and FW the pooled mean of control and heat-treated plants was used for QTL analysis since the measurement was taken before the heat treatment. Red highlights indicate QTL co-localized with QTL for HSIs for grain weight (GWS or SGW). Dta, days from sowing to anthesis; Gws, grain weight spike^{-1}; Gns, grain number spike^{-1}; Sgw, single grain weight; Gfd, grain-filling duration; Dtm, days from sowing to maturity; Chlc10, chlorophyll content 10 days after anthesis; Chlc13, chlorophyll content 13 days after anthesis; Chlc27, chlorophyll content 27 days after anthesis; Ausc, area under SPAD curve; Chlr13, linear rate of chlorophyll loss between SPAD 10 and 13 DAA points; Chlr27, chlorophyll loss rate determined by a linear regression of the three SPAD measurements (10, 13 and 27 DAA); Flse, days from anthesis to 95% flag leaf senescence; Shw, shoot dry weight; Ph, Plant height; Hi, harvest index; Fl, flag leaf length and Fw, flag leaf width.

Traits/QTL	Linkage group	Position	Exp[a]	Marker(s)	LOD	R^2	Additive[b]	Allele[c]
DTA (day)								
QDta.aww-2B	2B1	5.36	E2	*wsnp_Ra_c14112_22155451/ wsnp_Ex_c1358_2601510(R)*	3.80	6.91	0.722	D
QDta.aww-4B	4B	135.47	E1	*wsnp_Ex_c4148_7495656*	21.69	37.11	1.480	W
			E2		20.50	35.91	1.645	W
QDta.aww-7B	7B	34.87	E1	*wsnp_Ex_c24376_33619527*	6.11	14.45	0.924	D
GWS (g)								
QGws.aww-1A	1A2	0.00	E2-C	*wsnp_Ku_c40759_48907151(R)*	3.91	6.66	0.170	D
QGws.aww-2D	2D4	25.64	E1-C	*wsnp_Ku_c30494_40319867(R)*	5.35	7.85	0.086	D
	2D4		E1-H		5.53	7.76	0.093	D
QGws.aww-3B	3B1	11.02	E1-H	*wsnp_BE497169B_Ta_2_1(R)*	7.08	11.22	0.112	W
QGws.aww-4B1	4B	83.90	E1-C	*Rht-B1*	11.99	15.23	0.119	D
	4B		E1-H		12.48	15.08	0.129	D
	4B		E2-H		3.80	5.67	0.123	D
QGws.aww-4B2	4B	135.47	E1-C	*wsnp_Ex_c4148_7495656*	7.78	9.47	0.094	W
	4B		E1-H		4.05	4.41	0.070	W
	4B		E2-C		7.12	12.46	0.232	W
	4B	149.85	E2-H	*wsnp_BE403378B_Ta_2_1/ wsnp_CAP7_c5487_2464794*	7.05	15.56	0.203	W
QGws.aww-4D	4D	0.00	E1-C	*Rht-D1*	29.62	36.95	0.186	W
	4D		E1-H		23.79	28.71	0.179	W
	4D		E2-C		8.39	15.37	0.258	W
	4D		E2-H		12.44	23.05	0.248	W
QGws.aww-6B	6B2	27.34	E2-H	*wsnp_Ex_c42372_48966781(R)*	3.66	8.74	0.152	D
GNS								
QGns.aww-1A	1A2	0.00	E2-C	*wsnp_Ku_c40759_48907151(R)*	4.67	7.88	2.734	D
QGns.aww-4B	4B	135.47	E1-C	*wsnp_Ex_c4148_7495656*	5.53	10.34	1.478	W
	4B		E1-H		3.90	4.68	0.984	W
	4B		E2-C		8.96	15.45	3.829	W
	4B		E2-H		9.57	17.49	3.711	W
QGns.aww-4D	4D	0.00	E1-C	*Rht-D1*	10.72	21.38	2.125	W
	4D		E2-C		7.51	13.13	3.530	W
	4D		E2-H		10.25	18.88	3.855	W
	4D	7.95	E1-H	*wsnp_Ex_rep_c107564_91144523*	8.16	16.46	1.845	W
QGns.aww-6B	6B2	27.34	E1-H	*wsnp_Ex_c42372_48966781(R)*	4.48	4.72	0.988	D
SGW(mg)								
QSgw.aww-1A	1A1	36.53	E1-C	*wsnp_Ex_c200_391493(R)*	4.33	5.62	1.071	W
QSgw.aww-1B	1B	83.44	E2-H	*wsnp_Ku_c18227_27490539*	4.32	6.75	0.968	W
QSgw.aww-2D	2D4	32.43	E2-C	*wsnp_RFL_Contig2659_2346243*	3.53	6.08	0.858	D
	2D4		E2-H		3.53	7.46	1.018	D
QSgw.aww-3B1	3B1	11.02	E1-H	*wsnp_BE497169B_Ta_2_1(R)*	6.97	11.47	1.653	W
QSgw.aww-3B2	3B2	54.90	E2-H	*wsnp_Ex_c1097_2105209(R)*	3.80	5.91	0.906	W
QSgw.aww-4A	4A2	0.00	E1-H	*wsnp_Ex_c41074_47987860*	4.66	5.14	1.106	W
QSgw.aww-4B1	4B	83.90	E1-C	*Rht-B1*	14.17	21.48	2.094	D
	4B		E2-C		10.45	15.76	1.381	D
	4B		E2-H		6.09	10.22	1.191	D
	4B	86.26	E1-H	*wsnp_RFL_Contig4151_4728831*	25.96	33.04	2.806	D
QSgw.aww-4D	4D	0.00	E1-C	*Rht-D1*	14.30	20.52	2.047	W
	4D		E1-H		15.59	19.27	2.143	W
	4D		E2-C		9.08	12.94	1.252	W
	4D		E2-H		5.84	9.45	1.145	W
QSgw.aww-5A	5A2	116.62	E2-C	*wsnp_Ku_c14139_22353229(R)*	4.08	7.90	0.978	D
QSgw.aww-5B	5B2	20.84	E2-C	*wsnp_Ku_c10296_17072695(R)*	5.67	7.91	0.979	D
	5B2		E2-H		4.36	7.08	0.991	D
QSgw.aww-6A	6A	66.03	E1-C	*wsnp_Ex_c1104_2118684(R)*	4.24	5.46	1.055	D
	6A		E2-C		7.09	9.79	1.089	D
	6A		E2-H		3.32	4.95	0.829	D

[a]Experiment (Exp) indicated by E and the treatment indicated by C (control) and H (heat)
[b]Indicates the additive effect
[c]D and W indicate Drysdale or Waagan allele increased the trait value, respectively

Table 6.8 Continued.

Traits/QTL	Linkage group	Position	Exp	Marker(s)	LOD	R^2	Additive	Allele
GFD (day)								
QGfd.aww-1A	1A1	59.67	E2-C	*wsnp_Ex_c1997_3756118(R)*	6.32	7.33	0.517	D
QGfd.aww-3B	3B1	1.44	E1-H	*wsnp_Ex_c12875_20407926(R)*	6.37	13.07	0.758	W
QGfd.aww-4A1	4A1	0.00	E1-C	*wsnp_Ex_c11474_18507872*	4.66	9.43	0.528	D
QGfd.aww-4A2	4A2	42.27	E2-H	*wsnp_RFL_Contig25_2082245(R)*	4.61	4.21	0.494	D
QGfd.aww-4B1	4B	83.90	E2-H	*Rht-B1*	11.05	21.24	1.109	W
	4B	86.26	E2-C	*wsnp_RFL_Contig4151_4728831*	14.87	17.14	0.790	W
QGfd.aww-4B2	4B	108.33	E1-H	*wsnp_CAP12_rep_c4278_1949864(R)*	7.50	19.78	0.932	D
QGfd.aww-4B3	4B	127.49	E2-H	*wsnp_Ku_c11570_18860306(R)*	5.42	12.92	0.865	D
	4B	135.47	E2-C	*wsnp_Ex_c4148_7495656*	9.40	8.74	0.565	D
QGfd.aww-4D	4D	0.00	E2-C	*Rht-D1*	10.80	12.05	0.663	D
	4D		E2-H		10.12	14.70	0.923	D
QGfd.aww-5A	5A2	137.80	E1-C	*wsnp_Ex_rep_c101757_87064771*	5.31	10.51	0.557	W
QGfd.aww-6A	6A	70.74	E2-C	*wsnp_JD_rep_c62949_40140212*	6.99	6.86	0.500	D
QGfd.aww-7B1	7B	0.00	E1-H	*wsnp_CAP8_c334_304253*	5.86	11.29	0.704	D
	7B		E2-C		18.01	18.21	0.815	D
	7B		E2-H		5.98	8.20	0.689	D
QGfd.aww-7B2	7B	34.87	E1-C	*wsnp_Ex_c24376_33619527*	6.04	18.03	0.730	D
DTM (day)								
QDtm.aww-2A	2A	88.38	E1-H	*wsnp_Ex_c5984_10493714(R)*	3.80	7.12	0.687	W
QDtm.aww-4B1	4B	29.55	E1-C	*wsnp_Ex_c17561_26284693(R)*	5.00	10.74	0.964	W
QDtm.aww-4B2	4B	83.90	E2-H	*Rht-B1*	3.82	5.92	0.622	W
QDtm.aww-4B3	4B	135.47	E1-C	*wsnp_Ex_c4148_7495656*	4.59	7.87	0.825	W
	4B		E2-C		8.80	19.18	1.252	W
	4B		E2-H		12.76	22.18	1.205	W
	4B	141.25	E1-H	*wsnp_BE403378B_Ta_2_1*	7.06	14.01	0.965	W
QDtm.aww-4D	4D	0.00	E2-H	*Rht-D1*	5.36	8.19	0.732	D
QDtm.aww-7B1	7B	0.00	E1-H	*wsnp_CAP8_c334_304253*	6.31	12.71	0.919	D
	7B		E2-C		3.99	8.17	0.817	D
	7B		E2-H		5.19	8.07	0.727	D
QDtm.aww-7B2	7B	34.87	E1-C	*wsnp_Ex_c24376_33619527*	10.63	29.55	1.598	D
ChlC10DAA (SPAD units)								
QChlc10.aww-1A	1A2	0.00	E1	*wsnp_Ku_c40759_48907151(R)*	3.78	4.08	0.341	D
QChlc10.aww-2A1	2A	74.99	E1	*wsnp_Ex_c42720_49228237*	4.03	4.08	0.340	W
QChlc10.aww-2A2	2A	108.44	E2	*wsnp_Ex_c3808_6924802*	4.28	8.68	0.467	W
QChlc10.aww-2D	2D3	0.00	E1	*wsnp_Ex_c2258_4232538*	8.32	14.02	0.631	D
QChlc10.aww-3B	3B1	1.44	E1	*wsnp_Ex_c12875_20407926(R)*	10.68	17.92	0.714	W
	3B1		E2		7.36	16.75	0.649	W
QChlc10.aww-5A	5A2	3.76	E1	*wsnp_Ex_c1481_2831499*	6.31	12.12	0.587	D
ChlC13DAA (SPAD units)								
QChlc13.aww-2A	2A	77.95	E2-C	*wsnp_Ex_rep_c102538_87682273*	5.09	10.02	0.485	W
QChlc13.aww-2D	2D3	0.00	E1-C	*wsnp_Ex_c2258_4232538*	8.31	12.69	0.549	D
	2D3	5.76	E1-H	*wsnp_Ex_c7260_12463738(R)*	6.14	8.50	0.906	D
QChlc13.aww-3B	3B1	0.00	E2-H	*wsnp_Ra_c41135_48426638(R)*	15.95	34.34	1.952	W
	3B1	1.44	E1-C	*wsnp_Ex_c12875_20407926(R)*	7.67	14.64	0.590	W
	3B1		E1-H		26.02	41.97	2.013	W
	3B1		E2-C		8.85	19.36	0.674	W
QChlc13.aww-5A	5A2	1.50	E1-H	*wsnp_JD_c43389_30288993(R)*	4.56	6.25	0.777	D
	5A2	11.86	E1-C	*wsnp_Ex_c1481_2831499*	6.73	8.63	0.453	D
ChlC27DAA (SPAD units)								
QChlc27.aww-2A	2A	74.99	E1-C	*wsnp_Ex_c42720_49228237*	4.95	8.97	0.481	W
	2A	80.92	E2-C	*wsnp_Ra_c4503_8155485*	5.22	9.16	0.430	W
QChlc27.aww-2B	2B1	0.00	E2-C	*wsnp_Ra_c14112_22155451*	5.97	11.53	0.482	W
QChlc27.aww-2D	2D3	0.00	E1-C	*wsnp_Ex_c2258_4232538*	6.41	11.63	0.547	D
	2D3	5.04	E1-H	*wsnp_Ex_c20011_29041563*	5.38	6.65	0.870	D
QChlc27.aww-3B	3B1	0.00	E1-C	*wsnp_Ra_c41135_48426638(R)*	8.59	17.10	0.664	W
	3B1		E2-C		11.47	22.98	0.681	W
	3B1		E2-H		8.04	19.86	2.550	W
	3B1	1.44	E1-H	*wsnp_Ex_c12875_20407926(R)*	36.30	54.38	2.486	W
QChlc27.aww-4D	4D	2.90	E2-C	*wsnp_CAP11_c356_280910*	3.67	6.30	0.357	D

Table 6.8 Continued.

Traits/QTL	Linkage group	Position	Exp	Marker(s)	LOD	R^2	Additive	Allele
AUSC								
QAusc.aww-2A	2A	74.99	E1-C	*wsnp_Ex_c42720_49228237*	4.45	8.10	7.881	W
	2A	80.92	E2-C	*wsnp_Ra_c4503_8155485*	5.94	10.47	7.858	W
QAusc.aww-2B	2B1	0.00	E2-C	*wsnp_Ra_c14112_22155451*	4.27	7.92	6.834	W
QAusc.aww-2D	2D3	0.00	E1-C	*wsnp_Ex_c2258_4232538*	7.11	13.29	10.094	D
	2D3	5.76	E1-H	*wsnp_Ex_c7260_12463738(R)*	6.43	8.06	15.041	D
QAusc.aww-3B	3B1	0.00	E1-C	*wsnp_Ra_c41135_48426638(R)*	7.54	15.16	10.779	W
	3B1		E2-H		13.26	27.95	34.311	W
	3B1	1.44	E1-H	*wsnp_Ex_c12875_20407926(R)*	33.19	48.63	36.941	W
	3B1		E2-C		10.48	20.42	10.976	W
QAusc.aww-5A	5A2	1.50	E1-H	*wsnp_JD_c43389_30288993(R)*	4.38	5.37	12.281	D
QAusc.aww-6B	6B3	9.06	E2-H	*wsnp_Ex_c9038_15058444(R)/ wsnp_Ex_c11573_18650189(R)*	3.71	9.06	19.530	D
ChlR13								
QChlr13.aww-3B	3B1	0.00	E1-H	*wsnp_Ra_c41135_48426638(R)*	19.68	39.63	0.372	W
	3B1		E2-H		13.21	27.21	0.368	W
QChlr13.aww-6B	6B3	18.11	E2-H	*wsnp_Ex_c11573_18650189(R)*	4.79	8.94	0.211	D
ChlR27								
QChlr27.aww-1A	1A2	0.00	E2-C	*wsnp_Ku_c40759_48907151(R)*	4.59	11.25	0.035	W
	1A2		E2-H		4.36	10.68	0.096	W
QChlr27.aww-3B	3B1	0.00	E1-H	*wsnp_Ra_c41135_48426638(R)*	29.49	50.42	0.071	W
FLSe (day)								
QFlse.aww-1A	1A1	56.07	E2-C	*wsnp_Ku_c10292_17066821(R)*	3.84	5.35	0.887	D
QFlse.aww-3B	3B1	3.15	E1-H	*wsnp_BE497169B_Ta_2_1(R)*	5.54	14.18	2.056	W
QFlse.aww-4B1	4B	83.90	E1-C	*Rht-B1*	6.64	12.24	2.398	W
	4B	86.26	E2-C	*wsnp_RFL_Contig4151_4728831*	7.44	11.92	1.324	W
QFlse.aww-4B2	4B	135.47	E2-C	*wsnp_Ex_c4148_7495656 wsnp_BE403378B_Ta_2_1/*	16.43	27.88	2.024	D
	4B	149.85	E2-H	*wsnp_CAP7_c5487_2464794*	5.68	14.84	2.151	D
QFlse.aww-4D	4D	0.00	E1-C	*Rht-D1*	6.19	11.29	2.303	D
	4D		E2-C		12.49	19.09	1.675	D
	4D		E2-H		5.54	11.60	1.902	D
QFlse.aww-7B	7B	25.43	E1-C	*wsnp_JD_c1285_1848292/ wsnp_Ex_c24376_33619527*	8.10	26.45	3.526	D
ShW (g)								
QShw.aww-1A	1A1	48.15	E2-H	*wsnp_Ku_c23926_33870364(R)*	4.93	1.98	0.076	W
QShw.aww-2D1	2D3	3.60	E2-H	*wsnp_JD_c5919_7081809*	4.64	3.21	0.097	D
QShw.aww-2D2	2D4	18.84	E2-C	*wsnp_Ku_c30494_40319867(R)*	4.00	2.83	0.090	D
	2D4	25.64	E1-C	*wsnp_Ku_c30494_40319867(R)*	4.03	5.62	0.078	D
	2D4		E1-H		5.42	4.29	0.074	D
QShw.aww-3A	3A2	15.26	E1-H	*wsnp_Ex_c4069_7354375*	3.86	1.96	0.050	D
QShw.aww-3B1	3B1	11.02	E1-H	*wsnp_BE497169B_Ta_2_1(R)*	17.89	14.24	0.135	W
QShw.aww-3B2	3B1	40.49	E2-H	*wsnp_Ku_c3817_7009093/ wsnp_Ex_c44375_50444756(R)*	5.80	3.53	0.102	W
QShw.aww-4A	4A2	0.00	E1-H	*wsnp_Ex_c41074_47987860*	3.77	1.41	0.043	W
QShw.aww-4B1	4B	83.90	E1-C	*Rht-B1*	16.78	18.64	0.142	D
	4B		E1-H		34.23	23.13	0.173	D
	4B		E2-C		19.62	20.69	0.243	D
	4B		E2-H		32.64	26.48	0.278	D
QShw.aww-4B2	4B	135.47	E1-C	*wsnp_Ex_c4148_7495656*	4.44	5.10	0.074	W
	4B		E1-H		5.01	2.64	0.058	W
	4B		E2-C		11.14	9.75	0.167	W
	4B		E2-H		12.66	10.30	0.174	W
QShw.aww-4D	4D	0.00	E1-C	*Rht-D1*	39.92	47.26	0.227	W
	4D		E1-H		58.90	37.88	0.221	W
	4D		E2-C		50.45	48.65	0.373	W
	4D		E2-H		55.04	42.51	0.353	W
QShw.aww-6B	6B2	27.34	E2-H	*wsnp_Ex_c42372_48966781(R)*	6.02	4.45	0.114	D
QShw.aww-7B	7B	0.00	E1-C	*wsnp_CAP8_c334_304253*	4.07	2.94	0.057	D
	7B		E1-H		4.03	2.62	0.058	D

Table 6.8 Continued.

Traits/QTL	Linkage group	Position	Exp	Marker(s)	LOD	R^2	Additive	Allele
Plant height (cm)								
QPh.aww-1A	1A1	66.15	E2-C	*wsnp_BE517729A_Ta_2_1*	4.21	1.07	1.943	W
	1A1	67.59	E2-H	*wsnp_Ex_c5060_8985678*	4.90	1.48	2.309	W
QPh.aww-2A	2A	60.09	E1-H	*wsnp_BQ168780B_Ta_2_1*	4.30	1.28	2.084	D
QPh.aww-3A	3A2	15.26	E2-C	*wsnp_Ex_c4069_7354375*	7.12	1.45	2.265	D
	3A2		E2-H		6.58	2.10	2.754	D
	3A2	23.95	E1-H	*wsnp_Ex_c1141_2191485*	3.90	1.10	1.937	D
QPh.aww-4A	4A2	41.55	E1-C	*wsnp_Ex_rep_c68569_67411985(R)*	5.55	2.20	2.738	W
	4A2		E1-H		5.96	1.79	2.469	W
	4A2	42.27	E2-C	*wsnp_RFL_Contig25_2082245(R)*	8.99	1.79	2.515	W
	4A2		E2-H		7.34	2.33	2.901	W
QPh.aww-4B	4B	83.9	E1-C	*Rht-B1*	37.60	32.87	10.576	D
	4B		E1-H		46.25	36.78	11.185	D
	4B		E2-C		83.90	37.11	11.444	D
	4B		E2-H		44.92	35.88	11.379	D
QPh.aww-4D	4D	0	E1-C	*Rht-D1*	49.99	56.49	13.865	W
	4D		E1-H		56.50	56.43	13.855	W
	4D		E2-C		62.34	56.39	14.107	W
	4D		E2-H		54.00	52.64	13.784	W
HI (%)								
QHi.aww-3B	3B1	0.00	E2-C	*wsnp_Ra_c41135_48426638(R)*	8.67	11.53	0.940	D
QHi.aww-4B	4B	77.72	E2-C	*wsnp_Ex_c18433_27269748/ Rht-B1*	15.79	23.79	1.350	W
	4B		E2-H		11.99	25.36	1.218	W
QHi.aww-4D	4D	0.00	E2-C	*Rht-D1*	8.37	10.58	0.900	D
	4D		E2-H		10.82	19.43	1.066	D
QHi.aww-5A1	5A2	0.00	E1-H	*wsnp_CAP11_c923_558715(R)*	4.05	9.16	0.310	D
QHi.aww-5A2	5A2	126.88	E2-C	*wsnp_Ex_c3838_6981043*	5.42	6.71	0.717	D
QHi.aww-7A	7A2	33.74	E2-C	*wsnp_Ex_c2268_4251636*	3.62	5.47	0.648	W
QHi.aww-7B	7B	6.53	E1-H	*wsnp_JD_c1285_1848292*	4.36	9.88	0.321	W
Flag leaf length (cm)								
QFl.aww-2A	2A	0.00	E1	*wsnp_Ex_c2772_5130007*	3.88	7.57	0.301	W
QFl.aww-2B	2B1	126.34	E2	*wsnp_JD_c6010_7167159*	6.18	13.45	0.433	D
	2B1	133.11	E1	*wsnp_RFL_Contig1892_1042675(R)*	6.37	13.89	0.407	D
QFl.aww-4B	4B	126.05	E2	*wsnp_Ex_c39876_47057394*	6.92	13.61	0.436	D
QFl.aww-7B1	7B	0.00	E2	*wsnp_CAP8_c334_304253*	4.53	8.64	0.347	D
QFl.aww-7B2	7B	34.87	E1	*wsnp_Ex_c24376_33619527*	5.32	16.87	0.449	D
Flag leaf width (cm)								
QFw.aww-2A	2A	109.92	E2	*wsnp_Ex_c59095_60108185(R)*	5.42	6.76	0.026	W
QFw.aww-3A	3A2	30.42	E2	*wsnp_Ex_c25668_34932304*	5.43	5.96	0.024	D
QFw.aww-4A	4A2	47.31	E2	*wsnp_Ex_c55245_57821389*	4.74	5.99	0.024	D
QFw.aww-4B	4B	108.33	E1	*wsnp_CAP12_rep_c4278_1949864(R)*	10.06	20.44	0.033	W
	4B	117.19	E2	*wsnp_Ex_c39876_47057394*	16.87	31.20	0.056	W
QFw.aww-5A	5A2	131.2	E2	*wsnp_Ku_c23772_33711538*	5.71	9.13	0.030	W
QFw.aww-5B	5B2	62.54	E1	*wsnp_BE499835B_Ta_2_5(R)*	4.58	7.16	0.020	D
	5B2		E2		6.87	11.26	0.033	D

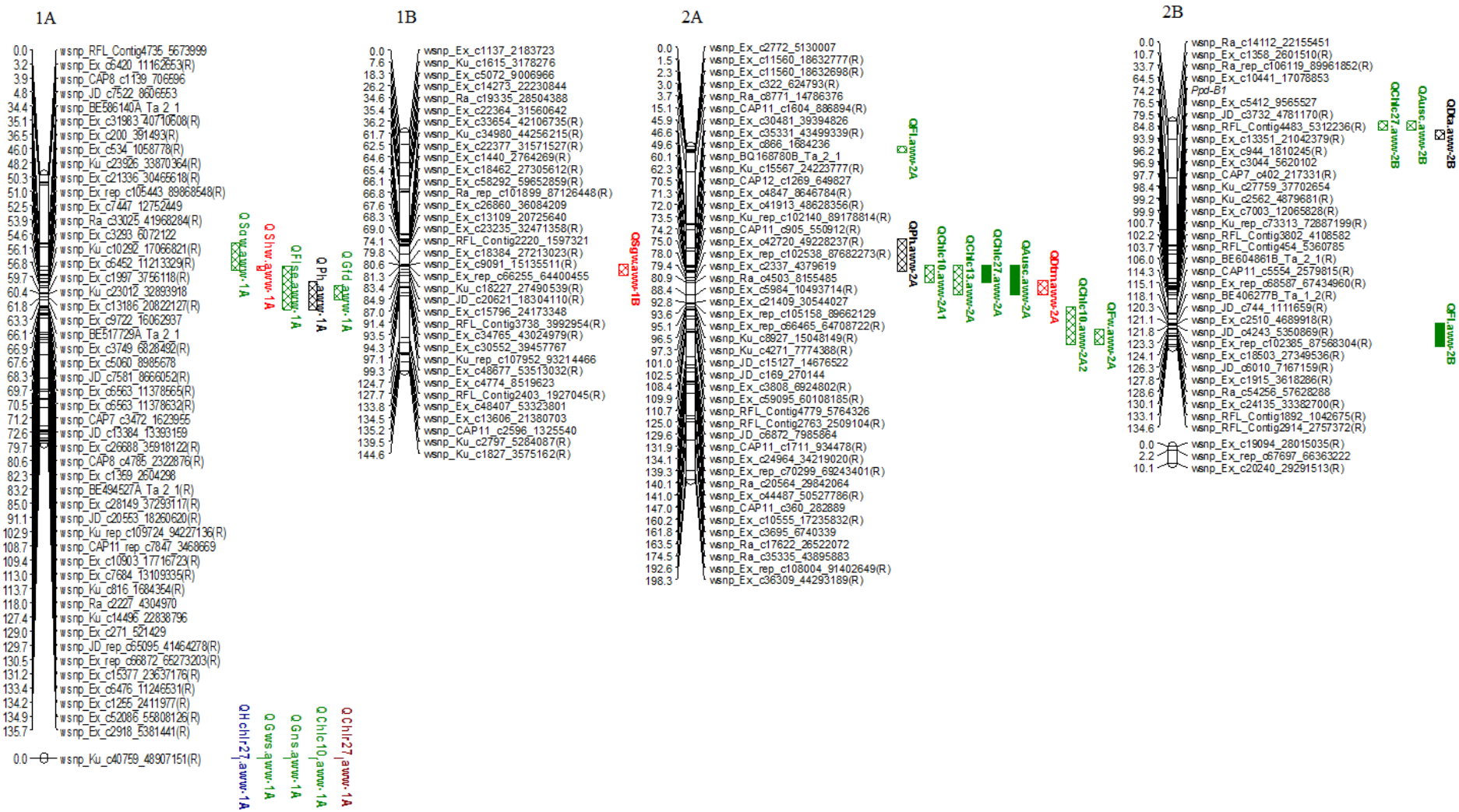


Figure 6. 2 Molecular marker linkage map and QTL detected for HSIs and absolute trait values in the Drysdale × Waagan DH population. The numbers to the left of each linkage group indicate cM distances from the top. QTL are presented as 1.5 LOD intervals. Blue: QTL for HSIs; black: QTL for DTA and PH; green, red, and brown: QTL detected for the absolute trait values under control, heat, and both control and heat conditions, respectively. Solid and hashed bars indicate QTL detected in both experiments or in one experiment only, respectively.

QTL at *wsnp_Ku_c40759_48907151(R)* on chromosome 1A, QHchlr27.aww-3B, QChlr27.aww-3B, QHi.aww-3B, and QShw.aww-3B1on chromosome 3B, and QFl.aww-7B1 on chromosome 7B were expressed in one experiment, but could not be presented with hashed bars due to the small size of the bars. Other QTL details are presented in Tables 7 and 8. Dta, days from sowing to anthesis; Gws, grain weight spike^{-1}; Gns, grain number spike^{-1}; Sgw, single grain weight; Gfd, grain-filling duration; Dtm, days from sowing to maturity; Chlc10, chlorophyll content 10 days after anthesis; Chlc13, chlorophyll content 13 days after anthesis; Chlc27, chlorophyll content 27 days after anthesis; Ausc, area under SPAD curve; Chlr13, linear rate of chlorophyll loss between SPAD 10 and 13 DAA points; Chlr27, chlorophyll loss rate determined by a linear regression of the three SPAD measurements (10, 13 and 27 DAA); Flse, days from anthesis to 95% flag leaf senescence; Shw, shoot dry weight; Ph, Plant height; Hi, harvest index; Fl, flag leaf length and Fw, flag leaf width; Hgws, HSI of grain weight spike^{-1}; Hgns, HSI of grain number spike^{-1}; Hsgw, HSI of single grain weight; Hgfd, HSI of grain-filling duration; Hdtm, HSI of days from sowing to maturity; Hchlc13, HSI of chlorophyll content 13 days after anthesis; Hchlc27, HSI of chlorophyll content 27 days after anthesis; Hausc, HSI of area under SPAD curve; Hchlr13, HSI of linear rate of chlorophyll loss between SPAD 10 and 13 DAA points; Hchlr27, HSI of linear rate of chlorophyll loss considering all of the three SPAD measurements (10, 13 and 27 DAA); Hflse, HSI of days from anthesis to 95% flag leaf senescence; Hshw, HSI of shoot dry weight; Hhi, HSI of harvest index.

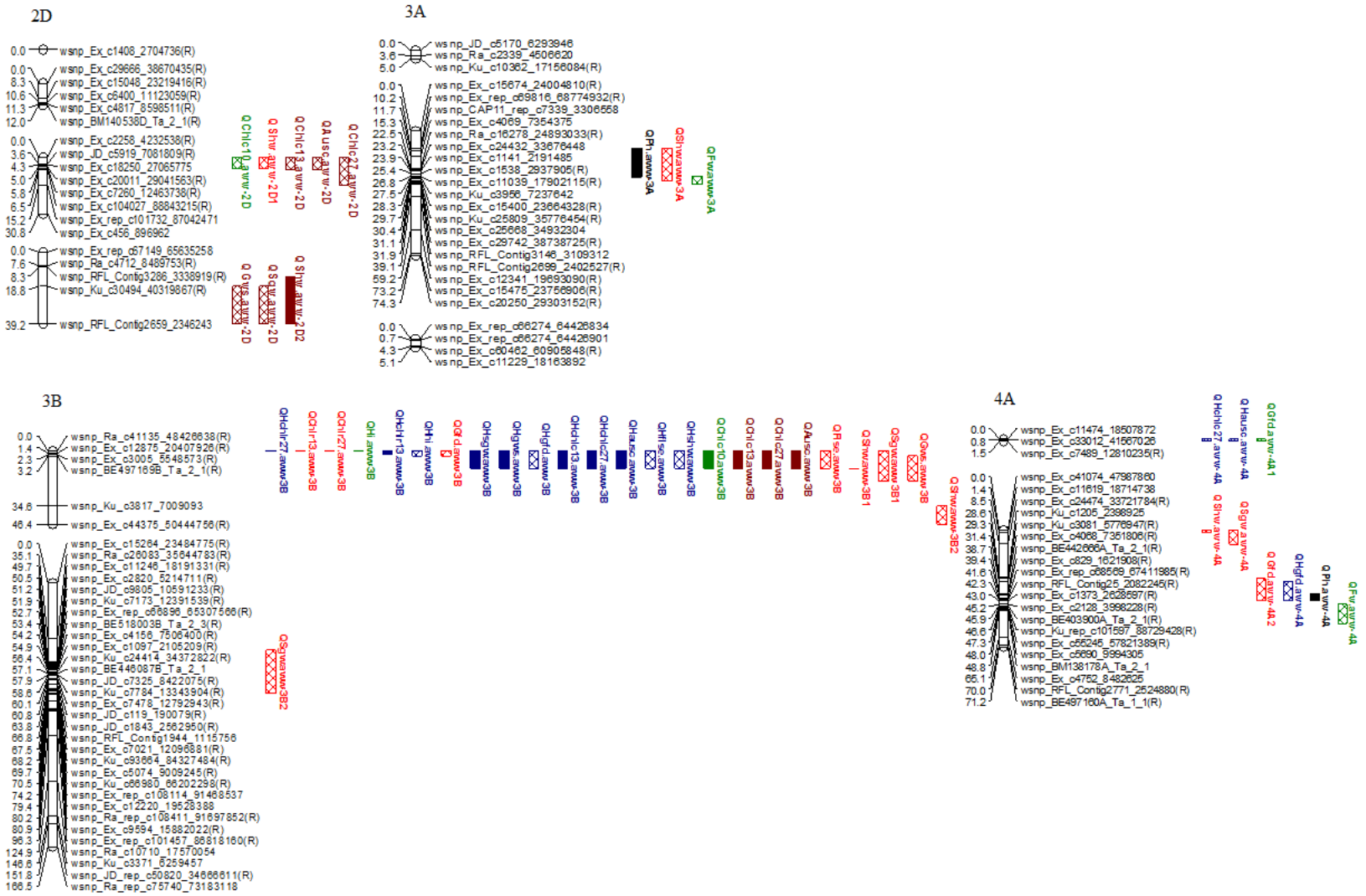


Figure 6.2 Continued.

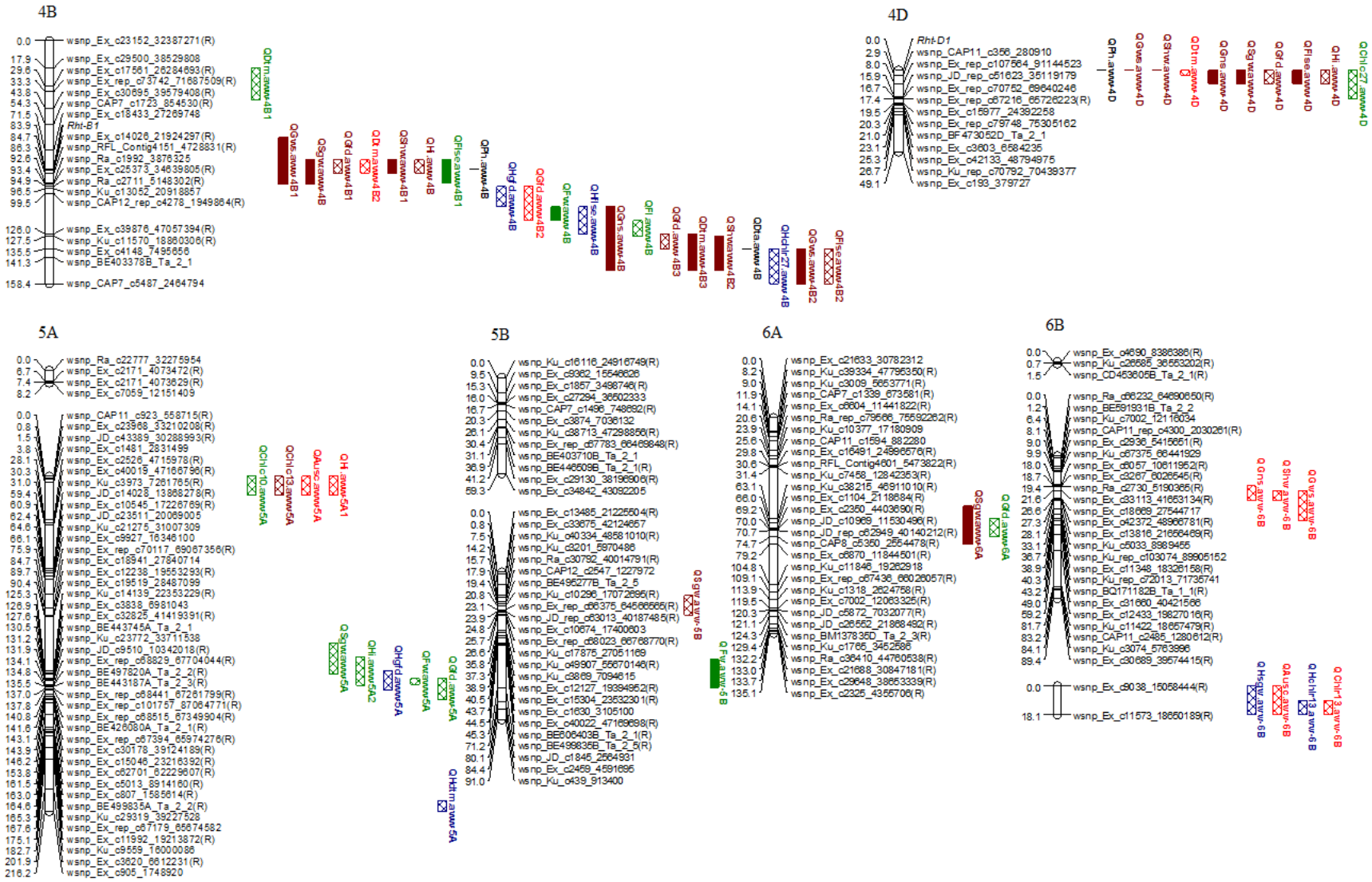


Figure 6.2 Continued.

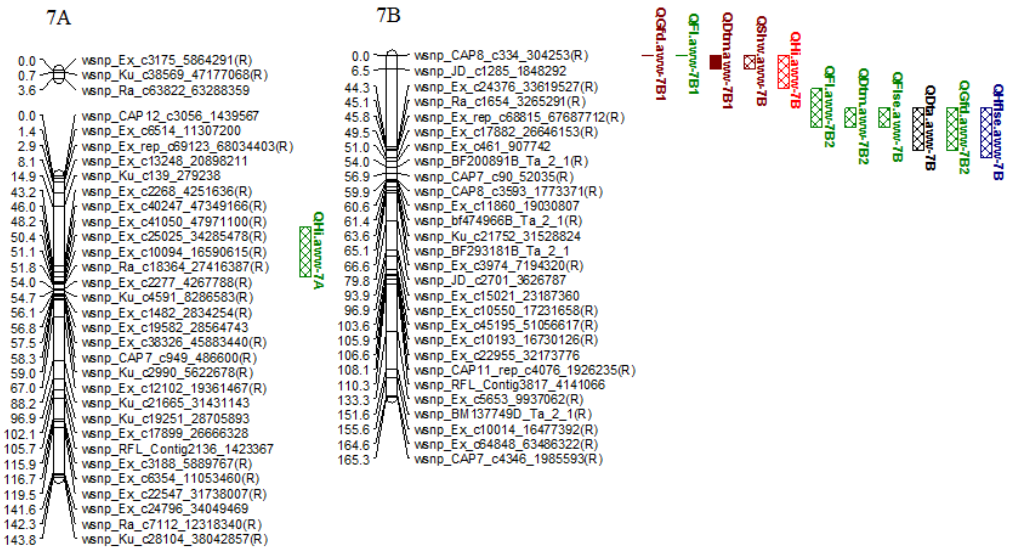


Figure 6.2 Continued.

6.4 Discussion

The Waagan and Drysdale parents showed some levels of contrast for SGW maintenance in response to a brief heat treatment in experiments presented in Chapters 3 and 4, while their contrast for this trait was relatively low in the first experiment and negligible in the second experiment in the current study. The reason for these conflicting results might be due to within-variety genetic variation in these parental lines (seed sources of the screening experiments, Chapters 3 and 4, differed from those of the current study) as presented in Chapter 5, and different growing conditions. Consistent with this idea in a separate experiment conducted under the same conditions as Experiment 1, but with the same seed source as the experiments described in Chapters 3 and 4, considerable contrast was observed between Drysdale and Waagan varieties (19.4 and 6.4% contrast for SGW and AUSC responses, respectively; Appendix 6.1a and b). Nevertheless, the heat-treatment significantly affected most of the studied traits in the Drysdale × Waagan DH population, including SGW in both experiments, allowing identification of QTL for heat responses (HSIs).

6.4.1 QTL mapping

HSIs and absolute trait values under control and heat conditions were both QTL-mapped. This can help us to understand tolerance mechanisms and have practical implications in breeding. In other words, if the lowest yielding lines under normal conditions are the most tolerant, it will create a problem for breeding, whereas a positive relationship between the tolerance and trait potential would make breeding simpler (indirect selection for tolerance through selecting genotypes with larger trait values, and varieties that perform well under both conditions). Flowering time and plant height were mapped since these traits have been reported to associate with yield components. Flag leaf length and width were also mapped since it has been previously suggested that they may influence plant productivity under heat stress conditions (Mason et al. 2010).

The population did not segregate for any known major locus for vernalisation or photoperiod response. Three minor QTL were detected for flowering time, on chromosomes 2B, 4B, and 7B, determining differences in time to anthesis of only 0.7 to 1.6 d. Loci affecting flowering time on these chromosomes have been previously reported or predicted. *Ppd-B1* (Beales et al. 2007) and loci affecting earliness *per se* have been reported on chromosome 2B (Kuchel et al. 2006; Shindo et al. 2003). Wheat chromosome arm 4BL corresponds to parts of barley chromosome 4H that contain flowering time loci *Vrn-H2* and *Eps-4HL* (Snape et al. 2001). Also, *Vrn3* (*FT*) and *VRT2* flowering time loci/genes were mapped on wheat group-7 chromosomes (Bonnin et al. 2008; Kane et al. 2005; Kane et al.

2007; Yan et al. 2006) and QTL affecting heading date and earliness *per se* have been reported on chromosome 7B (Hanocq et al. 2007; Kuchel et al. 2006) in a similar region to *Vrn3* (Bonnin et al. 2008). The detected QTL for flowering time in this study may represent allelic variation in the same or orthologous genes in wheat.

None of the QTL for HSI of grain weight (GWS and SGW) co-located with loci affecting DTA; QHchlr27.aww-4B and QHflse.aww-7B were the only HSI loci that co-located with DTA loci. A lack of correspondence of grain weight HSI loci with flowering time loci was not unexpected, since tillers were heat treated at the same tiller developmental stage (10 DAA), and there were also no major flowering time loci segregating in the population. Therefore, this study does not shed any light on whether phenology could affect heat tolerance.

Larger spikes with more spikelets and grains per spikelet might be expected to have less synchronous floret pollination, resulting in some florets being heat stressed much sooner after anthesis than 10 days, or even before anthesis. Exposure to heat stress at around meiosis to early grain development (< 10 DAA) can result in floret sterility/abortion (Saini and Aspinall 1982b; Tashiro and Wardlaw 1990a). A QTL for GNS under both control and heat conditions was detected on chromosome 4B (QGns.aww-4B; also co-locating with QDta.aww-4B). While there was no significant HSI QTL for GNS at this location, this QTL for GNS *per se* did show a weaker effect on average under heat conditions, consistent with the idea that the large GNS allele may have resulted in some floret sterility under heat. Overall, heat treatment did not have any significant impact on GNS, and there were no significant GNS HSI QTL detected, indicating that grain number had been set in all or the vast majority of florets at the time of heat treatment. Yield reduction in this experiment was therefore just a result of a reduction in SGW, which is in accordance with outcomes of previous heat experiments in which stress was applied ≥10 DAA (Bhullar and Jenner 1985; Stone and Nicolas 1995b; Tashiro and Wardlaw 1990a; Tashiro and Wardlaw 1990b). In this study, plants were heat-treated at such a late stage that it sheds no light on heat effects on floret fertility.

There were 11 QTL for heat tolerance (HSI; 5 and 6 QTL in Experiment 1 and 2, respectively). These QTL explained ~6 to 40% of the phenotypic variance depending on the trait and experiment. These results are in accordance with previous reports of heat tolerance in wheat being under control of multiple QTL/genes (Mason et al. 2010; Mason et al. 2011; Mason et al. 2013; Paliwal et al. 2012; Tiwari et al. 2013; Yang et al. 2002b). The only stably expressed HSI QTL region was on chromosome 3BS. The two experiments differed for temperature and day length conditions in the greenhouse which may have contributed to a scarcity of stable QTL across experiments. Furthermore, the non-stable HSI QTL were

relatively weak, giving a greater chance of not being significant, just due to chance. Both parents contributed to heat tolerance, which is not unexpected considering the quantitative nature of heat tolerance. However, favourable alleles for the stable 3BS QTL region were provided only by Waagan.

Three QTL were detected for grain weight response (QHgws.aww-3B, QHsgw.aww-3B and QHsgw.aww-6B). The SGW and GWS response QTL on 3BS had greater effects in Experiment 1 than in Experiment 2. This may have been due to several hot days which occurred in the greenhouse during the flowering/grain-filling period in Experiment 2 (Table 6.1), which may have reduced the contrast between the control and heat-treated plants.

QTL affecting grain weight in both control and heat conditions, but having a higher average additive effect under heat, were also detected on chromosomes 2D, 4B (at *Rht-B1*) and 5B. Also, QTL for grain weight *per se* under just heat (but not control) were located on chromosomes 1B, 3B (QSgw.aww-3B2), 4A, and 6B (QGws.aww-6B). While the heat/control differences at these loci were not strong enough to produce significant HSI QTL, these loci may represent weak tolerance loci that may still be of potential use to breeders in improving grain weight under heat-stress conditions.

In cases where HSI associates with higher performance *per se* of traits, it might provide the opportunity to improve both trait stability and performance *per se* simultaneously in breeding. The QTL region on 3BS has this potential. This region controls HSIs of GWS (QHgws.aww-3B) and SGW (QHsgw.aww-3B) as well as GWS and SGW *per se* only under high temperature conditions (QGws.aww-3B and QSgw.aww-3B1, respectively), with the favourable effects provided by Waagan.

Leaf senescence is a process which naturally accelerates during the final stage of development to translocate nutrients into growing grains. Leaf senescence can be hastened by environmental factors (e.g. heat and drought), resulting in enhanced loss of chlorophyll and photosynthetic capacity (Vijayalakshmi et al. 2010). Current photosynthesis and post-anthesis accumulation and remobilization of assimilates contribute a large proportion of final grain yield in wheat (Al-Khatib and Paulsen 1989; Evans 1975). Thus, a reduction in photosynthetic capacity due to accelerated senescence at early grain-filling, triggered by biotic and abiotic stresses, can deprive the grains of assimilates and cause a significant yield reduction (Lopes and Reynolds 2012; Reynolds et al. 1994; Reynolds et al. 2000; Rosyara et al. 2009; Rosyara et al. 2010b). In the present study, the brief heat treatment accelerated flag leaf senescence as reported in some other works (Al-Khatib and Paulsen 1984; Lopes and Reynolds 2012; Reynolds et al. 1994; Reynolds et al. 2000). QTL were detected for HSIs and

absolute values of stay-green related traits on chromosomes 1A, 2A, 2B, 2D, 3B, 4A, 4B, 4D, 5A, 6B, and 7B. Among these QTL, those on 3BS and 6BL had the largest effects on chlorophyll content *per se* and chlorophyll loss rate in particular in heat-treated plants. These two QTL co-localized with HSI QTL for grain weight (QHgws.aww-3B, QHsgw.aww-3B and QHsgw.aww-6B) with the stay-green allele (i.e., for delayed senescence and ability to maintain green leaf area during grain-filling, as indicated by larger chlorophyll content, slower chlorophyll loss rate, and longer FLSe) being the one that also maintained grain weight under heat conditions, indicating a genetic (and possible functional) link between grain weight responses and stay-green under heat. With few exceptions (Naruoka et al. 2012), individual studies have only looked at mapping stay-green (Kumar et al. 2010; Vijayalakshmi et al. 2010) or grain weight responses (Mason et al. 2010; Mason et al. 2011; Mason et al. 2013; Paliwal et al. 2012; Tiwari et al. 2013) under high temperature conditions, rather than both at once. The current study looked at both of these traits and showed a strong genetic link between heat stability of grain weight and stay-green related traits in response to a brief heat stress.

Only a little is known about the physiological mechanisms linking stay-green with better performance (e.g. yield) under stress conditions (Thomas and Ougham 2014). Stay-green under drought stress in sorghum is associated with increased xylem pressure potential, delayed loss of photosynthetic competence, and enhanced N uptake (Tuinstra et al. 1998; Vadez et al. 2013). In wheat, stay-green related traits have been found to be highly positively correlated with photosynthesis under high temperature conditions (Al-Khatib and Paulsen 1984; Gutiérrez-Rodriguez et al. 2000; Reynolds et al. 2000) and also to a root architecture which allows better extraction of water from deep in the profile post-anthesis under field conditions (Christopher et al. 2008). The QTL on 3BS also co-localized with QTL for heat stability of ShW, HI, and GFD, with alleles having effects in the same direction to stay-green related traits, indicating a possible functional link between these traits. The larger grain weight, ShW, HI, and GFD maintenance (smaller HSI) in stay-green lines in this study might be an indicator of their higher photosynthetic rate, longer photosynthetic competence as well as better overall plant health and plant canopy survival under heat conditions. The QTL on chromosomes 1A, 4A, 4B, and 7B for HSIs of stay-green related traits had weaker effects than those on 3BS and 6BL, and were unstable across experiments. As stated earlier, those on 4B (QHchlr27.aww-4B) and 7B (QHflse.aww-7B) co-localized with QTL affecting flowering time, where late flowering was associated with weaker stay-green. Such a relationship has been also reported in some previous studies (Blake et al. 2009; Tewolde et al. 2006), and suggests a functional link between flowering time and stay-green.

Several QTL were detected for performance *per se* of stay-green related traits, under just control conditions (1A at *wsnp_Ku_c10292_17066821(R)*, 2A, 2B, and 4B at *Rht-B1*) or heat conditions (6B). However, for most QTL, including the strongest (1A, 2D, 3B, 4B, 4D, and 5A), the effect was associated with performance *per se* under both control and heat conditions, and a higher additive effect was observed under heat, although (except for 1A and 3B loci), the differences between control and heat was not strong enough to manifest as HSI QTL. Stay-green QTL expressed under both control and heat conditions suggests a genetic tendency to senesce faster under heat may at least partly derive from an acceleration of senescence processes that normally occur, rather than the occurrence of a heat specific type of damage. By contrast, Vijayalakshmi et al. (2010) detected different chromosomal regions affecting senescence under different conditions (optimum vs. heat stress conditions) and concluded involvement of different sets of genes under these different conditions. The conflict between results of the current study and those of Vijayalakshmi et al. (2010) might result from the difference in magnitude and duration of heat treatment, choice of mapping population or the method used to quantify flag leaf senescence.

Although the superiority of semi-dwarf wheat genotypes under optimal growing conditions is widely accepted, their yield benefit in low yielding environments has been questioned. Nizam Uddin and Marshall (1989) and Kuchel et al. (2007) reported a better grain yield performance of semi-dwarfs than tall genotypes under both stress and non-stress conditions. Alghabari et al. (2014) tested tall and semi-dwarf near isogenic lines under heat and drought stress at booting/anthesis stage and detected no difference in their stress sensitivity. Law et al. (1981) and Law and Worland (1985) observed that *Rht* dwarfing alleles (*Rht-B1b*, *Rht-D1b*, and *Rht-B1c*) conferred higher levels of sterility caused by high temperatures during booting. Butler et al. (2005) reported a grain yield and grain weight advantage of tall lines, in comparison with semi-dwarf lines, under stress conditions. Semi-dwarfing may influence heat tolerance of wheat by affecting assimilate availability under stress conditions (Alghabari et al. 2014). Owing to differences in stem length, tall genotypes have larger stem reserves than semi-dwarfs; the dwarfing alleles at *Rht-B1* and *Rht-D1* have been estimated to decrease stem reserve storage by 35 and 39%, respectively (Borrell et al. 1993). In general, shoot weight has been shown to correlate positively with levels of water soluble carbohydrates (Blum et al. 1994; Ehdaie et al. 2008; Talukder et al. 2013). Under optimum conditions, pre- and post-anthesis stored assimilates in the wheat stem have been estimated to contribute to around 10 to 20% of the final grain yield (Austin et al. 1977; Borrell et al. 1993; Wardlaw and Porter 1967). However, several studies have shown that, under stress conditions that disturb

photosynthesis, there is a large increase in relative contribution of stem reserves to the final grain weight, ranging from 6 to 100% (Blum 1998 and references cited therein).

The current population segregated for both major semi-dwarf genes *Rht-B1* and *Rht-D1*, providing an opportunity to investigate the relationship between PH, ShW and HI variation on grain weight maintenance under heat. These two loci controlled up to 93.5% of the variation for PH. The non-dwarfing allele at each locus was associated with larger absolute GWS (QGws.aww-4B1 and QGws.aww-4D), SGW (QSgw.aww-4Band QSgw.aww-4D), and ShW (QShW.aww-4B1 and QShW.aww-4D) in both control and heat-treated plants and in both experiments. QTL for HI were also detected at both dwarfing loci in both treatments, but just in Experiment 2, with dwarfing alleles associated with larger HI. The non-dwarfing allele of *Rht-D1* was also associated with larger GNS (QGns.aww-4D) in all four treatments/experiments. On average, tall genotypes showed better SGW maintenance (smaller HSI; 0.66 and 0.93 for SGW in Experiments 1 and 2, respectively) than semi-dwarfs (larger HSI; 0.78 and 1.02 for SGW in Experiments 1 and 2, respectively) or 'double-dwarfs' carrying both dwarfing genes (1.57 and 0.99 for SGW in Experiments 1 and 2, respectively). However, the difference in effect was not great enough to manifest as a HSI QTL for SGW or GWS at either locus. Also, none of the other (minor) PH QTL coincided with any HSI QTL for SGW or GWS. While these results are indicative that increased stem carbohydrate stores provided by tall *Rht* alleles (and/or other associated traits) may allow better grain weight maintenance under heat, the effect is small compared to other genetic effects (e.g., associated with stay-green), at least under the current experimental conditions and in this population. Yield benefits associated with dwarfing genes can be affected by various factors including growth habit (spring vs. winter), genetic background, and environmental factors (Alghabari et al. 2014; Bush and Evans 1988). Stem reserves may also not be an advantage unless plants can efficiently mobilize the carbohydrate reserves to the growing grains or convert delivered sugars to starch in the grain (e.g. as a result of heat stable soluble starch synthase activity) (Blum 1998).

The only ShW HSI QTL was detected on chromosome 3BS and it co-localised with stay-green related traits and grain weight (GWS and SGW) HSI QTL. ShW at this locus was controlled in the same direction as HSIs of stay-green related traits and grain weight (i.e. tolerant lines tended to maintain both better chlorophyll and ShW). This indicates that the heat stability of stay-green related traits and consequently photosynthesis possibly may have allowed better supply of assimilates to support growth not only of the grain but also of the vegetative parts, under heat stress conditions. The data provided no evidence for differences in mobilization efficiency affecting tolerance, as could be concluded if the allele conferring

better SGW maintenance instead lowered ShW at maturity. In addition to co-control of responses (HSIs) and performance *per se* of ShW and grain weight on 3BS, QTL for performance *per se* of these traits under heat co-localized, on chromosomes 4A and 6B, and were independent of plant height QTL, which further indicates a genetic/functional link between larger stem weight *per se* and grain weight *per se* under high temperature conditions. At both loci, larger ShW was associated with larger grain weight under heat conditions. This relationship suggests that either greater ShW contributes to grain weight maintenance under heat or that similar processes control both traits.

In both experiments, HI response showed a strong positive association with GWS, while it was not associated, or showed relatively a weak negative association, with ShW response in Experiment 1 and Experiment 2, respectively (Table 5). The only QTL detected for HSI of HI was detected on 3BS in Experiment 2. This effect mainly derived from the response of GWS and SGW, since no QTL was detected for ShW response in Experiment 2 in this region (althoughQShw.aww-3B2 for larger ShW *per se* under heat conditions was detected close to this region in Experiment 2). Other notable QTL for HI were those on 5A and 7B. On 5A, larger HI *per se* under heat conditions co-localized with larger chlorophyll content *per se* which suggests a beneficial effect of stay-green on HI under heat conditions. On 7B, HI *per se* under heat conditions was negatively associated with ShW, GFD and DTM, and FL. As already discussed, larger ShW and more stem reserves are not necessarily beneficial unless plants have additional genetic factors to utilise the reserves. Therefore, the reverse association of these traits might be due to a lack of an ability to utilise the reserves.

It has been previously pointed out that the flag leaf dimensions (length and width) may also play a role in plant productivity under heat stress conditions (Mason et al. 2010). In the current study, none of the QTL for flag leaf dimensions co-localised with QTL for grain weight maintenance (HSI). However, QTL affecting heat stability (HSI) of either GFD or flag leaf stay-green related traits (or both) on 4B, 5A, and 7B co-localized with flag-leaf dimension QTL. On 4B, flag leaf dimensions also co-localized with performance *per se* of a number of other traits (GNS, DTM, GWS, DTA) under both control and heat conditions. This may suggest a contribution of these phenotypic characters to higher performance *per se* (absolute trait values), or control by common genes and processes.

6.4.2 Co-localisation with previously reported QTL

The 3BS QTL region showing strong and consistent control of heat tolerance was compared to the position of QTL for heat tolerance and/or heat related traits identified by others, by linking marker sequences to the chromosome 3B reference sequence (http://wheat-

urgi.versailles.inra.fr/; Figure 6.3). This exercise was limited by a lack of available sequence for some of the markers. Maccaferri et al. (2008) reported a major QTL on distal end of chromosome 3BS (*Xbarc133*/*Xgwm493*) affecting both grain yield and plant height in various environments (including stressed environments) in a durum wheat RIL population (Kofa × Svevo). Mason et al. (2010) detected a QTL on chromosome 3BS (*barc75*/*Xgwm493*) affecting heat tolerance (indicated by HSI) of grain number spike^{-1} and grain weight spike^{-1} in response to a brief heat stress (at 10 DAA), and flag leaf length *per se*, under controlled conditions, in a spring × winter wheat cross (Halberd × Cutter). Kumar et al. (2010) mapped a major QTL affecting stay-green (visually scored) in this region (associated with *Xgwm533*) in a bread wheat RIL population (Chirya3 × Sonalika) under high temperature field conditions. Wang et al. (2009) detected QTL on the distal end of 3BS (associated with *Xgwm533*) affecting maximum grain-filling rate, grain-filling duration, thousand grain weight, and flowering time in a winter bread wheat RIL population (HSM × Y8679) under field conditions. Bennett et al. (2012) detected QTL for several traits including yield, thousand grain weight, grains m^{-2}, normalized difference vegetation index (NDVI), chlorophyll content (measured by SPAD chlorophyll meter), and canopy temperature on chromosome 3BS in a spring bread wheat DH population (RAC875 × Kukri) in various field environments including drought and heat-stressed environments (SPAD and canopy temperature QTL were associated with *barc75*). Sharma (2013) found that short arm of chromosome 3B to be important genomic region associated with Fv/Fm parameter under heat stress conditions. As illustrated in Figure 6.3, the aforementioned regions overlapped with the QTL detected in this study, consistent with a hypothesis in which the same underlying gene(s) are responsible for the different reported QTL. The QTL peak region (0 to 3.15 cM, illustrated in Figure 6.3) represents ~1.5% of the total genetic length of chromosome 3B (not including the gap between linkage groups) in the current study, and ~5% of total physical (bp) length of chromosome 3B according to wheat 3B reference sequence.

The control of heat/drought tolerance by this region in independent studies and in different genetic backgrounds, and the pleiotropic nature of this locus affecting several heat tolerance related traits, suggests this region contains potentially useful and interesting genes that may be worthy of use in marker assisted selection, or as targets for positional cloning. For the Drysdale × Waagan population, the 3BS QTL should be tested in the field to determine its usefulness in breeding, as many factors are different in greenhouse vs. field (e.g. absence of any drought stress, wind, etc. under controlled conditions). This could be done by trialling the whole population using an early vs. late sowing approach or by trialling it at low vs. high temperature sites. Alternatively, homozygous nearly-isogenic lines for the QTL could be

selected from progeny of 3BS-heterozygous RILs (a Drysdale × Waagan RIL population of 3,000 lines has already been made) and the lines evaluated using the aforementioned field approaches.

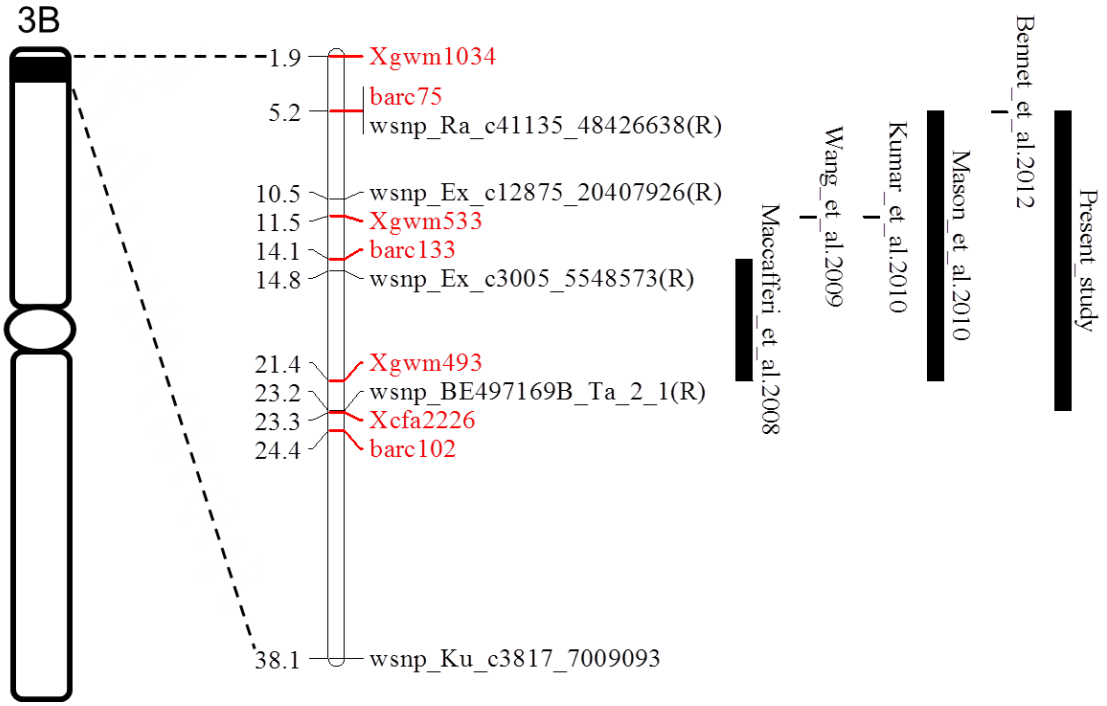


Figure 6. 3 Physical position of markers from the current study (black) and from previous studies (red) on the chromosome 3B reference sequence. Bars indicate QTL positions described for heat tolerance related traits in this and previous studies. The numbers to the left indicates Mbp distances from the top of the chromosome. For QTL detected by Wang et al. (2009), Kumar et al. (2010), and Bennett et al. (2012) flanking marker sequences were not available, and hence the most closely associated markers (in which the markers sequence were available) were examined.

Some of the other chromosome regions to which HSI QTL were located in this study have previously been reported to affect heat related traits. QTL for HSI of SGW and grain-filling rate under high temperature in the field were reported on 6B (Barakat et al. 2011; Tiwari et al. 2013), in a region showing QTL for HSIs and performance *per se* under heat conditions in the current study. There were QTL on chromosome 5AL for heat stability of GFD and DTM in the current study, and QTL affecting heat tolerance (HSI) and heat related traits have been previously mapped to the same chromosome arm. QTL for GFD, grain protein content, temperature depression of flag leaf and main spike, heat stability of GWS, GNS, and SGW and also visual wax score under high temperature conditions have been mapped on 5AL (Groos et al. 2003; Mason et al. 2010; Mason et al. 2011; Yang et al. 2002b). While the distal region of long arm of chromosome 5A carries the major vernalisation response gene *Vrn-A1*, which could potentially confer heat avoidance through its effect on flowering time, this cannot be the basis for the 5AL QTL in the current study, because the Drysdale × Waagan population does not segregate for functional variation at *Vrn-A1*.

Other heat tolerance QTL were mapped in the current study on chromosomes 1A (QHchlr27.aww-1A), 4A (QHgfd.aww-4A, QHchlc27.aww-4A, and QHausc.aww-4A), 4B (QHgfd.aww-4B, QHchlr27.aww-4B, and QHflse.aww-4B), and 7B (QHflse.aww-7B). QTL affecting heat tolerance have been reported on these chromosomes in earlier studies for various traits including HSIs of GNS (1A, 4A, and 4B), SGW (1A, 4A, and 7B) and yield (4A, 4B, 7B), and grain hardness (4B), canopy temperature depression (1A, 4A, 4B, and 7B), stay-green (1A), and NDVI (4A, 4B, and 7B) under high temperature conditions in field trials or controlled environments (Mason et al. 2010; Mason et al. 2011; Mason et al. 2013; Mohammadi et al. 2008b; Paliwal et al. 2012; Pinto et al. 2010). A lack of common markers between studies, and in some cases low marker density, would make comparison of these QTL locations difficult.

6.5.3 Comparison to results of screening 36 wheat genotypes for heat tolerance during grain-filling

A significant negative association was observed between grain weight (GWS and SGW) responses and flowering time in Chapter 3. In the current study flowering time also showed significant correlation with HSIs of several traits (Table 6.6). However, the Drysdale × Waagan population did not allow an effective test of these associations at genomic level, since it showed no major QTL for flowering time. A significant negative association was also observed between grain weight potential and grain weight response in Chapter 3 (i.e. larger-grained varieties tended to lose a greater proportion of grain weight under heat). However, none of the grain weight HSI QTL co-localized with QTL for grain weight *per se* under control conditions. Therefore, the grain weight potential vs. response association observed in Chapter 3 may have been due to chance co-occurrence of genes for the two traits in some of the sampled varieties.

A significant negative association was observed between plant height and grain weight response in Chapter 3, i.e., the taller genotypes tended to be more intolerant. However, dwarfing alleles at the *Rht-B1* and *Rht-D1* loci showed no significant association with single grain weight maintenance under heat in the Drysdale × Waagan population, and in fact, the trend was for the tall genotypes to be more tolerant. Thus, the negative association between plant height and grain weight response observed in Chapter 3 may have been due (as above) to chance co-occurrence of genes for the two traits in some of the varieties. Consistent with this idea, the highly susceptible and tall varieties Cadoux and Reeves were shown to be highly related in a similarity tree constructed using molecular marker data (data not shown).

A highly significant association was observed between chlorophyll retention and grain weight responses in Chapter 3. Moreover, a positive association was observed between responses of stay-green related traits and their potentials in Chapter 3, indicating a stronger response for genotypes with larger rate of natural senescence. Co-localization of QTL for the stay-green related traits and grain weight response provided strong evidence that these traits are indeed controlled by common gene(s) and physiological mechanisms. Likewise, the tendency of naturally faster senescing lines to lose chlorophyll faster in response to heat also seems to be due to common genetic control, since QTL alleles were found that conferred chlorophyll retention under both control and heat-stressed conditions and slower chlorophyll loss rates under heat. This supports the idea that natural senescence and accelerated senescence in response to the heat treatment were functionally related. However, QTL specific to either control or heat conditions were also detected which suggest some specific mechanisms under certain conditions.

6.5 Concluding remarks

Several QTL were detected for heat tolerance (HSI) and heat tolerance related traits. However, only reproducible effects may have value in plant breeding programs. The QTL on 3BS was detected in both experiments and this chromosome region has been reported to affect stress tolerance or heat tolerance related traits in several crosses in independent studies. This QTL may provide an appropriate target for fine mapping and marker assisted selection for improving heat tolerance in wheat, if it can be validated more widely in the field (and for this population). The pleiotropic nature of this locus provides an opportunity to simultaneously improve several traits which are related to larger yield performance under high temperature conditions. Chlorophyll content and chlorophyll loss rate showed high heritability under high temperature conditions (Table 6.4), and these traits can be scored easily (i.e. using SPAD chlorophyll meter). Consequently, these traits may provide an effective and early way to indirectly select tolerance (SGW stability under heat) in plants prior to maturity. Measurements at only two time points - before (10 DAA in this study) and after heat stress (13 DAA) - provided good discrimination between tolerant and intolerant genotypes, and could be sufficient. The measurement before the heat treatment can be used as a control value since there was not much change in chlorophyll content of control plants in a short period of time (10 to 13 DAA). Thus, control plants could be dispensed with. This could also be practical in the field where plants could be measured before a forecasted heat wave, and then after it. Based on the QTL analysis reported here, the association between stay-green related traits and tolerance across 36 bread wheat genotypes that was previously reported in Chapter 3 seems likely to be due to control by common gene(s) and physiological mechanisms. While

the *Rht-B1* and *Rht-D1* dwarfing loci influenced yield spike^{-1} and grain number and size, they did not affect the stability of these traits under heat, at least in Drysdale × Waagan population and under these conditions.

Chapter 7: Development of automated plant imaging and SPAD measurements for heat tolerance screening at the vegetative stage of wheat development

7.1 Introduction

In most regions wheat is grown outside of its optimal temperature for various developmental stages, and the situation will become more acute with climate change (Asseng et al. 2011; Shpiler and Blum 1986; Wollenweber et al. 2003). High temperatures at flowering and grain-filling are major concerns in many wheat growing regions (Asseng et al. 2011), while heat stress at the vegetative stage also constrains wheat production in some semi-tropical and tropical regions (Fischer and Maurer 1976; Fischer 1985b). High temperatures can adversely affect both vegetative (Shpiler and Blum 1986) and reproductive growth (Stone and Nicolas 1994; Wardlaw et al. 1989b). Yield impacts of high temperatures at flowering and grain-filling have been well documented (Dawson and Wardlaw 1989; McDonald et al. 1983; Saini and Aspinall 1982b; Saini et al. 1983; Stone and Nicolas 1995b; Wardlaw et al. 1989a). It has been estimated that each 1 ºC increase in temperature in the range 14-27 ºC in the 30 days before anthesis decreases wheat yields by 4%, presumably due to accelerated development and reduced photo-assimilate accumulation (Acevedo 1991; Fischer 1985a; Fischer and Maurer 1976). Photo-assimilates present in the stems and sheaths of wheat before anthesis contributes 10-40% of the final grain weight (Yang & Zhang 2006 and references cited therein). Decreased total leaf area in response to pre-anthesis drought stress has been correlated with reduced grain number in wheat (Frederick and Camberato 1995). Therefore high temperatures at the vegetative stage can compromise wheat grain yield production.

Tolerance at the reproductive stage has been the focus of the majority of work on heat tolerance in wheat, due to its well established relevance to the industry. However, assessments at this stage tend to be time consuming and costly, due to the need to grow plants to maturity. Techniques which are rapid and easy are desirable for identifying superior heat tolerant genotypes in breeding programs and for accelerating genetic studies of tolerance. Therefore, some efforts have been made to explore heat tolerance assays at the vegetative stage. For instance, several researchers measured cell membrane thermostability (CMS) and/or cell viability using a triphenyltetrazolium chloride assay on leaf tissue (Balota et al. 1993; Fokar et al. 1998; Saadalla et al. 1990a; Saadalla et al. 1990b; Reynolds et al. 1994) and found a significant positive correlation in these traits (i.e., CMS and cell viability) between the seedling and flowering developmental stages. Significant positive associations have been found between CMS and yield (Fokar et al. 1998; Reynolds et al. 1994; Saadalla et al. 1990a). Chlorophyll fluorescence has also been used to detect genotypic differences for heat tolerance

at various developmental stages, including during early growth (Al-Khatib and Paulsen 1990; Moffatt et al. 1990; Ristic et al. 2007; Sharma et al. 2012). While these traits show promise for tolerance selection, they are likely to reflect the effects of heat on only a few cellular/physiological process. By comparison, vegetative growth may provide a more comprehensive measure of heat tolerance, since it is expected to integrate the effects of heat on many molecular and physiological processes (e.g. enzyme kinetics, protein denaturation, membrane integrity, oxidative damage, net photosynthetic rate, cell division and hormone and water relations, etc.).

Recently, methods for high-throughput phenotyping such as non-destructive imaging and spectroscopy have been developed (Berger et al. 2010; Berger et al. 2012; Furbank and Tester 2011; Golzarian et al. 2011). In Australia, the Australian Plant Phenomics Facility (www.plantphenomics.org.au) (Furbank and Tester 2011), including the High Resolution Plant Phenomics Centre in Canberra and The Plant Accelerator in Adelaide, are now available to scientists for phenotyping (www.plantphenomics.org.au). Plant imaging has been used to investigate changes in growth dynamics and to discriminate genotypes for response to various abiotic stresses (e.g. salinity, drought, boron toxicity, and low irradiance) in several plant species including wheat (Berger et al. 2010; Hayes et al. 2013; Honsdorf et al. 2014; Pengelly et al. 2010; Rajendran et al. 2009). However, use of these techniques to assess heat tolerance in wheat has not been reported. Accordingly in this study, the potential to use automated plant imaging in The Plant Accelerator in wheat heat tolerance studies was investigated. More specific aims were to see whether genetic variability in heat tolerance could be detected with this system and to investigate the level of correlation between such variation and responses to heat at grain-filling.

7.2 Materials and Methods

Seven experiments were conducted to investigate the effects of a brief heat stress on growth and chlorophyll traits of spring bread wheat genotypes at the vegetative stage (~ 4 week old plants). The first experiment was used to determine heat treatment conditions and time points suitable for discriminating tolerant and intolerant genotypes. Using conditions chosen from the Experiment 1, 77 genotypes were then screened for heat tolerance in Experiments 2 and 3. In Experiment 4 and 5, three genotypes were characterised in more detail for a number of additional traits including leaf relative water content, leaf water potential, stomatal conductance, and water use efficiency. In Experiments 6 and 7, a sub set of 15 genotypes which were largely common between experiments 2, 3, and 4 were re-assayed at different times of the year to explore effects of pre-/post-treatment growing conditions on heat responses. Genotypes in common with the study of Chapter 3, in which a brief heat stress was

applied at 10 days after anthesis, were used to look for correlations between heat responses measured at vegetative and grain-filling stages of development.

7.2.1 Plant material

Seventy-seven spring bread wheat genotypes (*Triticum aestivum* L.) were used. These included cultivated varieties, synthetic derived lines, breeding lines, experimental lines and landraces. The majority of these genotypes were selected because they were parents of available mapping populations (listed in Appendix 1). Lyallpur-73 was selected because it was reported to be heat susceptible (Mufti 2005; Wardlaw et al. 1989a; Wardlaw et al. 2002). Seeds were obtained from Michael Francki (DAFWA), Dion Bennett (AGT), Dan Mullan (Intergrain), Bertus Jacobs (Longreach), Hugh Wallwork (SARDI), the Australian Winter Cereals Collection and local collections at the ACPFG or NSW-DPI Wagga Wagga. A list of the genotypes, their origin and the year of release (where applicable) is presented in Table 7.1.

Table 7. 1 List of genotypes used.

Genotype (origin, year of release)			
AUS1408 (South Africa, 1987)	Cranbrook (Australia, 1984)	Lyallpur-73 (Pakistan, 1973)	Spica (Australia, 1952)
AUS1490 (unknown)	Crusader (Australia, 2008)	Maringa (*Rht1*) (Brazil)[#]	SUN325B[‡]
Avocet (Australia, 1979)	Currawong (Australia, 1994)	Maringa (*Rht2*) (Brazil)[#]	Sunco (Australia, 1986)
Avocet-S (Australia)[¥]	Diamondbird (Australia, 1997)	Matong (Australia, 1982)	Sunstar (Australia, 1983)
Babax (Mexico, 1992)	Drysdale (Australia, 2002)	Millewa (Australia, 1979)	Tammarin Rock (Australia, 2004)
Batavia (Australia, 1991)	EGA Blanco (Australia, 2004)	Molineaux (Australia, 1988)	Tammin (Australia, 1994)
Berkut (Mexico, 2002)	Egret (Australia, 1973)	Morocco 426 (unknown)	Tasman (Australia, 1993)
Cadoux (Australia, 1992)	Ernie (USA, 1994)	Opata 85 (Mexico, 1993)	Trident (Australia, 1993)
Calingiri (Australia, 1997)	Excalibur (Australia, 1990)	Peake (Australia, 2007)	Vigour18 (Australia)[*]
Cappelle Desprez (France, 1992)	Frame (Australia, 1994)	PI624933-1[†]	W7985 Synthetic[¥]
Cascades (Australia, 1994)	Gladius (Australia, 2007)	PI625123-3[†]	Waagan (Australia, 2009)
CD87 (Australia, 1989)	Halberd (Australia, 1969)	PI625983-1[†]	Westonia (Australia, 1997)
Chara (Australia, 1998)	Hartog (Australia, 1982)	PI626580-4[†]	Whistler (Australia, 1998)
CM18 (China 1965)	Hereward (England, 1989)	QT7475 (Australia, 2006)[‡]	WW1842 (Australia)[*]
Cook (Australia, 1977)	Indis (South Africa, 1990)	RAC875 (Australia)[*]	WW2449(Australia)[*]
Correll (Australia, 2006)	Iraq 43 (Iraq)	Reeves (Australia, 1989)	Wyalkatchem (Australia, 2001)
CPI133814[¥]	Janz (Australia, 1989)	Roblin (Canada, 1986)	Young (Australia, 2005)
CPI133872[¥]	Katepwa (Canada, 1981)	Rosella (Australia, 1985)	
CPI33842[¥]	Krichauff (Australia, 1997)	Seri M82 (Mexico, 1982)	
CPI33859[¥]	Kukri (Australia, 1999)	Sokoll (Mexico, 2002)	

[Ɣ]Near isogenic line (NIL) for rust resistance, in Avocet background
[¥]Synthetic hexaploid
[#]*Rht* NIL in Maringa background
[†]Landrace from Iran
[‡]AUS1408-derived breeding line
[*]Breeding line

7.2.2 Plant establishment

In Experiment 1, pots were 8 × 18 cm with drain holes and contained 1 kg of the soil mix described in Chapter 3. In the other experiments, pots were 15 × 19 cm with drain holes, and contained 2 kg soil. Three seeds were sown per pot and seven days after sowing plants were thinned to one healthy seedling per pot, except in Experiment 5 where two seedlings were retained per pot.

7.2.3 Subsequent plant growth

7.2.3.1 Experiment 1

Two spring bread wheat cultivars with contrasting heat tolerance, namely Excalibur (tolerant; Bukovnik et al. 2009) and Lyallpur-73 (susceptible; Mufti, 2005, Wardlaw et al. 1989), were used to identify a high temperature regime and time points that would reveal genotypic differences in heat responses with minimal tissue death. Plants were grown in a naturally lit greenhouse (South Australian Research and Development Institute, University of Adelaide, Waite Campus, Adelaide) during July-September 2011 at approximately 24/18ºC, 8/16 h day/night, and watered every 2 days. At 29 days after sowing, one half of the plants were transferred to a growth chamber, 2 hours before the start of the day cycle of the chamber, to apply heat treatments. BioChambers GC-20 Bigfoot and Conviron BDW120 chambers were used to apply different heat regimes in Experiment 1, while for all other experiments only the BDW120 was used. Plants assigned to heat treatment were exposed to day/night temperatures of either 40/30 or 44/30°C, either for 6 hours or 2 days, before returning the plants to the greenhouse. The maximum temperature of the growth chamber was held for 8 h each day, with 2 h transition periods used either side to linearly ramp the temperature up and down. Lighting was at a maximum of 350-420 µM m^{-2} s^{-1} at pot level for 8 h each day. Average day/night relative humidity in the chamber was measured at 70/80%. Pots were placed in trays of water to ~ 2 cm while in the chamber to minimize drought stress. Plants were imaged with a LemnaTec 3D Scanalyzer stand-alone unit (LemnaTec, Würselen, Germany; http://www.lemnatec.de), located at the same greenhouse, starting from 27 days after sowing (before the heat treatment) and continuing after heat treatment every 2 days for up to 51 days after sowing. Relative chlorophyll content also was monitored using a portable SPAD chlorophyll meter (SPAD-502; Minolta Camera Co. Ltd., Japan) on the same days that images were taken.

7.2.3.2 Experiments 2 and 3

Experiments 2 and 3 were conducted during February-March and August-September 2012, respectively. These experiments used 77 genotypes: 35 and 52 in Experiments 2 and 3, respectively (10 genotypes in common). Heat treatments were the same in the two experiments. Plants were grown in a naturally lit greenhouse (The Australian Plant Accelerator, University of Adelaide, Waite Campus, Adelaide) at approximately 24/18 ºC (Table 7.2 shows measured conditions), 14/10 h day/night, and watered every 2 days (Figure 7.1a). At 21 days after sowing (DAS), plants were loaded onto the conveyor system of the fully-automated ‘Smarthouse’ plant imaging facility in The Plant Accelerator, University of

Adelaide, and imaged daily with a LemnaTec 3D Scanalyzer for up to 25 DAS (Figure 7.1b). Pots in the Smarthouse were watered automatically (pots sat on trays to allow uptake of drained water) to a pre-determined weight (35% w/w) to maintain 100% field capacity. At 25 DAS and after initial imaging, half of the plants were transferred to the growth chamber (BDW120, Conviron) at 26 DAS, 2 h before commencement of the day cycle, where they received a 2 d heat treatment (Figure 7.1c). Heat treatment was at 40/30 °C for 2 days as described in Experiment 1, except that the maximum temperature of the growth chamber was held for 7 h each day, and the lighting (mixture of metal halide and tungsten incandescent) was at a maximum of 600 µM m^{-2} s^{-1} at plant height for 7 h each day, with a 2 h transition period used either side to step the intensity at 460 µM m^{-2} s^{-1}. After heat treatment, plants were returned to the Smarthouse where they were further imaged along with the control plants (Figure 7.1), every one to two days, for up to 39 DAS. SPAD measurements were taken in Experiment 2 only, on the third fully expanded leaf, at 25 and 28 DAA (either side of heat treatment) and at 39 DAS.

Table 7. 2 Measured temperatures (ºC) during the growing period in the greenhouse/Smarthouse in Experiments 2, 3, 4, 5, 6, and 7. For Experiment 1 the greenhouse set temperatures are presented. The set temperature in the greenhouse/Smarthouse in other experiments was also 24/18ºC day/night temperature.

Experiment	Location	Month	Average day temperature	Average night temperature	Average minimum temperature	Average maximum temperature	Minimum temperature	Maximum temperature	Days > 30°C
Experiment 1	**Greenhouse**	**July-September**	24.0	18.0	-	-	-	-	-
Experiment 2	**Greenhouse**	**February**	23.9	20.2	19.3	26.6	18.6	29.7	0
		March	22.7	19.1	18.1	24.9	17.5	26.0	0
	Smarthouse	**March**	25.0	19.9	18.6	30.5	18.0	33.3	12
Experiment 3	**Greenhouse**	**August**	23.0	19.0	17.6	25.6	17.3	26.3	0
	Smarthouse	**August**	22.5	19.2	17.6	25.2	17.4	26.4	0
		September	24.1	20.3	18.7	28.2	17.5	33.3	4
Experiment 4 and 5	**Greenhouse**	**August**	23.1	19.1	17.4	26.1	17.6	25.6	0
		September	23.3	19.1	17.6	26.0	17.3	26.9	0
	Smarthouse	**September**	26.7	20.7	19.1	32.4	17.3	36.3	6
Experiment 6	**Greenhouse**	**February**	23.0	17.9	17.0	27.7	16.0	31.4	1
		March	22.6	18.0	17.0	27.2	15.3	29.9	0
	Smarthouse	**March**	24.8	20.2	19.1	29.5	17.8	36.0	7
Experiment 7	**Greenhouse**	**September**	23.6	19.6	18.0	27.0	15.9	31.3	2
		October	21.5	17.0	15.9	24.9	13.7	29.3	0
	Smarthouse	**October**	25.2	18.6	17.4	29.3	13.7	31.9	10

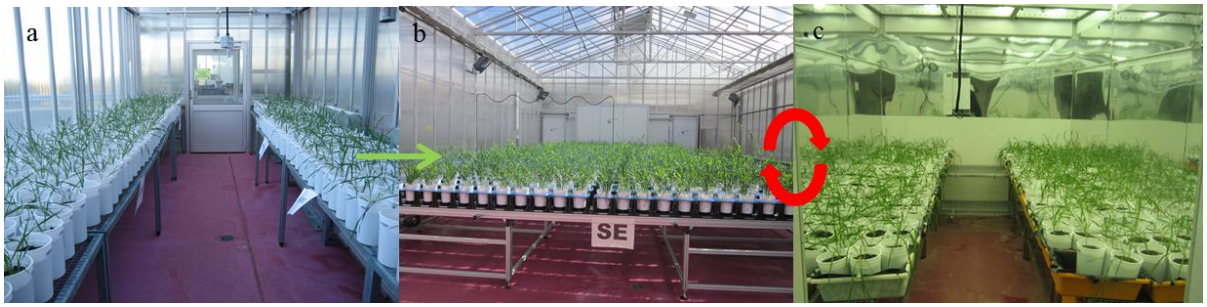


Figure 7. 1 Plants in the greenhouse (a), the Smarthouse (b) and a growth chamber under heat stress (c). For further explanation refer to Materials and Methods.

7.2.3.3 Experiments 4 and 5

Experiments 4 and 5 were conducted during August-September 2012 using the varieties Drysdale, Gladius and Waagan. The same procedures as in Experiments 2 and 3 were used, except plants were imaged once before treatment and for a shorter period after treatment (1 week) to reduce unnecessary cost. The method of watering also differed: Up to 25 DAS the pots were watered every 2 days, and from 25 DAS the pots were watered to weight to maintain field capacity (35% w/w), three times a day while being heat treated and once a day after the treatment. In Experiment 4, plants were imaged once before the treatment and 7 times after the treatment on a daily basis, and SPAD measurements were taken on the 3rd fully expanded leaf of each plant at 25, 28, and 34 DAS. Furthermore, in both heated and control plants, stomatal conductance was measured on the 3rd leaf. Plants in Experiment 5 were not imaged and were instead used to obtain destructive measurements of leaf relative water content and leaf water potential during the heat treatment. Pots were watered to field capacity as described above. On each of the two days of heat treatment, the 3rd fully expanded leaf from one of the two plants in each pot was taken to measure leaf relative water content. The 4th fully expanded leaf from the same plant was used to measure leaf water potential at the same time. Stomatal conductance, leaf relative water content, and leaf water potential measurements were performed during the middle of the first and second day of heat treatment.

7.2.3.4 Experiments 6 and 7

Experiments 6 and 7 were conducted during February-March and September-October 2013, respectively. The procedures were the same as in Experiments 2 and 3, except that SPAD measurements were not taken.

7.2.4 Measurements

7.2.4.1 Growth

The LemnaTec 3D Scanalyser used two cameras to obtain a top view image and two side views (90º rotated) per imaging session. Images were used to estimate plant size using an automated analysis algorithm ('grid') customized to the experiment, in the Scanalyser software. The algorithm was firstly used to analyse the complete set of images, to separate plant from non-plant pixels and to give a two-dimensional plant area for each image. At each time point, relative shoot area estimates were then derived by summing the pixels of the plant images taken for each plant from all three directions.

Growth data from Experiment 1 were compared to multiple mathematical models, but a single equation with a good fit to all the data could not be found, due to the different growth dynamics of control and heat-treated plants (data not shown). Therefore, the following equation was used to quantify instantaneous growth rate for specific time intervals:

Instantaneous growth rate = P2-P1/t2-t1

P1 and P2 is the projected shoot areas at time 1 and 2 expressed in pixels, and t1 and t2 are times 1 and 2 expressed in days, respectively.

In Experiments 2, 3, 6, and 7, relative growth rate (RGR) was calculated for each plant, for 21 or 22 to 25 DAS, 25-28 DAS and 28-39 DAS, corresponding to before, during and after heat treatment periods. In Experiment 4, RGR was only calculated for 25-28 DAS (during treatment) and for 28-34 DAS (after treatment). RGR was obtained using an exponential model fitted to plant shoot area data for the described time intervals:

$$y = ae^{bt}$$

Where y is the projected shoot area (pixels) of the plant at time t, a is the initial projected shoot area (the first measurement for each time interval described above), e is the Napier's number (mathematical constant with an approximate value of 2.718281828) and b is a rate constant (for each plant at each time interval) that determines the steepness of the curve (i.e. RGR).

7.2.4.2 Proportion of senescent area (PSA)

Pixels on the plant image were categorized as healthy (green) or senescent (yellow or brown) according to a subjective RGB range set for each of the two categories. PSA was defined as the ratio of senescent area to total plant area.

7.2.4.3 Relative chlorophyll content

Relative chlorophyll content of the 3rd fully expanded leaf was monitored using a portable SPAD chlorophyll meter (SPAD-502; Minolta Co. Ltd., Japan). Each value was the average of 10 measurements taken along the left hand side of the leaf between the mid-rib and leaf margin. SPAD values were normalized to the reading taken on the same plant at the first time point (measurement prior to heat treatment), to allow for variation in starting chlorophyll content between plants and genotypes.

AUSC (area under the normalized SPAD progress curve): This was calculated for each plant using the following equation, where X_i is the chlorophyll content (normalized SPAD units) on the i^{th} date, t_i determines date on which the chlorophyll content was measured, and n is the number of dates on which chlorophyll content was recorded.

$$AUSC = \sum_{i=1}^{n-1} [(\frac{X_i + X_{(i+1)}}{2}) \times (t_{(i+1)} - t_i)]$$

7.2.4.4 Stomatal conductance

Stomatal conductance was measured on the adaxial surface of the 3rd fully expanded leaf, half way between base and tip of the leaf, between the mid-rib and leaf margin, using a portable dynamic diffusion porometer (Delta-T AP4, Delta-T Devices Ltd, UK) at midday. The porometer was calibrated on each day of measurement as described in the porometer manual.

7.2.4.5 Water use efficiency (WUE)

WUE was defined as the ratio of projected shoot area produced over a particular time interval (in pixels) to total water transpired over that time (in mL) calculated using all of the pot weight measurements.

7.2.4.6 Leaf water potential (LWP)

LWP was measured using a Scholander pressure chamber (Model 3000, Soil moisture Equipment Corp, Santa Barbara, CA). For each measurement, the 4th fully expanded leaf was cut off with a razor blade at the base and immediately clamped in the pressure chamber with the cut surface protruding from the chamber. Leaf water potential was defined as being equal in magnitude, and negative, to the minimum air pressure in the chamber that was required to force sap out of the cut surface (Boyer 1967; Turner 1988).

7.2.4.7 Relative water content (RWC)

Leaf sections (250 mm^2) were collected from the middle of the 3rd fully expanded leaf at midday and the fresh mass (FM) immediately recorded. Samples were then immersed in 10 ml distilled water at 4 ºC for 24 h to completely hydrate, then weighed to determine the turgid mass (TM). Leaf samples were then oven-dried for at 65 ºC for 24 h and weighed to determine dry mass (DM). RWC was calculated using the equation:

$$RWC\ (\%) = 100 \times ((FM - DM)/(TM - DM))$$

7.2.5 Experimental design and data analysis

Each experiment used a completely randomised block split-plot design. There were 5 replications in Experiment 3, 12 in Experiment 4, and 6 in the others. Each replicate was split into 2 plots. Genotype was considered as the main-plot (one for each genotype) and treatment as the 2 sub-plots (control vs. heat). In the other words, control and heat-treated plants of each genotype were physically neighboured in each replicate in both the greenhouse and Smarthouse. Consequently, they were imaged at around the same time (within ~1 min). Each replicate was kept intact between the greenhouse and Smarthouse, although the positions of the replicates were re-randomised in the Smarthouse. Experiments 1, 4 and 5 were analysed by ANOVA in GenStat 16 (http://www.vsni.co.uk/genstat). Other experiments were analysed using a linear mixed model using ASReml-R software (Butler et al. 2009) available in the R statistical computing environment (R Development Core Team 2014). Statistical differences between means were tested using LSDs. Pairwise Pearson correlation tests was used to study relationships between traits measured under control or heat conditions, and between response ratios of various traits ($R_{H/C}$ = Mean trait $value_{Heat\ treatment}$ / Mean trait $value_{Control}$). Principal component analysis (PCA) was used to compare behaviours of plants across the four large experiments (Experiments 2, 3, 6, and 7). R language was used for correlation and PCA analyses, and for figure preparation (R Development Core Team 2014).

7.3 Results

7.3.1 Experiment 1

The 6 h heat treatment at 40 ºC did not significantly impact the measured traits in either genotype (Figures 7.2a and b, 3a and b, 4a and b, 5a and b), the only exception being that absolute growth rate in Lyallpur-73 was significantly larger at 49 DAS in heat-treated plants than in the unheated control plants (Figure 7.2b).

Two days at 40/30 ºC day/night had no significant impact on Excalibur for any of the measured traits, except instantaneous growth rate at 35 and 45 DAS and chlorophyll content

of the 3rd fully expanded leaf at 43 and 45 DAS which both showed significantly smaller values in heat-treated plants than control plants (Figures 7.2c, 7.3a, 7.4a, and 7.5a). By contrast, the same treatment significantly affected most of the traits at most of the time points in Lyallpur-73. In this genotype, it significantly reduced instantaneous growth rate at 33 to 47 DAS, projected shoot area at 37 to 49 DAS and the proportion of senescent area at 31 to 49 DAS (Figures 7.2d, 7.3b, and 7.4b). The chlorophyll content of the 3rd fully expanded leaf of Lyallpur-73 plants was significantly reduced by the heat treatment just at 33 DAS (Figure 7.5b).

Instantaneous growth rate, projected shoot area, and proportion of senescent area were significantly changed by treatments of either duration at 44/30 ºC day/night treatment in both genotypes and at most of the time points, with the longer treatment having a larger impact and Lyallpur-73 showing a greater response than Excalibur (Figures 7.2e-h, 7.3a and b and 7.4a and b). For chlorophyll content of the 3rd fully expanded leaf, Excalibur was not significantly affected by heat treatment at 44/30 ºC day/night for 6 h while Lyallpur-73 was only significantly affected by this treatment at 31 and 33 DAS (Figure 7.5a and b). The longer duration (2 days) at 44/30 ºC day/night significantly reduced the chlorophyll content of the 3rd fully expanded leaf of both genotypes by 31 and 33 DAS. Thereafter, Excalibur showed no effect of the heat treatment, while Lyallpur-73 showed significantly smaller chlorophyll content up to 39 DAS, relative to control (Figure 7.5a and b).

In general, the dynamics of the 44/30 ºC day/night treatment differed from that observed for the 40/30 ºC day/night treatment. At 44 ºC for either duration, there was a drastic immediate reduction in 'growth rate' in both genotypes, which was largely due to wilting and tissue death leading to a large reduction in plant size. This indicates that plants suffered from foliar dehydration despite good soil water availability and high humidity in the growth chamber during the heat treatment. After treatment, the growth rate recovered in both genotypes but remained generally smaller than in control plants (Figures 7.2e-h). The 44/30 ºC day/night treatments for both durations delayed the time from sowing to maximum growth rate ~ one week in both genotypes. The longer duration of 44/30 ºC day/night treatment drastically enhanced the responses, particularly in Lyallpur-73.

In summary, the two days heat treatment at 40/30 ºC day/night was the condition that best discriminated the tolerant (Excalibur) genotype from the intolerant (Lyallpur-73) one while avoiding drastic wilting and tissue death. Therefore, this condition (40/30 ºC day/night for 2 days) was used to apply heat stress in the subsequent experiments. Subsequently, stress was

also applied at 25 DAS instead of 29 DAS, so that growth rate measurements could be taken more in the log growth phase.

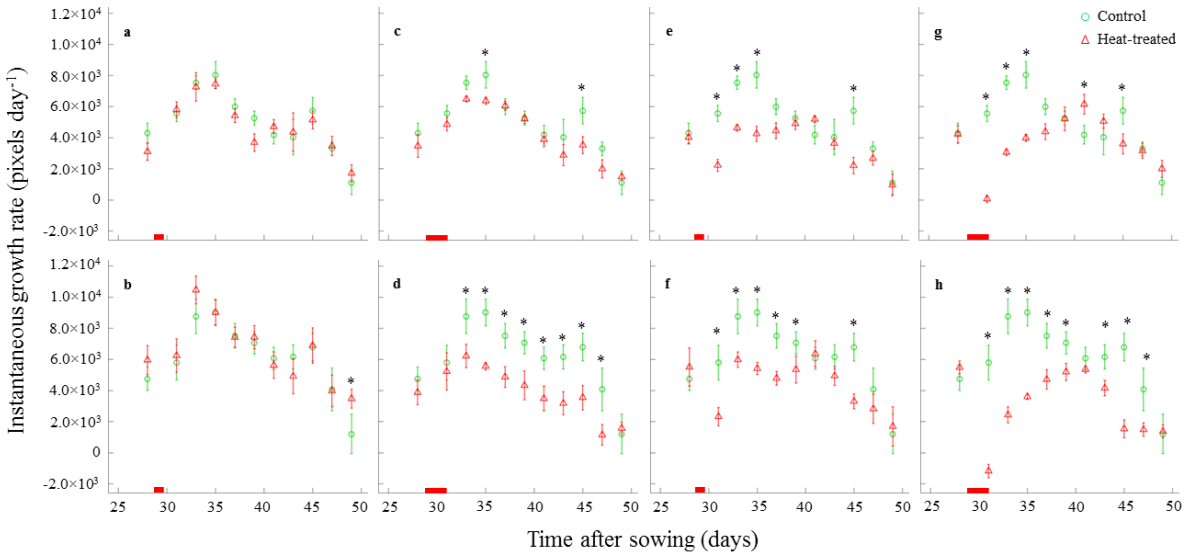


Figure 7. 2 Instantaneous growth rate of Excalibur (a, c, e and g) and Lyallpur-73 (b, d, f and h) plants grown under control conditions or with brief heat treatments: a, b) 40/30ºC day/night for 6 hours, c, d) 40/30 ºC day/night for 2 days, e, f) 44/30 ºC day/night for 6 hours, and g, h) 44/30 ºC day/night for 2 days. Horizontal red bars on x-axes represent the periods of high temperature treatment. Error bars represent S.E. (n=5 to 6). Asterisks indicate a significant difference between treatments at $p < 0.05$.

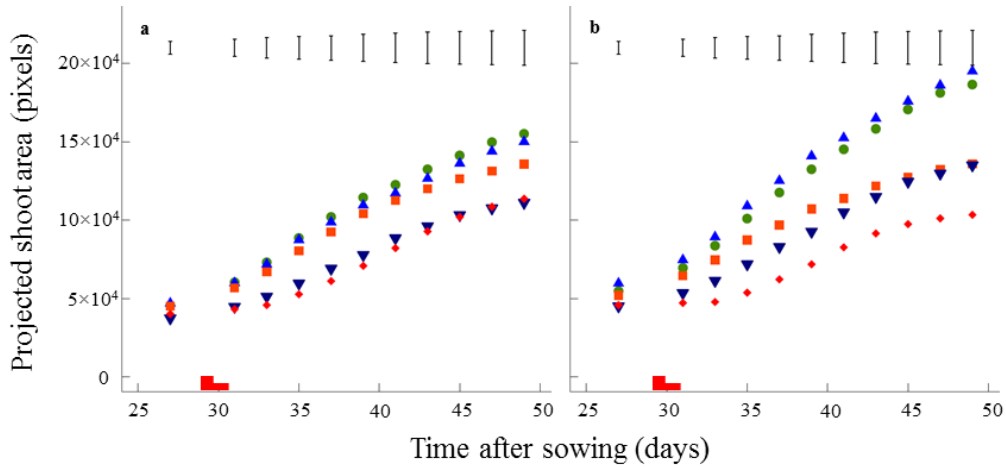


Figure 7. 3 Growth of Excalibur (a) and Lyallpur-73 (b) plants grown under control conditions (green circles), and exposed to heat treatments of 40/30ºC day/night for 6 hours (blue triangles), 40/30 ºC day/night for 2 days (orange squares), 44/30 ºC day/night for 6 hours (inverted navy-blue triangles) and 44/30 ºC day/night for 2 days (red diamonds). Bars represent LSD ($\alpha = 0.05$). n=5 to 6 plants per gentype/treatment. Horizontal bars on the x-axes represent the periods of high temperature treatment.

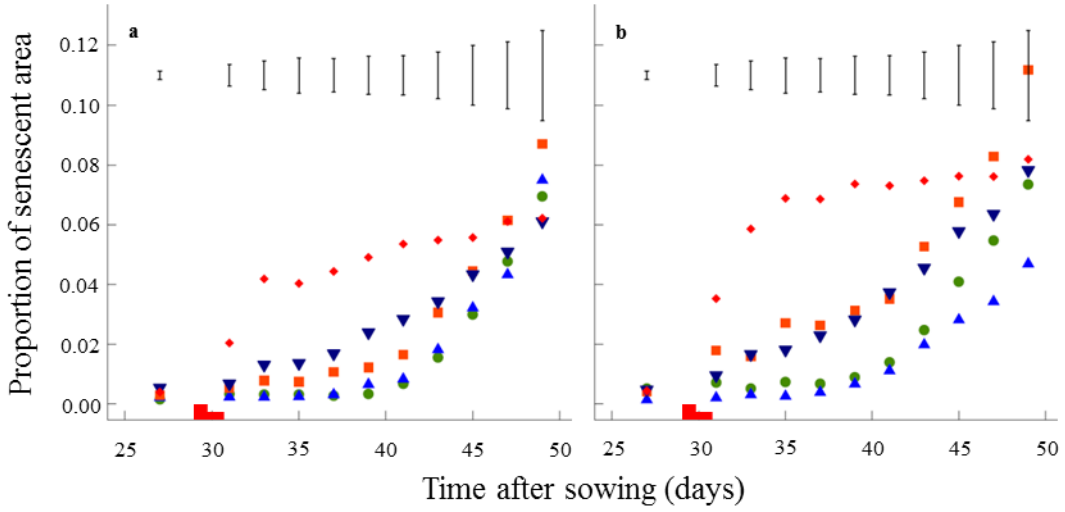


Figure 7. 4 Proportion of senescent area in Excalibur (a) and Lyallpur-73 (b) plants grown under control conditions (green circles) and exposed to heat treatments of 40/30ºC day/night for 6 hours (blue triangles), 40/30 ºC day/night for 2 days (orange squares), 44/30 ºC day/night for 6 hours (inverted navy-blue triangles) and 44/30 ºC day/night for 2 days (red diamonds). Bars represent LSD (α = 0.05). n=5 to 6 plants per gentype/treatment. Horizontal bars on the x-axes represent the periods of high temperature treatment.

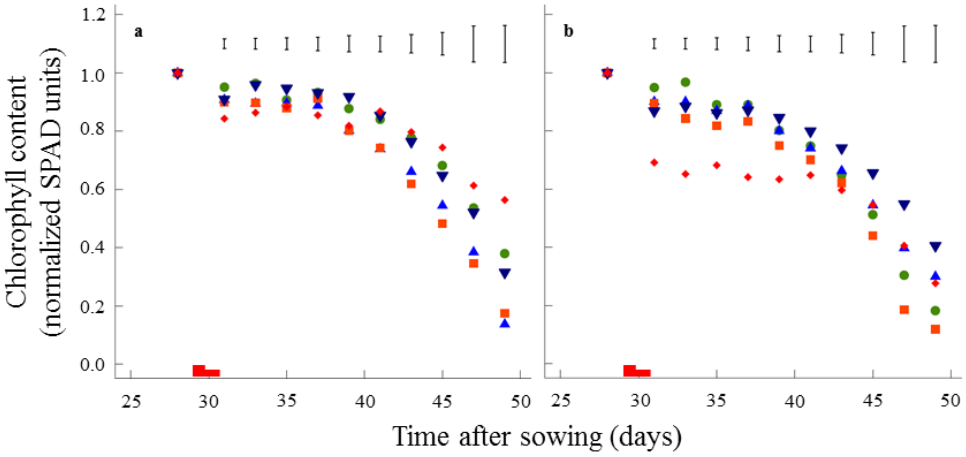


Figure 7. 5 Chlorophyll content of 3rd fully expanded leaf of Excalibur (a) and Lyallpur-73 (b) plants grown under control conditions (green circles) or exposed to heat treatments 40/30ºC day/night for 6 hours (blue triangles), 40/30 ºC day/night for 2 days (orange squares), 44/30 ºC day/night for 6 hours (inverted navy-blue triangles) and 44/30 ºC day/night for 2 days (red diamonds). Bars represent LSD (α = 0.05). n=5 to 6 plants per gentype/treatment. Horizontal bars on the x-axes represent the periods of high temperature treatment.

7.3.2 Experiments 2 and 3

In these two experiments, 77 spring bread wheat genotypes were evaluated for heat tolerance. The means for control and heat-treated plants of each genotype are presented in Tables 7.3, 7.4, and 7.5. A significant genotype effect was observed for all of the measured traits in both experiments (p=0.01 for ChC28DAS, p=0.02 for AUSC, and p<0.001 for other traits). Control plants and those assigned to the heat treatment showed no significant differences prior to heat exposure (Tables 7.3, 7.4, and 7.5) for any of the traits in either experiment.

The number of tillers on the plants at the time of heat treatment varied among the genotypes ($p<0.001$). In Experiment 2, genotypes had between 3 to 9 tillers, with the majority having 5 to 7 (Table 7.3). In Experiment 3, most of the genotypes had 2 tillers while a few had 1 or 3 (Table 7.4). Therefore at the time of heat treatment, plants were at a more advanced growth stage in Experiment 2 than in Experiment 3, due to seasonal effects.

Figure 7.6 illustrates an example of the change in plant size (projected shoot area) over time and fitted growth model before and after treatment in two contrasting varieties under control and heat conditions. During-treatment growth rates were calculated using the projected shoot areas measured directly before and after treatment (i.e. 25 and 28DAS). Genotypes varied for RGR before the treatment (at 22-25DAS; RGRBT) ($p<0.001$ in both experiments), showing values from 0.16 to 0.22 pixel pixel^{-1} day^{-1} in Experiment 2 and from 0.10 to 0.17 pixel pixel^{-1} day^{-1} in Experiment 3. Overall mean RGRBT in Experiment 2 was 26% higher than in Experiment 3 due to the more advanced developmental stage of the plants in the former experiment. Heat exposure reduced overall mean values of RGR during treatment (at 25-28 DAS; RGRDT) by ~20% and 2% relative to controls in Experiments 2 and 3, respectively. The heat effect was significant for RGRDT in Experiment 2 ($p<0.001$), but a lack of a significant genotype-by-treatment interaction in this experiment ($p=0.880$) indicated that the genotypes responded similarly. Nevertheless, the magnitude of differences (relative to control) ranged from ~-7 to -40%. The heat effect on RGRDT was insignificant ($p=0.099$) in Experiment 3 although there was a significant genotype-by-treatment interaction ($p=0.008$). Genotypes varied from showing 22% increase to 25% decrease in RGRDT, and 23 genotypes showed an increase while 30 genotypes showed a decrease, but the effect was significant in only four genotypes. Heat treatment significantly ($p<0.001$) reduced the overall mean of RGR at 28-39 DAS (RGRAT) by 4 and 3% in Experiments 1 and 2, respectively. The significant genotype-by-treatment interaction in both Experiments ($p=0.006$ and 0.042 in Experiments 2 and 3, respectively) for this trait may suggest a significant difference between genotypes for magnitude and direction of RGR response after heat treatment. Genotypes varied from showing 4 to -16% change in RGRAT in Experiments 2 and 7 to -12% in Experiment 3. The effect was significant in 7 and 10 genotypes in Experiments 2 and 3, respectively.

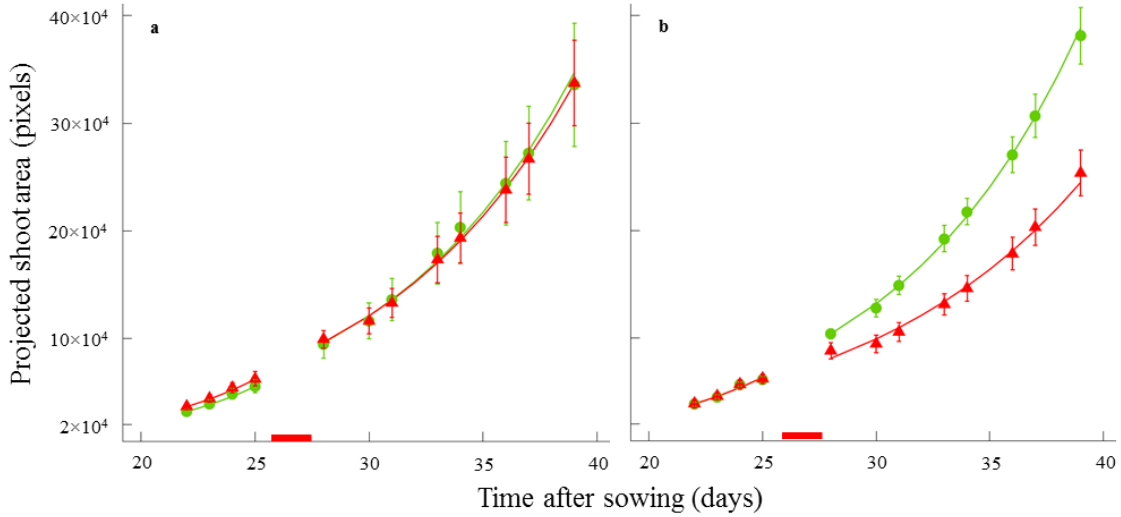


Figure 7. 6 Growth of a tolerant (Young; a) and an intolerant (Reeves; b) variety grown under control conditions (green circles), and with heat treatments of 40/30ºC day/night for 2 days (red triangles) in Experiment 2. Error bars represent S.E.. Lines represent fitted growth models on control (green) and heat-treated plants (red). Horizontal bars on the x-axes represent the periods of high temperature treatment.

Due to different patterns of change in proportion of senescent area (PSA) among genotypes, no attempt was made to model PSA. Instead, values at 25, 28 and 39 DAS (corresponding to directly before and after treatment and at the end of the Experiments) were used to investigate the heat effect on senescent area. In Experiment 2, heat stress significantly ($p<0.001$) increased the overall mean proportion of senescent area just after treatment (PSA28DAS), by 22%, and the genotype-by-treatment interaction was significant ($p = 0.003$). Genotypes increased in PSA28DAS (betweem 3 and 133%), with the exception of EGA-Blanco for which heated plants had 15% smaller PSA28DAS compared to controls. An insignificant ($p=0.411$) difference was observed between heated and unheated plants for the same trait in Experiment 3. Overall means of PSA39DAS were 1.4 and 2.3% larger in heated plants than in controls in Experiments 2 and 3, respectively. However in both experiments, the heat effect and genotype-by-treatment interaction were insignificant ($p=0.393$ and 0.121 in Experiment 2, and $p = 0.173$ and 0.962 in Experiment 3, for the heat effect and genotype-by-treatment effect, respectively). Compared to controls, PSA39DAS was larger in heat-treated plants in 16 and 30 genotypes, smaller in 15 and 14 genotypes and indistinguishable in 4 and 8 genotypes, in Experiments 2 and 3, respectively.

Table 7. 3 Means and LSDs for mean comparisons for relative growth rate before treatment (RGRBT), during treatment (RGRDT), and after treatment (RGRAT), proportion of senescent area before treatment (PSA25DAS), after treatment (PSA28DAS) and at the end of the experiments (PSA39DAS), tillers number (Tiller No) and Zadoks' growth stage (ZGS) estimated using tiller number at the time of heat treatment, in Experiment 2.

Genotype	RGRBT	RGRDT			RGRAT			PSA 25 DAS	PSA28DAS			PSA39DAS			Tiller No	ZGS
		C	H	Mean	C	H	Mean		C	H	Mean	C	H	Mean		
Berkut	0.173	0.194	0.147	0.171	0.119	0.117	0.118	0.031	0.027	0.034	0.031	0.032	0.03	0.031	7.309	Z27
Cadoux	0.161	0.156	0.113	0.135	0.131	0.118	0.125	0.034	0.027	0.063	0.045	0.027	0.041	0.034	3.801	Z24
CD87	0.17	0.151	0.132	0.142	0.105	0.1	0.103	0.028	0.035	0.04	0.038	0.03	0.026	0.028	6.513	Z26
Correll	0.178	0.201	0.17	0.186	0.128	0.123	0.126	0.025	0.022	0.027	0.025	0.027	0.024	0.026	5.842	Z26
Cranbrook	0.189	0.184	0.154	0.169	0.126	0.124	0.125	0.031	0.032	0.033	0.033	0.029	0.032	0.031	5.731	Z26
Crusader	0.168	0.175	0.138	0.157	0.126	0.121	0.124	0.028	0.025	0.028	0.027	0.027	0.03	0.029	4.854	Z25
Drysdale	0.183	0.188	0.148	0.168	0.123	0.116	0.12	0.028	0.028	0.035	0.032	0.028	0.028	0.028	5.898	Z26
EGA-Blanco	0.173	0.174	0.145	0.16	0.121	0.117	0.119	0.023	0.026	0.022	0.024	0.024	0.022	0.023	5.708	Z26
Egret	0.188	0.185	0.146	0.166	0.125	0.124	0.125	0.028	0.026	0.03	0.028	0.028	0.029	0.029	6.864	Z27
Excalibur	0.206	0.184	0.147	0.166	0.119	0.118	0.119	0.030	0.030	0.035	0.033	0.029	0.030	0.030	9.288	Z29
Frame	0.181	0.192	0.154	0.173	0.13	0.128	0.129	0.027	0.027	0.036	0.032	0.027	0.026	0.027	5.669	Z26
Gladius	0.171	0.176	0.164	0.17	0.114	0.103	0.109	0.02	0.021	0.023	0.022	0.02	0.02	0.02	4.943	Z25
Halberd	0.188	0.191	0.137	0.164	0.135	0.133	0.134	0.03	0.03	0.035	0.033	0.032	0.029	0.031	5.323	Z25
Hartog	0.188	0.179	0.14	0.16	0.121	0.113	0.117	0.035	0.031	0.039	0.035	0.036	0.033	0.035	6.559	Z27
Janz	0.188	0.175	0.151	0.163	0.124	0.118	0.121	0.03	0.029	0.032	0.031	0.028	0.03	0.029	6.085	Z26
Katepwa	0.202	0.182	0.144	0.163	0.136	0.134	0.135	0.039	0.037	0.042	0.04	0.042	0.036	0.039	7.057	Z27
Krichauff	0.188	0.18	0.154	0.167	0.112	0.105	0.109	0.027	0.024	0.028	0.026	0.027	0.026	0.027	7.235	Z27
Kukri	0.173	0.188	0.153	0.171	0.12	0.114	0.117	0.027	0.023	0.03	0.027	0.028	0.027	0.028	6.622	Z27
Lyallpur-73	0.199	0.184	0.151	0.168	0.121	0.12	0.121	0.028	0.027	0.029	0.028	0.029	0.027	0.028	10.809	Z29
Millewa	0.196	0.182	0.165	0.174	0.124	0.113	0.119	0.026	0.024	0.03	0.027	0.025	0.026	0.026	6.195	Z26
Molineaux	0.2	0.199	0.157	0.178	0.134	0.127	0.131	0.027	0.026	0.031	0.029	0.027	0.027	0.027	6.328	Z26
Opata 85	0.192	0.205	0.142	0.174	0.117	0.12	0.119	0.027	0.025	0.027	0.026	0.026	0.027	0.027	7.177	Z27
RAC875	0.176	0.163	0.121	0.142	0.112	0.11	0.111	0.023	0.02	0.025	0.023	0.022	0.024	0.023	5.592	Z26
Reeves	0.163	0.169	0.102	0.136	0.123	0.107	0.115	0.039	0.038	0.065	0.052	0.038	0.04	0.039	5.259	Z25
Sokoll	0.197	0.182	0.139	0.161	0.129	0.128	0.129	0.026	0.024	0.034	0.029	0.026	0.025	0.026	5.418	Z25
Sunco	0.219	0.176	0.131	0.154	0.13	0.124	0.127	0.039	0.035	0.04	0.038	0.038	0.041	0.04	7.746	Z28
Sunstar	0.167	0.178	0.142	0.16	0.113	0.115	0.114	0.025	0.029	0.03	0.03	0.026	0.023	0.025	8.228	Z28
Tamarin Rock	0.174	0.172	0.138	0.155	0.119	0.109	0.114	0.042	0.035	0.038	0.037	0.04	0.044	0.042	5.215	Z25
Tasman	0.178	0.18	0.157	0.169	0.115	0.113	0.114	0.025	0.028	0.033	0.031	0.025	0.026	0.026	7.349	Z27
Trident	0.173	0.165	0.145	0.155	0.125	0.119	0.122	0.025	0.023	0.025	0.024	0.026	0.025	0.026	5.108	Z25
W7985 Synthetic	0.171	0.152	0.111	0.132	0.13	0.127	0.129	0.03	0.023	0.028	0.026	0.027	0.033	0.03	3.462	Z23
Waagan	0.174	0.187	0.15	0.169	0.121	0.126	0.124	0.029	0.027	0.033	0.03	0.027	0.03	0.029	6.064	Z26
Westonia	0.183	0.184	0.151	0.168	0.113	0.105	0.109	0.025	0.024	0.028	0.026	0.025	0.025	0.025	6.144	Z26
Wyalkatchem	0.191	0.182	0.137	0.16	0.113	0.095	0.104	0.029	0.031	0.045	0.038	0.028	0.032	0.03	8.487	Z28
Young	0.178	0.168	0.154	0.161	0.115	0.117	0.116	0.03	0.028	0.030	0.029	0.033	0.029	0.031	5.285	Z25
Mean	0.183	0.179	0.143		0.121	0.117		0.029	0.028	0.033		0.029	0.029		6.319	
LSD (0.05)																
Genotype (G)	0.026		0.022			0.006		0.006		0.007			0.013		0.91	
Treatment (T)	n.s.		0.005			0.002		n.s.		0.002			n.s.		n.s.	
G × T	n.s.		n.s.			0.008		n.s.		0.011			n.s.		n.s.	

Table 7. 4 Means and LSDs for mean comparisons for relative growth rate before treatment (RGRBT), during treatment (RGRDT), and after treatment (RGRAT) and relative senescent area before (PSA25DAS), after (PSA28DAS) and end of the experiments (PSA39DAS), tillers number (Tiller No), and estimated Zadoks' growth stage (ZGS) estimated using tiller number at the time of heat treatment, in Experiment 3.

Genotype	RGRBT	RGRDT			RGRAT			PSA 25 DAS	PSA28DAS			PSA39DAS			Tiller No	ZGS
		C	H	Mean	C	H	Mean		C	H	Mean	C	H	Mean		
AUS1408	0.135	0.179	0.177	0.178	0.137	0.134	0.136	0.034	0.035	0.030	0.033	0.044	0.045	0.045	1.842	Z21
AUS1490	0.135	0.164	0.181	0.173	0.123	0.123	0.123	0.043	0.031	0.031	0.031	0.044	0.037	0.041	1.709	Z21
Avocet	0.132	0.158	0.161	0.160	0.134	0.125	0.130	0.031	0.025	0.020	0.023	0.034	0.027	0.031	1.593	Z21
Avocet S	0.143	0.160	0.164	0.162	0.122	0.120	0.121	0.029	0.024	0.021	0.023	0.033	0.035	0.034	2.109	Z21
Babax	0.123	0.176	0.150	0.163	0.125	0.120	0.123	0.030	0.030	0.028	0.029	0.040	0.038	0.039	1.667	Z21
Batavia	0.152	0.177	0.161	0.169	0.129	0.124	0.127	0.031	0.024	0.032	0.028	0.039	0.032	0.036	1.767	Z21
Calingiri	0.122	0.160	0.164	0.162	0.112	0.104	0.108	0.029	0.019	0.019	0.019	0.032	0.033	0.033	1.693	Z21
CappelleDesprez	0.133	0.174	0.163	0.169	0.121	0.128	0.125	0.043	0.048	0.020	0.034	0.065	0.062	0.064	2.009	Z21
Cascades	0.139	0.165	0.160	0.163	0.121	0.121	0.121	0.031	0.027	0.026	0.027	0.038	0.038	0.038	2.567	Z21
CD87	0.121	0.160	0.175	0.168	0.113	0.104	0.109	0.022	0.021	0.019	0.020	0.036	0.046	0.041	2.093	Z21
Chara	0.114	0.155	0.173	0.164	0.122	0.107	0.115	0.034	0.031	0.031	0.031	0.030	0.032	0.031	1.559	Z21
CM18	0.118	0.173	0.139	0.156	0.114	0.114	0.114	0.025	0.025	0.023	0.024	0.025	0.029	0.027	1.167	Z20
Cook	0.132	0.175	0.173	0.174	0.129	0.119	0.124	0.035	0.041	0.036	0.039	0.042	0.051	0.047	2.359	Z21
Correll	0.129	0.155	0.178	0.167	0.125	0.112	0.119	0.027	0.023	0.022	0.023	0.027	0.025	0.026	1.409	Z21
CPI133814	0.129	0.162	0.144	0.153	0.129	0.128	0.129	0.046	0.024	0.035	0.030	0.050	0.050	0.050	2.042	Z21
CPI133872	0.108	0.115	0.093	0.104	0.109	0.105	0.107	0.039	0.030	0.030	0.030	0.036	0.035	0.036	1.059	Z20
CPI33842	0.116	0.134	0.101	0.118	0.121	0.117	0.119	0.041	0.031	0.029	0.030	0.028	0.031	0.030	1.193	Z20
CPI33859	0.105	0.130	0.110	0.120	0.117	0.113	0.115	0.046	0.031	0.042	0.037	0.050	0.052	0.051	0.930	Z20
Currawong	0.137	0.163	0.172	0.168	0.127	0.129	0.128	0.027	0.021	0.022	0.022	0.034	0.036	0.035	1.609	Z21
Diamondbird	0.136	0.145	0.164	0.155	0.112	0.110	0.111	0.031	0.029	0.029	0.029	0.043	0.042	0.043	1.793	Z21
Ernie	0.142	0.178	0.155	0.167	0.120	0.122	0.121	0.052	0.040	0.041	0.041	0.054	0.054	0.054	2.559	Z23
Excalibur	0.148	0.174	0.155	0.165	0.132	0.128	0.130	0.035	0.040	0.024	0.032	0.035	0.035	0.035	2.230	Z22
Hartog	0.144	0.171	0.151	0.161	0.125	0.120	0.123	0.036	0.035	0.032	0.034	0.041	0.044	0.043	1.942	Z21
Hereward	0.141	0.154	0.138	0.146	0.119	0.115	0.117	0.042	0.042	0.030	0.036	0.050	0.050	0.050	2.367	Z21
Indis	0.147	0.171	0.150	0.161	0.121	0.117	0.119	0.028	0.026	0.026	0.026	0.030	0.031	0.031	1.942	Z21
Iraq 43	0.118	0.180	0.155	0.168	0.134	0.144	0.139	0.042	0.043	0.029	0.036	0.039	0.031	0.035	1.867	Z21
Janz	0.132	0.135	0.165	0.150	0.119	0.115	0.117	0.030	0.029	0.030	0.030	0.032	0.033	0.033	1.609	Z21
Krichauff	0.128	0.153	0.185	0.169	0.116	0.109	0.113	0.032	0.026	0.027	0.027	0.033	0.034	0.034	1.742	Z21
Lyallpur-73	0.135	0.166	0.153	0.160	0.122	0.114	0.118	0.035	0.034	0.032	0.033	0.036	0.041	0.039	2.230	Z21
Maringa (Rht1)	0.131	0.157	0.167	0.162	0.122	0.117	0.120	0.030	0.028	0.029	0.029	0.032	0.032	0.032	1.630	Z21
Maringa (Rht2)	0.155	0.164	0.180	0.172	0.122	0.116	0.119	0.019	0.024	0.029	0.027	0.032	0.032	0.032	1.959	Z21
Matong	0.142	0.183	0.162	0.173	0.128	0.126	0.127	0.032	0.029	0.029	0.029	0.034	0.039	0.037	1.830	Z21
Morocco 426	0.175	0.209	0.187	0.198	0.140	0.137	0.139	0.028	0.028	0.025	0.027	0.046	0.047	0.047	1.393	Z21
Peake	0.124	0.153	0.171	0.162	0.122	0.117	0.120	0.027	0.030	0.035	0.033	0.031	0.031	0.031	1.559	Z21
PI624933-1	0.139	0.162	0.157	0.160	0.141	0.138	0.140	0.033	0.041	0.030	0.036	0.050	0.051	0.051	2.230	Z21
PI625123-3	0.149	0.181	0.165	0.173	0.133	0.137	0.135	0.032	0.026	0.026	0.026	0.040	0.038	0.039	2.109	Z21
PI625983-1	0.139	0.187	0.166	0.177	0.134	0.133	0.134	0.031	0.036	0.032	0.034	0.048	0.057	0.053	2.559	Z22
PI626580-4	0.141	0.166	0.175	0.171	0.118	0.119	0.119	0.024	0.020	0.021	0.021	0.027	0.025	0.026	1.409	Z21
QT7475	0.136	0.142	0.166	0.154	0.123	0.113	0.118	0.029	0.024	0.030	0.027	0.034	0.040	0.037	1.730	Z21
Roblin	0.133	0.158	0.159	0.159	0.126	0.113	0.120	0.035	0.034	0.029	0.032	0.038	0.040	0.039	1.742	Z21
Rosella	0.135	0.161	0.160	0.161	0.123	0.124	0.124	0.024	0.029	0.020	0.025	0.035	0.033	0.034	1.742	Z21
Seri M82	0.130	0.168	0.178	0.173	0.118	0.109	0.114	0.025	0.028	0.029	0.029	0.031	0.036	0.034	1.730	Z21
Spica	0.128	0.166	0.152	0.159	0.129	0.130	0.130	0.034	0.034	0.033	0.034	0.039	0.042	0.041	1.630	Z21
SUN325B	0.136	0.166	0.162	0.164	0.122	0.113	0.118	0.028	0.023	0.028	0.026	0.032	0.027	0.030	1.659	Z21
Sunco	0.150	0.164	0.168	0.166	0.128	0.113	0.121	0.033	0.028	0.025	0.027	0.035	0.042	0.039	2.530	Z21
Tammin	0.132	0.159	0.164	0.162	0.116	0.116	0.116	0.023	0.026	0.034	0.030	0.031	0.032	0.032	1.867	Z21
Vigour18	0.127	0.156	0.148	0.152	0.111	0.108	0.110	0.038	0.023	0.029	0.026	0.041	0.042	0.042	1.493	Z21
Whistler	0.146	0.153	0.128	0.141	0.111	0.115	0.113	0.023	0.020	0.026	0.023	0.032	0.027	0.030	1.942	Z21
WW1842	0.132	0.163	0.156	0.160	0.111	0.109	0.110	0.032	0.034	0.032	0.033	0.037	0.039	0.038	1.842	Z21
WW2449	0.145	0.155	0.159	0.157	0.132	0.133	0.133	0.026	0.018	0.028	0.023	0.033	0.035	0.034	1.667	Z21
Wyalkatchem	0.139	0.144	0.163	0.154	0.114	0.102	0.108	0.024	0.020	0.028	0.024	0.029	0.031	0.030	1.993	Z21
Young	0.145	0.163	0.168	0.166	0.128	0.121	0.125	0.032	0.024	0.027	0.026	0.037	0.042	0.040	1.442	Z21
Mean	0.135	0.162	0.159		0.123	0.119		0.032	0.029	0.028		0.037	0.038		1.814	
LSD (0.05)																
Genotype (G)	0.017		0.019			0.006		0.010		0.009			0.006		0.963	
Treatment (T)	n.s.		n.s.			0.001		n.s.		n.s.			n.s.		n.s.	
G × T	n.s.		0.027			0.009		n.s.		n.s.			n.s.		n.s.	

Chlorophyll content of the 3rd fully expanded leaf was measured only in Experiment 2 (Table 7.5). Prior to heat treatment, control plants showed no significant differences in this trait relative to the plants destined for heat treatment (ChC25DAS; $p = 0.682$). A significant genotype and heat treatment effect ($p<0.001$ in both cases) was observed for both ChC28DAS and AUSC, while the genotype-by-treatment effect was insignificant in both cases ($p=0.133$ and 0.260 for ChC28DAS and AUSC, respectively). ChC28DAS was reduced by an average of 3.1% in the heat-treated plants relative to controls. ChC28DAS was smaller in heat-treated plants than controls for the majority of genotypes (32 genotypes; varied from -0.4 to -8.3%), but in 3 genotypes the value in heat-treated plants was larger (by 0.6 to 2.5%). The AUSC (an

indication of chlorophyll content from 25 to 39 DAS in this experiment) for heat-treated plants was 2.9% smaller than for controls. Genotypes had between 0.7 and 8.9% smaller AUSC in heat-treated plants compared with controls, with the exception of 3 genotypes in which heat-treated plants had a larger AUSC than controls (by 0.3 to 3.4%). Overall this tends to indicate a significant heat-induced chlorophyll loss in heat-treated plants in the majority of genotypes.

Table 7. 5 Mean values and LSDs for mean comparisons for chlorophyll content of 3rd fully expanded leaf at 25 (ChC 25 DAS) and 28 (ChC 28 DAS) days after sowing, and area under SPAD curve (AUSC), in Experiment 2.

Genotype	ChC25DAS	ChC28DAS			AUSC		
		C	H	Mean	C	H	Mean
Berkut	49.3	1.034	1.006	1.02	14.285	13.979	14.132
Cadoux	53.7	1.019	0.934	0.977	14.176	12.917	13.547
CD87	50.0	1.023	0.989	1.006	14.318	13.644	13.981
Correll	54.0	1.007	0.964	0.986	13.987	13.463	13.725
Cranbrook	47.7	1.048	1.006	1.027	14.346	13.86	14.103
Crusader	50.0	1.03	0.981	1.006	14.537	13.533	14.035
Drysdale	49.4	1.037	1.015	1.026	14.444	14.138	14.291
EGA-Blanco	49.9	1.016	1.012	1.014	14.044	13.782	13.913
Egret	48.6	1.056	0.999	1.028	14.392	13.803	14.098
Excalibur	49.4	1.009	1.002	1.006	13.945	13.794	13.87
Frame	54.1	0.969	0.983	0.976	13.48	13.941	13.711
Gladius	53.1	1.016	0.989	1.003	14.058	13.769	13.914
Halberd	46.9	1.011	0.969	0.99	13.951	13.499	13.725
Hartog	48.6	1.035	1.003	1.019	13.904	13.55	13.727
Janz	47.2	1.034	0.98	1.007	14.454	13.775	14.115
Katepwa	47.4	1.055	1.003	1.029	14.627	14.131	14.379
Krichauff	52.7	1.004	0.988	0.996	14.008	13.761	13.885
Kukri	50.1	1.001	0.982	0.992	13.669	13.552	13.611
Lyallpur-73	50.0	1.022	0.984	1.003	14.102	13.67	13.886
Millewa	48.1	1.012	0.955	0.984	13.918	13.27	13.594
Molineaux	50.3	1.031	0.948	0.99	14.203	13.269	13.736
Opata 85	51.1	1.044	1.018	1.031	14.256	14.161	14.209
RAC875	53.9	1.019	0.964	0.992	14.25	13.551	13.901
Reeves	47.2	1.038	0.969	1.004	14.329	13.176	13.753
Sokoll	52.4	1.029	1.012	1.021	14.224	14.102	14.163
Sunco	47.5	1.028	1.004	1.016	14.311	13.884	14.098
Sunstar	49.1	1.011	0.977	0.994	14.013	13.791	13.902
Tamarin Rock	46.7	1.005	0.983	0.994	13.913	13.461	13.687
Tasman	48.4	1.007	1.013	1.01	13.994	14.026	14.01
Trident	54.2	1.028	1.016	1.022	14.191	14.056	14.124
W7985 Synthetic	59.4	1.031	1.012	1.022	14.397	14.107	14.252
Waagan	48.0	1.012	1.037	1.025	14.308	14.516	14.412
Westonia	50.8	1.011	0.986	0.999	14.038	13.672	13.855
Wyalkatchem	48.0	1.03	0.978	1.004	14.121	13.666	13.894
Young	49.8	1.008	0.993	1.001	14.159	13.898	14.029
Mean	50.2	1.022	0.990		14.153	13.747	
LSD (0.05)							
Genotype (G)	1.9		0.033			0.459	
Treatment (T)	n.s.		0.008			0.110	
G × T	n.s.		n.s.			n.s.	

7.3.3 Experiments 4 and 5

In these experiments the varieties Gladius, Drysdale and Waagan were used. These varieties were chosen for further analysis because they were parents of available mapping

populations (Gladius/Drysdale and Drysdale/Waagan) and because they contrasted for RGR responses either during or after treatment in Experiment 2.

In Experiment 4, heat treatment significantly reduced RGR during and after treatment in all three genotypes, but unfortunately there was no significant difference between Gladius and Drysdale, or between Drysdale and Waagan for absolute RGR values (Figure 7.7A and B). In general, the levels of contrast between genotypes for the aforementioned traits were considerably different from those seen in Experiment 2, possibly due to the different sowing time and developmental stage at the time of treatment relative to Experiment 2 (it being earlier for Experiment 4). Drysdale vs. Waagan differed by 3.57 and 0.77%, and Gladius vs. Drysdale by 2.2 and 0.66%, for RGRDT and RGRAT responses, respectively (while in Experiment 2 Drysdale vs. Waagan showed 1.3 and 10% contrast, and Gladius vs. Drysdale showed 14.4 and 3.3% differences for RGRDT and RGRAT responses, respectively).

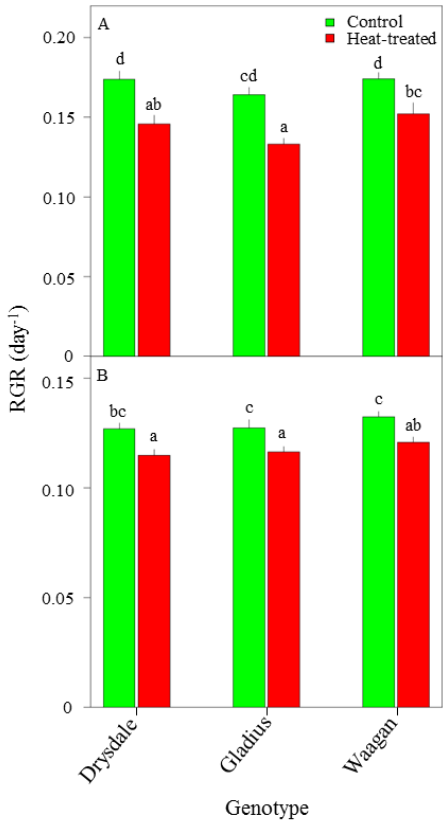


Figure 7. 7 Relative growth rate (RGR) of control and heat-treated plants of three wheat genotypes during treatment (RGRDT; A) and after treatment (RGRAT; B) in Experiment 4. Error bars show S.E. (n =11 to12). Means with the same letter were not significantly different ($p > 0.05$) in LSD tests.

Under control conditions, Gladius and Waagan showed significantly smaller ChC28DAS and AUSC than Drysdale (Figure 7.8A and B). However, under heat conditions, there was no significant difference between genotypes for ChC28DAS, but the heat response was larger in Drysdale compared with the others. Under heat, AUSC in Drysdale was significantly larger than in Gladius while there was no significant difference between Drysdale and Waagan.

Nevertheless, the mean heat response of Drysdale was larger than that of the two other genotypes. Heat stress produced an insignificant effect on proportion of senescent area (PSA) measured at 28 and 34 DAS (Figure 7.8C and D). Drysdale showed a larger value for both PSA28DAS and PSA34DAS compared with two other genotypes, under either control or heat conditions. None of the differences between Drysdale and Waagan were significant while Gladius and Drysdale differed significantly for RSA28DAS under control conditions and for PSA34DAS under both conditions.

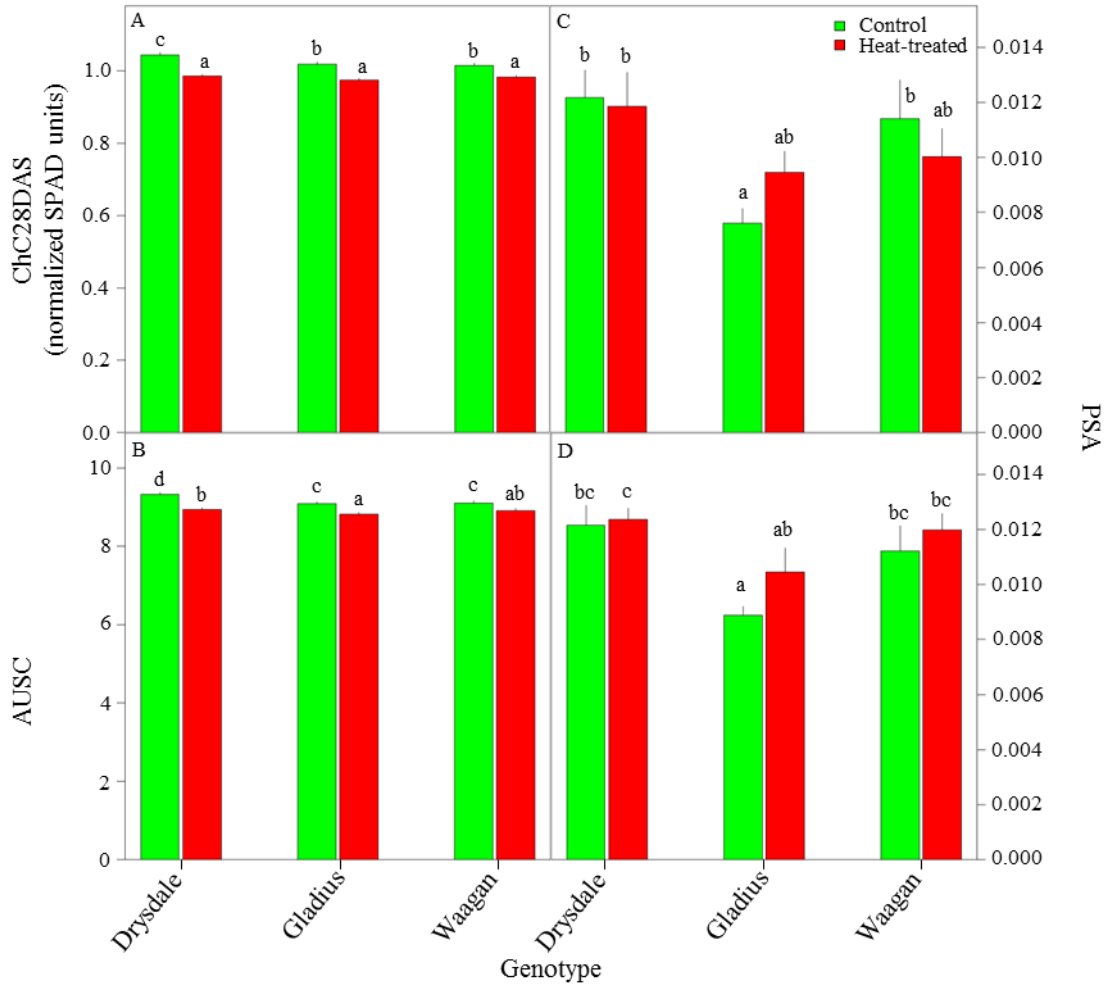


Figure 7. 8 Chlorophyll content of the 3rd fully expanded leaf at 28 days after sowing (ChC28DAS; A), area under SPAD curve (AUSC, B), proportion of senescent area (PSA) at 28 DAS (PSA28DAS; C) and 34 DAS (PSA34DAS; D) in heat-treated and control plants of three wheat genotypes. Error bars show S.E. (n =11 to 12 plants). Means with the same letter were not significantly different ($p > 0.05$) in LSD tests.

On the first day of heat exposure, heat treatment had decreased relative water content (RWC) and leaf water potential (LWP) and increased stomatal conductance (g_s) in all three genotypes (Figure 7.9A, C and E). The effect was significant for LWP and g_s in all genotypes. Compared with the other varieties, Drysdale showed the biggest reduction in RWC and LWP, and smallest increase in g_s. Measurements taken on the second day of treatment (Figure 7.9B, D and F) showed the same features, except there was also a significant effect of heat on RWC.

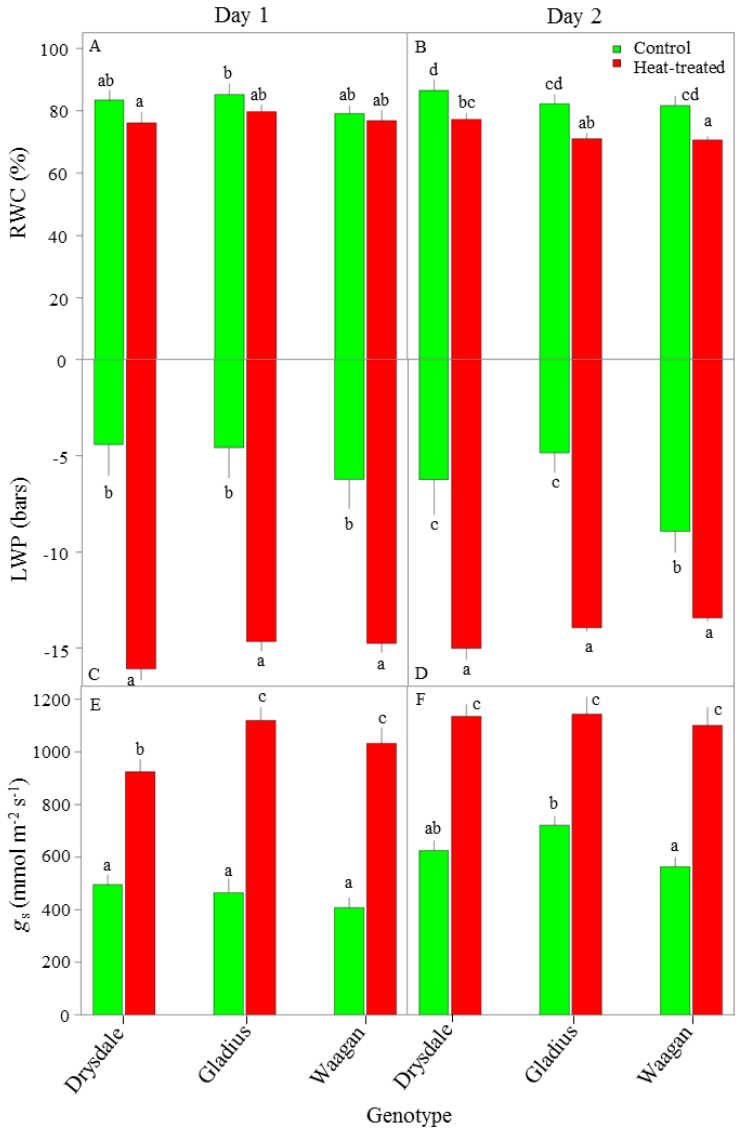


Figure 7. 9 Leaf relative water content (RWC; A and B), leaf water potential (LWP; C and D) and stomatal conductance (g_s; E and F) in Drysdale, Waagan, and Gladius wheat varieties at first (A, C, E) and second day (B, D, F) of the heat treatment. Error bars show S.E. (n = 6 to 12). Means with the same letter were not significantly different ($p > 0.05$) in LSD tests.

In all genotypes, the treatment decreased water use efficiency as measured during the treatment (WUE) (Figure 7.10A). Heat treated plants also showed smaller WUE than controls after the treatment; however, the differences were not significant (Figure 7.10B). In both cases, responses of WUE to the heat treatment were the smallest in Waagan and greatest in Gladius.

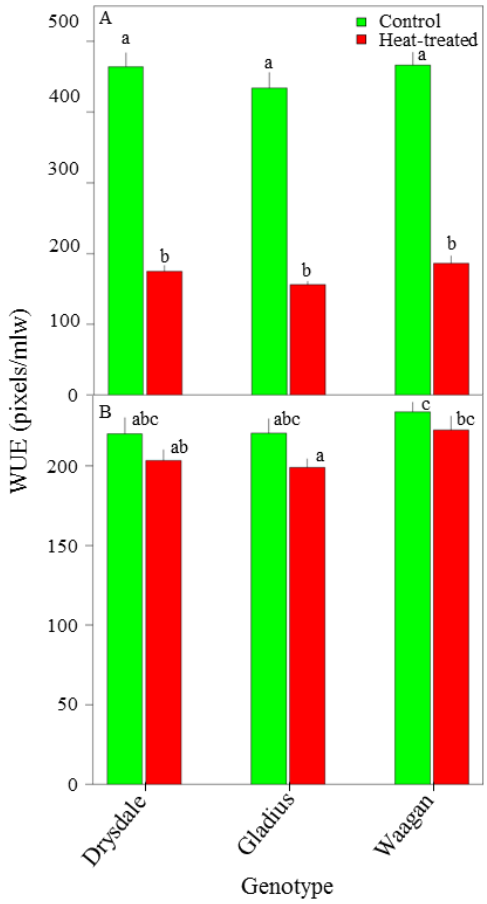


Figure 7. 10 Water use efficiency (WUE, pixels/mlw, ml of water) during treatment (A) and after treatment (B) in Drysdale, Waagan, and Gladius wheat varieties. Error bars show S.E. (n =11 to 12). Means with the same letter were not significantly different ($p > 0.05$) in LSD tests.

7.3.4 Experiments 6 and 7

Due to the inconsistency of heat responses of the 10 genotypes common to Experiments 2 and 3, and of Drysdale, Gladius and Waagan in Experiments 2 and 4, a subset of 15 genotypes were re-tested in Experiments 6 and 7 to investigate the influence of pre-/post- treatment conditions on the heat responses. All of the 15 genotypes were present in Experiment 2 and 10 of them were in Experiment 3. These experiments were performed (in different years) on dates coinciding as close as possible to those used for Experiments 2 and 3. Experiment 6 was done on exactly the same dates as Experiment 2 while Experiment 7 was ~ 40 days later than Experiment 3 due to limitations of Smarthouse availability. A significant genotypic effect was observed for all of the traits measured in both experiments ($p<0.001$). There were also significant heat effects on RGRDT, RGRAT and PSA39DAS in both experiments, and a significant genotype-by-treatment interaction was observed for RGRDT and RSA39DAS in Experiment 6 and for all of the traits measured after heat application in Experiment 7. Heat treatment had a similar effect on the overall means of RGRDT and RGRAT. It significantly reduced overall means of RGRDT by ~14% and of RGRAT by 3%, in both experiments (Table 7.6). Response of RGRDT to heat in genotypes ranged from -5 to -40%, and 9 to -

30%, in Experiments 6 and 7, respectively, and the effect of the heat treatment was significant in 10 genotypes in each experiment (Table 7.6). However, the RGRDT responses were not consistent between the experiments, since some genotypes appeared to show a significant response to the heat treatment in one experiment and an insignificant response in the other experiment (Table 7.6). RGRAT responses to heat in the genotypes varied from 11 to -11%, and from 19 to -14%, in Experiments 6 and 7, respectively. The effect was significant in 6 genotypes in Experiment 7 (Table 7.6). The overall heat effect was not significant for PSA28DAS in either experiment, however, genotypes varied from showing 33 to -23% and 49 to -37% change in PSA28DAS in Experiments 6 and 7, respectively. The effect was significant just in 2 genotypes in Experiment 7 (Table 7.6). Genotypes varied from showing 59 to -6% and 110 to -22% changes in PSA39DAS in response to the heat treatment with the majority of the genotypes showing an increase in the trait values in both experiments. The effect was significant in 3 and 2 genotypes in Experiments 6 and 7, respectively (Table 7.6).

Table 7. 6 Means and LSDs for mean comparisons for relative growth rate before treatment (RGRBT), during treatment (RGRDT), and after treatment (RGRAT) and for relative senescent area before (RSA28DAS), after (RSA28DAS) treatment, and at the end of the experiments (RSA39DAS), tillers number (Tiller No), and estimated Zadoks' growth stage (ZGS) estimated using tiller number at the time of heat treatment.

Genotype	RGRBT	RGRDT			RGRAT			PSA 25 DAS	PSA28DAS			PSA39DAS			Tiller No	ZGS
Experiment 6		Control	Heat	Mean	Control	Heat	Mean		Control	Heat	Mean	Control	Heat	Mean		
Cadoux	0.169	0.183	0.109	0.146	0.146	0.139	0.143	0.02	0.017	0.019	0.018	0.022	0.035	0.029	-	-
CD87	0.176	0.148	0.141	0.145	0.113	0.106	0.110	0.021	0.024	0.025	0.025	0.032	0.040	0.036	-	-
Correll	0.186	0.186	0.162	0.174	0.130	0.128	0.129	0.015	0.017	0.013	0.015	0.017	0.019	0.018	-	-
Drysdale	0.191	0.171	0.141	0.156	0.123	0.117	0.120	0.018	0.019	0.018	0.019	0.021	0.024	0.023	-	-
Excalibur	0.200	0.178	0.155	0.167	0.125	0.125	0.125	0.021	0.021	0.021	0.021	0.022	0.026	0.024	-	-
Gladius	0.182	0.165	0.155	0.160	0.123	0.116	0.120	0.017	0.018	0.015	0.017	0.018	0.020	0.019	-	-
Hartog	0.183	0.178	0.150	0.164	0.124	0.120	0.122	0.019	0.019	0.017	0.018	0.024	0.029	0.027	-	-
Janz	0.179	0.172	0.161	0.167	0.123	0.121	0.122	0.021	0.018	0.020	0.019	0.022	0.023	0.023	-	-
Krichauff	0.174	0.183	0.162	0.173	0.124	0.118	0.121	0.017	0.019	0.016	0.018	0.024	0.024	0.024	-	-
Lyallpur-73	0.196	0.169	0.160	0.165	0.099	0.110	0.105	0.019	0.021	0.017	0.019	0.023	0.022	0.023	-	-
Reeves	0.161	0.161	0.128	0.145	0.128	0.125	0.127	0.028	0.021	0.028	0.025	0.022	0.027	0.025	-	-
Sunco	0.184	0.183	0.167	0.175	0.136	0.126	0.131	0.029	0.026	0.029	0.028	0.029	0.027	0.028	-	-
Waagan	0.194	0.188	0.151	0.170	0.121	0.121	0.121	0.022	0.022	0.018	0.020	0.020	0.026	0.023	-	-
Wyalkatchem	0.169	0.172	0.151	0.162	0.110	0.108	0.109	0.016	0.025	0.021	0.023	0.024	0.024	0.024	-	-
Young	0.193	0.181	0.154	0.168	0.142	0.127	0.135	0.021	0.02	0.017	0.019	0.021	0.025	0.023	-	-
Mean	0.182	0.175	0.150		0.124	0.120			0.02	0.020		0.023	0.026		-	-
LSD (0.05)																
Genotype (G)	0.011		0.013			0.007		0.005		0.004			0.004			
Treatment (T)	n.s.		0.005			0.003		n.s.		n.s.			0.001			
G × T	n.s.		0.018			n.s.		n.s.		n.s.			0.005			
Experiment 7																
Cadoux	0.203	0.176	0.124	0.150	0.129	0.123	0.126	0.010	0.016	0.016	0.016	0.012	0.013	0.012	2.083	Z21
CD87	0.175	0.177	0.163	0.170	0.120	0.108	0.114	0.006	0.012	0.008	0.010	0.011	0.022	0.017	3.879	Z23
Correll	0.194	0.172	0.148	0.160	0.111	0.122	0.116	0.005	0.011	0.008	0.009	0.011	0.008	0.010	3.179	Z22
Drysdale	0.184	0.166	0.138	0.152	0.118	0.108	0.113	0.008	0.012	0.011	0.012	0.012	0.010	0.011	3.022	Z22
Excalibur	0.199	0.167	0.133	0.150	0.089	0.106	0.098	0.012	0.015	0.014	0.014	0.014	0.015	0.015	4.579	Z24
Gladius	0.205	0.167	0.151	0.159	0.120	0.109	0.115	0.005	0.009	0.010	0.010	0.007	0.009	0.008	2.826	Z22
Hartog	0.196	0.175	0.151	0.163	0.118	0.114	0.116	0.011	0.015	0.015	0.015	0.011	0.018	0.015	3.267	Z22
Janz	0.182	0.164	0.138	0.151	0.112	0.109	0.110	0.011	0.017	0.015	0.016	0.011	0.011	0.011	3.256	Z22
Krichauff	0.187	0.171	0.153	0.162	0.119	0.116	0.118	0.006	0.009	0.008	0.008	0.008	0.009	0.008	3.921	Z23
Lyallpur-73	0.184	0.189	0.150	0.170	0.103	0.107	0.105	0.009	0.016	0.014	0.015	0.014	0.013	0.014	4.758	Z24
Reeves	0.204	0.170	0.134	0.152	0.116	0.111	0.113	0.012	0.017	0.026	0.021	0.014	0.017	0.016	2.987	Z22
Sunco	0.186	0.182	0.139	0.160	0.130	0.129	0.129	0.012	0.020	0.013	0.016	0.015	0.014	0.014	4.260	Z23
Waagan	0.180	0.170	0.155	0.163	0.112	0.110	0.111	0.010	0.014	0.014	0.014	0.010	0.012	0.011	2.953	Z22
Wyalkatchem	0.179	0.176	0.138	0.157	0.102	0.098	0.100	0.008	0.013	0.013	0.013	0.011	0.011	0.011	4.554	Z24
Young	0.191	0.181	0.198	0.190	0.136	0.117	0.126	0.007	0.010	0.011	0.011	0.011	0.012	0.012	2.925	Z22
Mean	0.190	0.174	0.148		0.116	0.112			0.014	0.013		0.012	0.013			
LSD (0.05)																
Genotype (G)	0.013		0.017			0.006		0.002		0.003			0.002		0.355	
Treatment (T)	n.s.		0.006			0.002		n.s.		n.s.			0.001		n.s.	
G × T	n.s.		0.024			0.008		n.s.		0.004			0.003		n.s.	

7.3.5 Associations between heat responses of traits within each experiment

To explore the relationships between heat responses of the traits in Experiments 2, 3, 6, and 7, pairwise correlation tests were performed (Table 7.7). Generally, RGR response was negatively associated with the proportion of senescent area, indicating that genotypes that suffered a greater reduction in RGR also tended lose more green area in response to the heat treatment. Similarly, chlorophyll loss response measured using a SPAD chlorophyll meter in Experiment 2 was positively correlated with relative growth rate response. Accordingly, chlorophyll loss measured using a SPAD chlorophyll meter was also negatively associated with the proportion of senescent area, suggesting that heat-induced chlorophyll loss and tissue death may be controlled by common processes/genes. This relationship also demonstrates a correlation between the different methods used to measure senescence (SPAD chlorophyll meter vs. imaging).

Pairs of experiments performed at the same/similar time of the year (Experiments 2 & 6 and Experiments 3 & 7; Table 7.7) showed some similar patterns of correlation between heat responses (within each experiment). For instance, in Experiments 2 and 6 which were both conducted in late summer/early autumn, RGRDT was not correlated with RGRAT and tended to be negatively correlated with PSA28DAS and PSA39DAS. By contrast, in Experiments 3 and 7 which were performed in late winter/early spring, RGRDT tended to be negatively correlated with RGRAT and showed weak positive correlations to PSA28DAS and PSA39DAS.

Table 7. 7 Correlations between heat responses among studied traits in Experiments 2, 3, 6, and 7. RGRDT, relative growth rate during treatment; RGRAT, relative growth rate after treatment; RSA28DAS, relative senescent area after treatment; RSA39DAS, relative senescent area at the end of the experiments; ChC 28 DAS, chlorophyll content of 3rd fully expanded leaf at 28 days after sowing; AUSC, area under SPAD curve.

Experiment/Trait	RGRDT	RGRAT	PSA28DAS	PSA39DAS	ChC28DAS	AUSC
Experiment 2						
RGRDT	-					
RGRAT	0.09	-				
RSA28DAS	-0.47***	-0.46**	-			
RSA39DAS	-0.34*	-0.33	0.65	-		
ChC28DAS	0.30	0.46**	-0.42**	-0.29	-	
AUSC	0.28	0.50***	-0.43**	-0.38*	0.91***	-
Experiment 3						
RGRDT	-					
RGRAT	-0.43***	-				
RSA28DAS	0.18	-0.14	-			
RSA39DAS	0.08	-0.38**	0.01	-		
Experiment 6						
RGRDT	-					
RGRAT	0.17	-				
RSA28DAS	-0.29	-0.20	-			
RSA39DAS	-0.81***	-0.23	0.28	-		
Experiment 7						
RGRDT	-					
RGRAT	-0.45	-				
RSA28DAS	0.08	-0.21	-			
RSA39DAS	0.29	-0.34	-0.04	-		

Values are Pearson correlation coefficients, with asterisks showing significance levels: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

7.3.6 Associations between Experiments 2, 3, 6, and 7

To compare the results of the 4 large experiments (2, 3, 6, and 7), traits common between the experiments, and their heat responses, were subjected to principal component analysis (PCA). The percentage variance explained by the first 3 principal components (PCs) and the variables' loadings are shown in Table 7.8. The first and second principal components together explained 51.49% of the total variance. The bi-plot of individuals using the first 2 PCs showed a separation of the 4 experiments which suggests a large impact of the pre-/post-stress conditions on the trait values and responses of genotypes (Figure 7.11). Experiments 2 and 6, which were conducted in similar environments (same season, but different years), were grouped relatively close to one another. PC1 was mainly explained by senescence related traits, while PC2 was mainly explained by RGR from 25 to 28 DAS in heat-treated plants (RGRDT.H), RGR during treatment response (RGRDT.R), PSA28DAS under heat conditions (PSA28DAS.H) and tiller number at the time of treatment (TN; Figure 7.12). Thus, PC2 was mainly explained by the effect of heat during the treatment (direct effects) and TN, indicating that plants of a more advanced growth stage tended to suffer greater direct effects of heat on RGR (RGRDT.H and RGRDT.R).

Table 7. 8 Variables' loadings for the first three principal components (PCs) of the principal component analysis (PCA) performed on data for common traits and responses, from Experiments 2, 3, 6, and 7. Percentages of variation explained by the corresponding principal components are shown in parentheses. RGRBT, relative growth rate before treatment; RGRDT, relative growth rate during treatment; RGRAT, relative growth rate after treatment; RSA25DAS, relative senescent area 25 days after sowing (before treatment); RSA28DAS, relative senescent area after treatment; RSA39DAS, relative senescent area at the end of the experiments and TN, tillers number. C, H or R following trait names indicate traits measured in control or heated plants, or trait responses, respectively.

Variable	PC1 (32.09%)	PC2 (19.40%)	PC3 (14.75%)
RGRBT	-0.33	0.31	0.11
RGRDT.C	-0.14	0.31	0.40
RGRAT.C	0.15	0.15	0.41
RSA25DAS	0.42	0.15	-0.06
RSA28DAS.C	0.38	0.17	0.03
RSA39DAS.C	0.43	0.04	0.05
TN	-0.17	0.43	-0.04
RGRDT.H	0.05	-0.32	0.32
RGRAT.H	0.14	0.16	0.54
RSA28DAS.H	0.29	0.33	-0.26
RSA39DAS.H	0.42	0.03	0.01
RGRDT.R	0.14	-0.48	0.02
RGRAT.R	-0.02	0.03	0.24
RSA28DAS.R	0.02	0.27	-0.37
RSA39DAS.R	-0.09	-0.03	-0.08

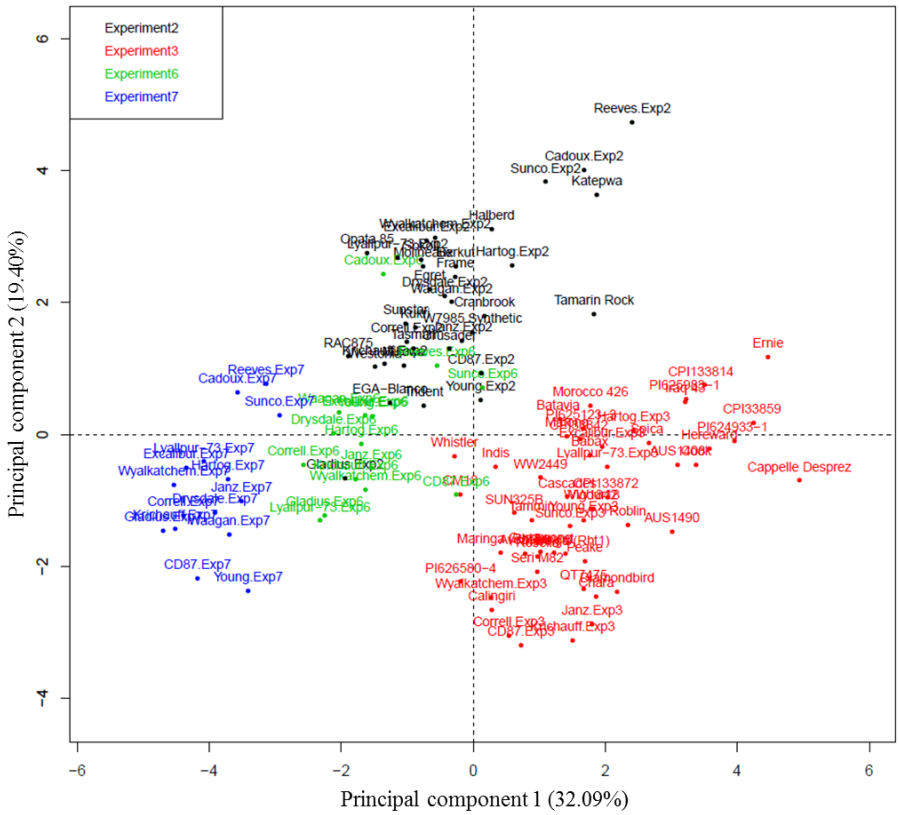


Figure 7. 11 Principal component analysis plot of genotypes in Experiments 2, 3, 6 and 7 based on the traits common between experiments. The first 2 principal components, which accounted for the highest proportion of variation (51.49%), are presented.

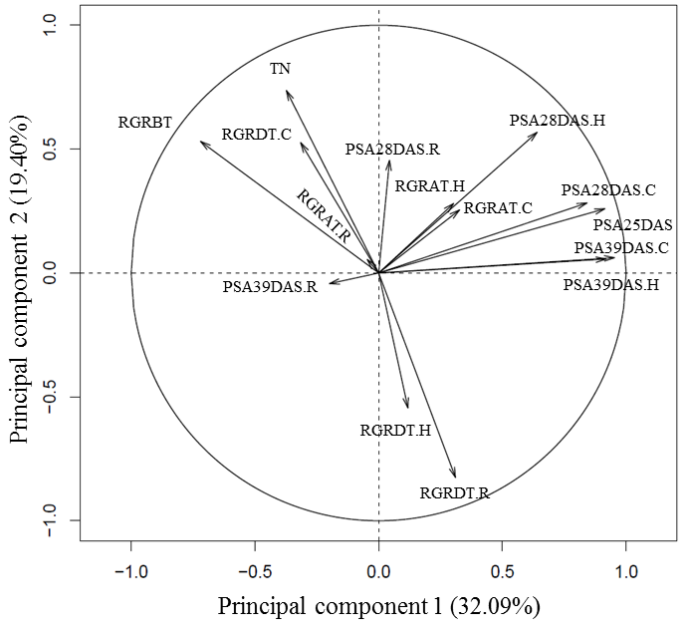


Figure 7. 12 Projection of trait variables from principal component analysis (PCA), using traits in control plants (C suffix), traits in heat-treated plants (H suffix), and heat/control ratios of traits (R suffix). RGRBT, relative growth rate before treatment; RGRDT, relative growth rate during treatment; RGRAT, relative growth rate after treatment; RSA25DAS, relative senescent area 25 days after sowing (before treatment); RSA28DAS, relative senescent area after treatment; RSA39DAS, relative senescent area at the end of the experiments and TN, tillers number.

7.3.7 Association of traits and trait responses in genotypes common between Experiments 2, 3, 6 and 7

Correlations between traits in control and heat-treated plants as well as heat responses, for genotypes common between Experiments 2, 3, 6, and 7, are presented in Table 7.9 (response ratios in the experiments are presented in Appendix 7.1). Tiller number was positively and significantly associated between experiments, reflecting genetic control of tillering. Significant correlations were observed between Experiments 2 and 6 for direct (RGRDT and PSA28DAS) and after (RGRAT) effects of heat treatment which suggests some level of reproducibility at least in experiments performed at the same time of year. Of all responses, that of RGRDT showed the highest reproducibility, being significantly correlated between Experiments 2 and 6 and between Experiments 2 and 7, i.e. the tolerance rankings of the genotypes showed some consistency for this trait (Appendix 7.2).

Table 7. 9 Correlations between studied traits in control and heat-treated plants and heat responses among genotypes common between Experiments (Exp.) 2, 3, 6, and 7. RGRBT, relative growth rate before treatment; RGRDT, relative growth rate during treatment; RGRAT, relative growth rate after treatment; RSA25DAS, relative senescent area 25 days after sowing (before treatment); RSA28DAS, relative senescent area after treatment; RSA39DAS, relative senescent area at the end of the experiments and TN, tillers number.

Condition/Response		Control				Heat				Response			
Trait	Experiment	Exp. 2	Exp. 3	Exp. 6	Exp. 7	Exp. 2	Exp. 3	Exp. 6	Exp. 7	Exp. 2	Exp. 3	Exp. 6	Exp. 7
RGRBT	Experiment 2	-											
	Experiment 3	0.69*	-										
	Experiment 6	0.46	0.48	-									
	Experiment 7	-0.28	0.57	-0.17	-								
RGRDT	Experiment 2	-				-				-			
	Experiment 3	-0.02	-			0.28	-			0.34	-		
	Experiment 6	0.50	0.08	-		0.73**	0.17	-		0.53*	0.24	-	
	Experiment 7	-0.20	0.43	-0.01	-	0.42	0.24	0.33	-	0.70**	0.15	0.33	-
RGRAT	Experiment 2	-				-				-			
	Experiment 3	0.62	-			0.65*	-			0.41	-		
	Experiment 6	0.48	0.58	-		0.58*	0.67*	-		0.15	0.14	-	
	Experiment 7	0.11	0.00	0.64**	-	0.54*	0.19	0.71**	-	0.22	0.13	0.57*	-
PSA25DAS	Experiment 2	-											
	Experiment 3	0.44	-										
	Experiment 6	0.79***	0.29	-									
	Experiment 7	0.82***	0.60	0.70**	-								
PSA28DAS	Experiment 2	-				-				-			
	Experiment 3	0.02	-			-0.09	-			0.50	-		
	Experiment 6	0.66**	-0.23	-		0.61*	-0.32	-		0.48	-0.36	-	
	Experiment 7	0.65**	0.50	0.36	-	0.72**	0.73*	0.48	-	0.39	0.40	0.26	-
PSA39DAS	Experiment 2	-				-				-			
	Experiment 3	0.67*	-			0.33	-			-0.16	-		
	Experiment 6	0.47	0.40	-		0.42	0.72*	-		0.55*	0.05	-	
	Experiment 7	0.67**	0.23	0.27	-	0.32	0.79**	0.78***	-	-0.24	0.58	0.32	-
TN	Experiment 2	-											
	Experiment 3	0.70*	-										
	Experiment 6	NA[Y]	NA	-									
	Experiment 7	0.95***	0.79**	NA	-								

Values are Pearson correlation coefficients, with asterisks showing significance levels: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.
[Y]Not available

7.3.8 Associations of heat responses between vegetative and grain-filling stages of development

Correlations between heat responses at grain-filling and vegetative stages of development were investigated to see if there was evidence for common genetic/physiological control of tolerance at the different growth stages (Table 7.10). Grain weight spike^{-1} (GWS) and single grain weight (SGW) each showed a highly significant positive association with response of RGRDT and a significant negative association with response of PSA28DAS (Table 7.10). In other words genotypes that showed higher tolerance (smaller GWS and SGW reduction) in

response to brief heat treatment at grain-filling also tended to show smaller growth rate reduction and produce less senescent area under heat treatment at the vegetative stage. A significant positive association was also observed between responses of RGRDT and harvest index (HI; Table 7.10). Chlorophyll content response of the flag leaf measured shortly after treatment (ChlC13-16DAA) at the grain-filling stage showed a significant positive association with the responses of chlorophyll content measured on the 3rd fully expanded leaf (ChC28DAS and AUSC) at the vegetative stage (Table 7.10), which indicates a possible link between chlorophyll responses to heat stress across these developmental stages. Responses of ChlC13-16DAA and AUSC measured at the grain-filling stage were both negatively associated with response of PSA28DAS (Table 7.10). In other words, genotypes that showed larger heat-induced chlorophyll loss at the grain-filling stage also tended to develop a larger proportion of senescent area in response to heat at the vegetative stage.

Table 7. 10 Genotypic correlations between response ratios of traits ($R_{H/C}$ = Mean trait value$_{Heat\ treatment}$ / Mean trait value$_{Control}$) measured at grain-filling (listed on x axis) and at the vegetative stage of development (listed on y axis). Trait responses of 32 genotypes that were common to the grain-filling experiment (Chapter 3) and vegetative stage analyses (current Chapter) were used to perform the correlation test. For genotypes that were evaluated at the vegetative stage across several experiments, the analysis was based on average values over the experiments. GWS, grain weight spike^{-1}; SGW, single grain weight; GFD, grain-filling duration; ChC13-16DAA, chlorophyll content before treatment at grain-filling stage; AUSC, area under SPAD curve; FlSe, days from anthesis to 95% flag leaf senescence; ShW, shoot dry weight of plants heated at grain-filling stage; HI, harvest index; RGRBT, relative growth rate before treatment; RGRDT, relative growth rate during treatment; RGRAT, relative growth rate after treatment; RSA25DAS, relative senescent area 25 days after sowing (before treatment); RSA28DAS, relative senescent area after treatment; RSA39DAS, relative senescent area at the end of the experiments; ChC28DAS, chlorophyll content of 3rd fully expanded leaf at 28 days after sowing.

Trait	GWS	SGW	GFD	ChlC13-16DAA	AUSC	FlSe	ShW	HI
RGRDT	0.50**	0.54**	0.19	0.32	0.31	0.19	0.28	0.36*
RGRAT	-0.30	-0.24	0.06	0.02	-0.08	-0.06	-0.07	-0.33
PSA28DAS	-0.41*	-0.50**	-0.13	-0.47**	-0.40*	-0.15	-0.29	-0.24
PSA39DAS	0.03	-0.18	-0.09	-0.14	-0.14	0.08	-0.03	0.08
ChlC28DAS	0.19	0.33	0.20	0.42*	0.29	0.05	0.22	-0.01
AUSC	0.10	0.28	0.25	0.38*	0.28	0.05	0.19	-0.10

Values are Pearson correlation coefficients, with asterisks showing significance levels: * $p < 0.05$ and ** $p < 0.01$.

7.4 Discussion

High temperatures adversely affect wheat growth, development, and ultimately yield. So far, a lack of rapid, easy and high-throughput screening methods has limited the potential to identify heat tolerant genotypes and desirable recombinants in segregating populations that can be used for genetic improvement. Heat tolerance is usually measured at maturity by evaluating yield and its components (Bennett et al. 2012; Mason et al. 2013; Pinto et al. 2010; Reynolds et al. 1994; Stone and Nicolas 1995b; Wardlaw et al. 1989b). This approach is time consuming, laborious and costly. Therefore, alternative approaches for heat tolerance evaluation were explored by screening 77 bread wheat genotypes were screened for growth

rate and senescence responses at the vegetative stage (~4 week old plants) using The Plant Accelerator automated plant imaging platform. The brief episode of very high temperature had large impacts on growth and senescence. There was also considerable variation for these heat responses among wheat genotypes (Appendix 7.3). Furthermore, the observed variation for growth and senescence responses measured directly after treatment significantly correlated with the variation observed for grain weight and chlorophyll content heat responses at the reproductive stage (Chapter 3). Overall, the findings demonstrated this straightforward automated plant imaging assay for young wheat plants could be used for obtaining an early indication of tolerance that would be expressed at a later (reproductive) stage. This could be useful in heat tolerance research or in reducing numbers of lines in an early-generation of a heat tolerance breeding program.

Although some genotypes responded similarly in different experiments (e.g., Cadoux and Reeves performed poorly for all of the traits under heat stress in all experiments; Appendices 5 and 6), the overall correlation of heat responses of genotypes common between experiments (Table 7.9; Appendix 7.1) and ranking of genotypes in different experiments (Appendix 7.2) indicated discrepancies for heat responses from experiment to experiment. Therefore the results of this study should be used with caution. Discrepancies between heat responses in different experiments could stem from various factors such as differences in growth stage and physiological status of the plants at the time of treatment and variable pre-/post-treatment growing conditions (i.e. seasonal and across-year light and temperature differences in the greenhouse and Smarthouse) which could affect plant growth and development and consequently the heat responses. Consistent with this idea, Experiments 2 and 6, which were conducted in similar environments (same time of year in different years), were grouped relatively close to one another in a PCA analysis considering all of the studied traits (Figure 7.11). Overall, these results indicate that precise control over growing conditions before and after heat treatment is critical for consistent performance of this assay. Currently, the temperature in the Smarthouse is somewhat uneven and uncontrolled - the evaporative cooling system can control temperature only to some extent, and the room is large (230 square meters and 4.5 meters height), providing opportunity for spatial variation in temperature. Often the choice where to put an experiment on the conveyor system is limited, due to the existence of other experiments in the Smarthouse. Improvements in Smarthouse and greenhouse temperature control, by introducing refrigerative cooling and better air circulation, and/or use of growth chambers for pre-/post- heat-treatment growth of plants, could potentially improve the performance of the assay.

Of all heat responses, that of RGRDT showed highest reproducibility (Table 7.9; Appendices 7.4 and 7.5) and also significantly correlated with grain weight heat responses at reproductive stage (Table 7.10). Measuring this trait is relatively easy, as it relies on only two measurements i.e., directly before and after treatment. Relationships among the studied traits revealed by the PCA analysis suggested that variability in RGRDT response between the experiments might be due to differences in RGR before the treatment (RGRBT) and developmental stage of the plants at the time of heat treatment (Figure 7.12). The strong negative association of TN and RGRBT with absolute value and response of RGR during treatment (RGRDT.H and RGRDT.R; Figure 7.12, note the opposite direction of TN and RGRBT relative to RGRDT.H and RGRDT.R) indicates that plants with a more advanced growth stage at the time of heat treatment and having larger RGRBT (i.e. being in a more active stage of growth) showed greater reductions in RGRDT than those that were in an earlier developmental stage. Before treatment, plants in Experiments 2, 6 and 7 experienced longer photoperiod and higher temperatures than those in Experiment 3. This resulted in a more advanced growth stage by the time of heat treatment in Experiments 2, 6 and 7, producing more comparable RGRDT heat responses between these experiments (at least in the case of Experiment 2 vs. Experiment 6 and Experiment 2 vs. Experiment 7 the correlation was significant). Overall, these results indicate that to increase data reproducibility and genotypic discrimination for growth rate heat response during treatment, timing of the heat stress should not be based on Julian days (time after sowing) but rather on a developmental stage, namely late tillering (e.g. Zadoks stage 25 to 27) (Zadoks et al. 1974). Late tillering has previously been reported to be a wheat developmental stage that is highly heat sensitive, as evidenced by heat-induced reductions in dry matter accumulation (Fischer and Maurer 1976).

After exposure to 40 ºC for 2 days, plants of all genotypes were able to recover their RGR when they were returned to regular conditions (compare RGDT to RGRAT in Appendix 7.3), which held true in all of the experiments. This suggests that the effects of brief exposure to 40 ºC were largely reversible and that plants may have effective mechanisms to repair damage caused by such a heat treatment. By contrast, exposure to 44 ºC (for 6 h or 2 d) produced more lasting effects, including a reduced growth rate, increased proportion of senescent area (tissue death) and a developmental delay (increased time from sowing to maximum growth rate by ~7 days; Figure 7.2). This level of stress that was greater than what the plants had the ability to completely recover from.

Results presented here indicate that there may be different genes controlling during and after treatment growth rate responses. In general, genotypes may be classified into four

groups according to their RGR responses during and after treatment (Appendix 7.3): 1) those that showed strong heat tolerance both during and after treatment (e.g. AUS1490 and Currawong), 2) those that showed high heat sensitivity during and after treatment (e.g. Cadoux and Reeves), 3) those that were highly affected during heat treatment but which largely recovered after heat treatment (e.g. Opata 85 and Sunstar) and 4) those that showed weak response during heat treatment but a considerable response after heat treatment (e.g. Chara and QT7475). Further research is required to confirm these differences and to identify mechanisms underlying this variation.

Turgor is required for plant growth (e.g. cell expansion) (Reynolds et al. 2012); therefore, studying various water related traits such as leaf water potential and leaf relative water content may help us understand the effect of a stress on growth and performance. Heat stress increases evaporative demand and transpiration. Increased transpiration can help to lower plant temperature relative to air temperature (evaporative cooling) and thus helps the plant cope better with high air temperatures. However, if the demand for water exceeds the supply, dehydration can result. In this study, heat stress increased evaporative demand and consequently transpiration as shown by the significant increase in stomatal conductance (Figure 7.9E and F) during heat treatment. Heat stress also considerably reduced leaf relative water content (RWC; Figure 7.9A and B) and leaf water potential (LWP; Figure 7.9C and D) which indicates a negative impact of heat stress on plant water status. Although this study was conducted under high relative humidity and soil moisture, the impaired water status of the leaves under heat suggests that the hydraulic conductivity of the soil, roots and/or vascular system were unable to match the higher evaporative demand. Similarly, in other plant species such as sugarcane (Wahid 2007; Wahid and Close 2007) and tomato (Morales et al. 2003), high temperatures combined with high soil moisture and relative humidity were reported to lower leaf water and osmotic potentials, leaf relative water content, and root hydraulic conductance. Heat induced alterations in plant water status may disturb cellular and physiological processes, leading to growth suppression.

Water use efficiency was smaller in the heat-treated plants during and after treatment, although the latter effect was not statistically significant (Figure 7.10a and b). A decline in growth and increase in water use resulted in a large reduction in water use efficiency during treatment, while the reduced water use efficiency after treatment derived from the heat-derived reduction in growth (Appendix 7.4a-d). In general, heat-treated plants were unable to take advantage of the available water to produce biomass, perhaps due to heat damage to various physiological processes as described below.

Heat stress during either the vegetative or reproductive developmental stages can negatively affect different aspects of the photosynthetic machinery, reducing photosynthetic capacity of the whole plant (Al-Khatib and Paulsen 1990; Bird et al. 1977; Todd 1982). At the seedling stage, variation among wheat genotypes for heat stress effects on photosynthetic rate was closely correlated with biomass production responses (Al-Khatib and Paulsen 1990). In the current study, heat stress significantly reduced chlorophyll content (measured by a SPAD chlorophyll meter in Experiments 1, 2 and 4) and viable leaf area (proportion of non-senescent area based on colour coding of images). These are changes that were likely to reflect a smaller photosynthetic capacity. Generally, the correlation between heat responses of senescence related traits and growth rate (Table 7.7) indicated that genotypes with increased chlorophyll loss and proportion of senescent area under heat conditions suffered more growth rate reduction.

Stomatal closure usually occurs during water stress conditions which in turn can adversely affect CO_2 acquisition and limit photosynthesis (Prasad et al. 2008; Shah and Paulsen 2003). Although plants in this study also experienced some level of water stress while being heat-treated, as evidenced by their decreased LWP and RWC, their stomata were open during this time as shown by their increased stomatal conductance relative to control plants. This suggests that under these conditions of good soil moisture supply, heat impacted on photosynthesis not by limiting CO_2 supply but by injuring the photosynthetic apparatus (e.g. photosystem II, and by causing chlorophyll loss). Heat and water stress have also been shown to impair photosynthetic metabolic activities. For instance, they both impair rubisco and rubisco-activase activities required for photosynthesis (reviewed by Prasad et al. 2008).

By contrast, high temperatures generally increase respiration rate (Almeselmani et al. 2012; Prasad et al. 2008; Wahid et al. 2007). Thus, under high temperature, increased respiration, and possibly also increased energy expended on protective mechanisms (e.g. production of heat shock proteins), could reduce photosynthate available for growth, contributing to growth reductions observed under heat.

There is variation among wheat genotypes relating to many of the aforementioned processes (i.e. thermo-stability of photosynthetic apparatus, photosynthetic rate, change in respiration, etc.) under high temperatures condition (Al-Khatib and Paulsen 1989; Al-Khatib and Paulsen 1990; Al-Khatib and Paulsen 1999; Almeselmani et al. 2012). Such variation could have contributed to differences in heat responses of growth rate observed in this study.

Apart from the effects of heat stress on water and energy balance, other mechanisms could be involved in the growth rate responses. Cell division and expansion are components of plant

growth (Prasad et al. 2008) and have both been shown to be adversely affected by various stresses such as drought (Alves and Setter 2004; Tardieu et al. 2000) and salinity (Munns and Tester 2008). By contrast, rates of cell division and expansion and developmental processes such as leaf initiation rate have a temperature optimum, which varies according to the species; the optimum is around 28 °C in wheat (Parent and Tardieu 2012). The stress temperatures used in this study (40/30 ºC and 44/30 ºC day/night) were well above this optimum, consistent with the reduced growth rates observed.

In sugarcane sprouts, changes in levels of primary (free proline, glycinebetaine and soluble sugars) and secondary metabolites (anthocyanin, carotenoids and soluble phenolics) were shown to relate to net assimilation capacity and the heat stress responses of various growth attributes such as relative shoot growth rate, relative leaf expansion rate and shoot dry weight (Wahid 2007). Ashraf and Hafeez (2004) found differences between maize and pearl millet for shoot dry weight, relative growth rate and net assimilation rate responses under very high temperatures at the vegetative stage, with pearl millet being more tolerant for the aforementioned traits. The observed differences in heat responses of maize and pearl millet were attributed to their differences in nutrient uptake patterns under heat stress conditions (Ashraf and Hafeez 2004). In pearl millet, high temperature caused a significant increase in uptake of N, P, and K^+, and had no effect on the uptake of Ca^{2+}, Mg^{2+}, Na^+ and S. By contrast, high temperature reduced uptake of N, S, Mg^{2+} and Na^+, and increased uptake of K^+ and Ca^{2+} in maize (Ashraf and Hafeez 2004). An additional mechanism controlling heat responses could be alterations in content of hormones such as abscisic acid and ethylene (Wahid et al. 2007). Transcriptome analysis of heat tolerant and susceptible wheat genotypes following heat treatment at the seedling stage suggested that genes for heat shock proteins, transcription factors, calcium signalling and metabolism pathways, and other factors, were involved in responses to heat (Qin et al. 2008). Eventual cloning of loci determining varietal differences in heat responses of growth rate in wheat should help elucidate which if any of the aforementioned physiological/molecular processes are involved in controlling this variation.

An overall significant association was observed between the responses of growth and senescence at the vegetative stage with those of grain weight and senescence at the reproductive stage. This may indicate some physiological/genetic link between heat responses at the different developmental stages. Several other researchers of wheat and other species had similar findings. For instance, Al-Khatib and Paulsen (1990) found a positive association between heat responses of photosynthetic rate at vegetative and reproductive stages of wheat development. Senthil-Kumar et al. (2003) found some associations between thermo-tolerance

at the seedling and mature plant stages in sunflower hybrids. In wheat, cell membrane thermostability (CMS) at the seedling stage was significantly correlated with either CMS at flowering time and/or with yield under high temperature conditions (Balota et al. 1993; Fokar et al. 1998; Saadalla et al. 1990a; Saadalla et al. 1990b; Reynolds et al. 1994). There are also suggestions that developmental stages differ in heat tolerance. For instance, several Arabidopsis *hot* mutants were only defective in thermo-tolerance when assayed as 2.5-day-old seedlings and not as more advanced plants (Hong et al. 2003). By contrast, *npr1* mutants and *NahG* transgenic plants were more heat-tolerant as 2.5-day-old seedlings but heat-susceptible as 10-day-old seedlings (Clarke et al. 2004). Schapendonk et al. (2007) found no association between photosynthesis measured at vegetative (end of tillering) and grain-filling (10-14 DAA) stages among three wheat genotypes contrasting for heat tolerance, growing under high temperature conditions. In the current study, some genotypes behaved differently at the two stages, consistent with the idea that some tolerance genes could be stage-specific. A possibility could be genes for enzymes that synthesize starch in the developing grains, that can only express during grain-filling (Hawker and Jenner 1993; Jenner 1994; Keeling et al. 1993; Keeling et al. 1994; Zahedi et al. 2003).

In total, 56 pairs of parents of existing mapping populations were assayed for vegetative heat tolerance. These varied from showing no contrast to showing 31 (Sunco/QT7475), 14 (Chara/WW2449), 56 (Chara/WW2449), and 52% (CD87/Katepwa) contrast for responses of RGRDT, RGRAT, PSA28DAS, and PSA39DAS, respectively (Appendix 7.5). However, due to inconsistencies observed between the experiments (Appendices 7.1 and 7.2), re-evaluation of selected contrasting parents for heat tolerance is desirable. Drysdale/Waagan and Gladius/Drysdale pairs showed some consistency among the experiments for their contrast in RGR response (Appendix 7.2) and therefore look most promising for genetic studies. Gladius/Drysdale contrasted for during treatment response but showed lower contrast for after treatment response, while the reverse held true for Drysdale/Waagan (Appendices 7.1 and 7.2). Use of the two corresponding mapping populations may therefore reveal loci, genes and mechanisms controlling variation in RGR responses during the heat treatment and for recovery of RGR after heat treatment relief.

There are possible additional issues, related to the roots, that may have confound the results here and in experiments conducted at grain-filling stages in Chapters 3, 4 and 6. As in many studies of heat tolerance by other researchers, pots were placed in trays of shallow water (~ 2-cm depth) for the brief heat treatment in the chamber to minimize drought stress (exceptions being pots in Experiments 4 and 5 in this chapter, which were watered to field

capacity while they were under heat treatment). This raises the concern that plant performance was affected by water logging. Water logging can reduce root and shoot biomass, photosynthesis, chlorophyll content and yield (Ballesteros et al. 2014; Collaku and Harrison 2002; Huang et al. 1994; Lee et al. 2007; Malik et al. 2001). Therefore, experiments combining different watering methods with heat and control temperature treatments are needed to check whether waterlogging was confounding these experiments. In addition, similar to most of the previous studies related to heat stress, root temperature was not controlled or monitored. Soil in pots is more prone to over-heating than soil in the field, and water and nutrient uptake by roots is known to be sensitive to heat stress (Farooq et al. 2011; Martínez-Ballesta et al. 2009). Further experiments would also be needed to determine whether root heating was also a factor contributing to the observed responses. See Passioura (2006) for other complications related to pot experiments that may worth considering in future works.

7.5 Concluding remarks

Heat stress can adversely impact wheat plants at different growth stages. Results showed that a brief episode of severe high temperatures can considerably affect growth and senescence of young wheat plants and that these responses show genetic variation. However, more work is required to find procedures that will result in greater reproducibility. Further work is also required to identify the physiological/molecular processes and genetic loci controlling the variation in heat responses at the vegetative stage. Overall, the significant association of heat responses during treatment at the vegetative stage with responses of grain weight and chlorophyll at the grain-filling stage may suggest a possible physiological/genetic link between heat responses at different developmental stages, with implications for developing more efficient heat tolerance screening methods. Such methods may assist breeding for heat tolerance and identification of heat tolerance genes.

Chapter 8: Conclusions, contribution to knowledge, and future work

Heat stress is a major constraint for wheat production worldwide and with current climate change trends the situation is predicted to worsen (IPCC 2007; Ortiz et al. 2008; Hansen et al. 2012). To increase wheat production under such conditions to meet the worlds' growing demand, new superior heat tolerant wheat varieties will be required. To breed these, knowledge of heat tolerance mechanisms, identification of tolerance sources and development of reliable screening tools will all be essential. To this end, the present work contributes to the understanding of physiological mechanisms of heat tolerance and its genetic basis in hexaploid wheat, and identifies assays with potential to assist heat tolerance studies and in breeding programs. This chapter summarizes the major findings of this project and discusses possibilities for further research.

This study provides information on variability for heat tolerance traits among a small sample of Australian and exotic genotypes in the greenhouse/chamber (Chapters 3, 4 and 7). Some of the identified tolerant genotypes might be useful as sources of heat tolerance in breeding. However, field validation of the heat tolerance sources would be required. Genotypes of contrasting tolerance identified here could also be used as the basis for further research into genetic, physiological, and biochemical aspects of heat tolerance.

The Drysdale × Waagan population was chosen for genetic analysis of heat tolerance in the current study because the parent varieties were found to contrast for tolerance. Parents of other existing populations were also found to contrast for heat tolerance (SGW response), i.e., Young vs. Wyalkatchem, Janz vs. Frame, Sunco vs. Tasman, and Sokoll vs. Krichauff (Appendix 3.2a). Therefore, these populations might be useful in discovering new heat tolerance QTL. Crosses could also be made between the most tolerant and intolerant genotypes to produce new populations for genetic studies.

In the Drysdale × Waagan population, QTL for stay-green and grain weight maintenance under brief episodes of high temperature coincided on chromosome arms 3BS and 6BL (Chapter 6). Moreover, these traits were significantly associated in the study of 36 bread wheat genotypes (Chapter 3), indicating the possibility that stay-green may be dominant in determining wheat productivity (grain weight maintenance) in heat-stressed environments in the broader germplasm. Currently there is no consensus in the literature about the role of photosynthate source activity/availability in maintaining grain weight after heat stress at grain-filling (Al-Khatib and Paulsen 1984; Al-Khatib and Paulsen 1990; Bhullar and Jenner 1983; Jenner 1991a; Jenner 1994; Nicolas et al. 1984; Reynolds et al. 2000; Shah and Paulsen 2003; Wardlaw et al. 1980; Wardlaw and Wrigley 1994). The correlations and QTL results

presented here support the hypothesis that common gene(s) and therefore molecular mechanisms can determine those traits. The ability to maintain carbohydrate supply by the vegetative tissues may contribute to grain weight maintenance under heat stress conditions, or alternatively it may make no contribution (as findings in Chapter 4 seem to suggest), with the correlation simply reflecting common genetic control of senescence in the leaves and grain. Therefore, selection for stay-green related traits, which were also highly heritable under high temperature (H^2 = 0.81-0.87; Chapter 6), may offer a means of selecting for high wheat productivity (high grain yield) in terminal heat stress environments.

Of the various methods used to define stay-green, flag leaf chlorophyll content measured directly before and after heat stress (normalised difference between control and heat-treated plants for chlorophyll content *per se*) using a portable SPAD chlorophyll meter showed the most promise as an easy selection procedure for heat tolerance and provided good discrimination between tolerant and intolerant genotypes (Chapters 3 and 6). This method represents an inexpensive and simple way to indirectly select heat tolerance (grain weight maintenance), that can be done earlier in the plant's development (10 to 13 DAA) than direct assessment of grain weight at maturity (~ 50 DAA). In the field, plants could be measured just before a forecasted heat wave, and then just after it. Generally, chlorophyll content hardly changed from 10 to about 30 DAA in control plants (Appendix 3.1), although it varied according to genotype. Therefore, the measurement before the heat treatment or heat wave, within this developmental window, could serve as the 'control' value and obviate the need for a comparison to non-stressed plants. This is important, since non-stressed control plants would be difficult to obtain in the field at the same time as stressed plants. The QTL on chromosome 3BS stably influenced chlorophyll content *per se* in non-stressed plants in addition to heat tolerance (HSIs) of several traits, with the Waagan allele conferring both larger chlorophyll content *per se* and higher heat tolerance (smaller HSIs). This suggested that use of a SPAD chlorophyll meter to select for larger chlorophyll content at a single time point (e.g., 10, 13 or 27 DAA as used in this study) in non-stressed plants could also be used to indirectly select for heat tolerance, at least in relation to effects controlled by the 3BS locus.

Results of this study also provided evidence of tolerance mechanisms independent of stay-green (Chapters 3 and 4). For instance, Millewa and Sunco which both appeared to be heat tolerant for grain weight maintenance but relatively prone to chlorophyll loss under heat conditions (Chapter 3), both showed large WSC mobilization efficiency under heat conditions (Chapter 4). Populations derived by crossing these varieties with other varieties could be used to seek QTL for tolerance that is independent of stay-green. Furthermore, the populations

could be used to determine what physiological traits are associated with such tolerance QTL (e.g., mobilization of stem carbohydrate reserves).

A molecular genetic map was constructed for the Drysdale × Waagan DH population (Chapter 5). The map could be used for mapping other agronomically important traits (e.g. floret sterility effects of heat, drought tolerance, etc.), and to support map-based cloning of the gene(s) controlling the target trait(s). The map construction work described in this study highlighted the importance of tracking which lines are derived from different individual parent plants. The study described a method to avoid disruption to the map caused by parent heterogeneity, which could be applied in similar situations. Large gaps (30.8 to 37.8 cM) were observed in the map in some regions, and some chromosomes had few markers (e.g. chromosome 3D). A targeted approach may not help to fill at least some of the gaps, if those regions in the parents were identical by common descent. The marker density and genome coverage of the Drysdale × Waagan genetic map could be further improved by applying other tools to the population, such as a 90K SNP array (Wang et al. 2014) or genotyping-by-sequencing (Poland et al. 2012).

The instability of most of the detected heat tolerance QTL suggested that there may be a genotype by environment interaction for heat tolerance. Some of the QTL may have been inconsistently detected simply because they had smaller effects. However, the 3BS QTL showed a strong and consistent effect on heat tolerance for various traits. Its location corresponded with some QTL reported to affect stress or heat tolerance related traits in several other crosses (Bennett et al. 2012; Kumar et al. 2010; Maccaferri et al. 2008; Mason et al. 2010; Wang et al. 2009), suggesting the same heat/stress tolerance gene(s) on 3B may be found in multiple varieties. Although the QTL analysis of this study improved our understanding of the genetic basis of heat tolerance and highlighted the potential importance of the 3BS locus in affecting heat tolerance in wheat, the conclusions are currently restricted to the studied conditions (two experiments under controlled conditions; Chapter 6) which differ in many ways from those encountered in the field. Therefore as a follow up study, the Drysdale × Waagan mapping population could be tested in the field using an early vs. late sowing approach, or by trialling it at various low and high temperature sites, to test if the effect of the 3BS QTL is manifested under hot field conditions. Alternatively, homozygous nearly-isogenic lines for the QTL could be selected from progeny of 3BS-heterozygous Drysdale × Waagan RIL plants and tested under the aforementioned field conditions. The usefulness of the 3BS QTL effect could be further explored by seeing if it expresses when crossed into a range of heat-intolerant backgrounds. If this QTL could be validated in the field

and in different genetic backgrounds it could be considered worthy of marker assisted selection in breeding, i.e., by converting the closest identified SNPs, *wsnp_Ra_c41135_48426638(R)*, *wsnp_Ex_c12875_20407926(R)* and *wsnp_BE497169B_Ta_2_1(R)*, to high-throughput single marker assays such as competitive allele-specific PCR (KASP; LGC Genomics, London, UK; www.lgcgenomics.com) or high-resolution melting (HRM) (Liew et al. 2004; Wittwer et al. 2003) assays.

Cloning of the gene(s) underlying the 3BS QTL could provide the opportunity to transform (genetically modify) current varieties with the favourable allele(s) to make them more heat tolerant, when conditions for the deployment of such technologies in wheat become more commercially practical. The 3BS region was associated with heat stability and absolute values for several traits. It is not clear at this stage whether this association is due to pleiotropy of the same gene or the effects of distinct but closely linked genes. Fine mapping or cloning of the responsible gene(s) would allow this question to be addressed. The cloned gene(s) could also provide a tool to investigate the molecular mechanisms controlling heat tolerance (e.g. using reverse genetic approaches). The availability of the sequence of chromosome 3B (Choulet et al. 2014, Mayer et al. 2014; http://wheat-urgi.versailles.inra.fr/) could help facilitate fine mapping and isolation of the tolerance gene(s) on 3BS. SPAD measurements could provide a rapid means of phenotyping large mapping populations used in fine mapping, at least for the stay-green effect.

There is also a large contrast (~ 60%) between Drysdale and Waagan parents for floret fertility response to a brief heat stress at meiosis/booting with Drysdale and Waagan being intolerant and tolerant, respectively (Dr. Nick Collins, personal communication). Recently, the Drysdale × Waagan DH population was scored for floret sterility induced by a brief heat stress at meiosis/booting and QTL analysis is underway (Dr. Nick Collins, personal communication). This will allow comparison of the grain-filling and fertility responses to heat at the genetic level. For example, it would be interesting to see if the 3BS QTL also affects tolerance to heat induced floret sterility. If this is the case, it would imply a common mechanism for heat tolerance at the different developmental stages, and would make this region useful for selecting heat tolerance at both developmental stages. Positive associations have been reported between stem water soluble carbohydrate content/metabolism and maintenance of both floret fertility/grain set and grain weight under high temperature (Dreccer et al. 2014; Talukder et al. 2013), providing evidence that there may indeed be common mechanisms involved in tolerance at the two stages.

The loci on chromosomes 3BS and 6BL explained a considerable proportion of the variation for grain weight HSI (11 to 21%; Chapter 6) in the Drysdale × Waagan DH population but additional work would be required to quantify their contribution to heat tolerance within wheat in general. The SNPs directly around each of these two loci could be converted to KASP or HRM assays which could then be used to determine which wheat genotypes have the tolerant 'Waagan' haplotype (marker-allele combination) for the chromosome segment, and by inference, the tolerance allele. Correlation between this haplotype (or others at the loci) and heat tolerance could then be used to estimate the contribution of each locus to heat tolerance in the wider germplasm. Suitable material for use in such a study could be 307 genotypes, including 140 elite hexaploids and 128 landraces, which have been subjected to heat tolerance screening in the field using irrigated normal vs. late sowings in central NSW in an ACPFG/NSW-DPI collaboration (Dr. Nick Collins, personal communication).

Recently, genome-wide association mapping has been used to map traits in wheat and barley (Breseghello and Sorrells 2006; Crossa et al. 2007; Emebiri et al. 2010; Neumann et al. 2011). This approach could provide a broader picture of heat tolerance loci present in wheat, by exploiting collections of wild accessions, landraces, breeding populations or varieties to discover other loci. In addition to potentially detecting higher number of positive alleles per locus, it does not require specific crosses and population development (saving time and resources) and potentially also provides higher mapping resolution (Cavanagh et al. 2008; Gupta et al. 2005; Soto-Cerda and Cloutier 2012; Zhu et al. 2008). However, there are also some limitations using this approach, e.g., low power to detect effects of rare alleles, increased rate of false positives when there is genetic structure in the population that is not dealt with appropriately (Cavanagh et al. 2008; Gupta et al. 2005; Soto-Cerda and Cloutier 2012; Zhu et al. 2008). Other mapping population types such as Nested Association Mapping (McMullen et al. 2009; Yu et al. 2008) and Multi-parent Advanced Generation Inter-cross (Cavanagh et al. 2008) could also be considered for use in wheat heat tolerance studies. These populations utilise multiple parents and complex crossing strategies, combining the advantages of both linkage and association mapping methodologies, while avoiding difficulties associated with uncontrolled genetic structure (Cavanagh et al. 2008). Nevertheless, large amounts of resources and time are required to phenotype these populations due to their large size.

There are contradictory reports linking GA-insensitive dwarfing alleles to abiotic stress tolerance (Alghabari et al. 2014; Blum et al. 1997; Butler et al. 2005; Law et al. 1981; Law

and Worland 1985). Results of this study showed no effect of the major dwarfing loci (*Rht-B1* and *Rht-D1*) on the single grain weight response to a brief episode of severe heat at grainfill, in the Drysdale × Waagan population, under controlled conditions (Chapter 6). The situation may be different in the field. Nonetheless, this study found no evidence of there being an advantage in selecting for either type (tall or short) in breeding for heat tolerance.

Association was observed between heat responses at vegetative and grain-filling stages of wheat development, suggesting a degree of common physiological/genetic links for heat responses between these stages (Chapters 3 and 7). Therefore, automated plant imaging of seedlings may have potential utility in heat tolerance studies in wheat to screen for heat tolerance. As shown here, discriminating heat tolerant and intolerant genotypes for growth rate response during heat treatment only needs plant size to be measured at two time points, i.e., directly before and after treatment. The Plant Accelerator facility could be dispensed with by instead taking destructive measurements of shoot weight at those time points. This vegetative growth assay may have the potential help to facilitate breeding for heat tolerance or mapping of heat tolerance loci. As a follow up, QTL analysis could be done in a single mapping population to test for a genetic link between tolerance at the vegetative and grain-filling stages of wheat development, as it could be argued that the observed associations between vegetative and grain-filling stage could have merely occurred by chance in the small sample of genotypes studied. Populations derived from parents contrasting for heat tolerance at both developmental stages could be used for this purpose (e.g., Drysdale × Waagan and Gladius × Drysdale mapping populations).

For heat tolerance at the vegetative stage, genotypes responded differently between experiments (Chapter 7). It was likely this was due to differences in growth stage and/or physiological status of the plants at the time of heat treatment, caused by seasonal and across-year light and temperature differences in the greenhouse and Smarthouse. This highlights the importance of the effect of growing environment (conditions pre-/post- heat treatment) on reproducibility of heat responses. Interestingly, the same major tolerance locus on 3BS for grain weight and chlorophyll was detected in two trials conducted at contrasting times of the year (Chapter 6). Therefore, at least for the 3BS locus, experiments conducted at the reproductive stage seem to give reproducible results regardless of the time of the year, enabling screening to be undertaken continually throughout the year. To improve data reproducibility at the vegetative stage, plants could be heat-treated according to their developmental stage (preferably late tillering stage, i.e. Zadoks stage 25 to 27) (Zadoks et al. 1974) rather than according to the Julian days (time after sowing) as done in the current study.

Currently, the temperature in the Smarthouse is somewhat uneven and uncontrolled, due to the evaporative cooling system and the large size of the room (230 square meters and 4.5 meters height). Often the choice of where to put an experiment on the conveyor system is limited due to the existence of other experiments in the Smarthouse. As precise control over temperature is critical in heat tolerance evaluation, as illustrated by the results of this study, this may limit the utility of The Plant Accelerator in vegetative heat tolerance studies, at least for assays in which the plants are monitored for a long time (~ 2 weeks) after heat treatment in the Smarthouse.

It may worth trying field-based high-throughput phenotying equipment such as phenomobiles, phenotowers and polycopters equipped with a range of remote sensing technologies (Araus and Cairns 2014; White et al. 2012; www.plantphenomics.org.au) for monitoring crop performance at the vegetative and reproductive stages under heat stressed field conditions. Relationships between vegetative growth and grain yield responses in the field could then be examined, and results compared to those obtained using the greenhouse/chamber assays. Currently, field assays for heat tolerance are very time consuming, laborious, and expensive. The aforementioned high-throughput field-based equipment may help to alleviate some of the difficulties of current field assays, if association between traits (e.g. biomass) measured using these new technologies and grain yield could be established under high temperature conditions.

References

ACEVEDO, E. 1991. Imporovement of winter cereal crops in mediterranean environments: use of yield, morphological, and physiological traits. *Breeding for drought resistance in wheat*, 188.

AKBARI, M., WENZL, P., CAIG, V., CARLING, J., XIA, L., YANG, S., USZYNSKI, G., MOHLER, V., LEHMENSIEK, A. & KUCHEL, H. 2006. Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. *Theoretical and Applied Genetics,* 113**,** 1409-1420.

AL-KHATIB, K. & PAULSEN, G. M. 1989. Enhancement of thermal injury to photosynthesis in wheat plants and thylakoids by high light intensity. *Plant Physiology,* 90**,** 1041.

AL-KHATIB, K. & PAULSEN, G. M. 1990. Photosynthesis and productivity during high-temperature stress of wheat genotypes from major world regions. *Crop science,* 30**,** 1127-1132.

AL-KHATIB, K. & PAULSEN, G. M. 1999. High-temperature effects on photosynthetic processes in temperate and tropical cereals. *Crop science,* 39**,** 119-125.

AL-KHATIB, K. & PAULSEN, G. M. 1984. Mode of high temperature injury to wheat during grain development. *Physiologia Plantarum,* 61**,** 363-368.

ALGHABARI, F., LUKAC, M., JONES, H. & GOODING, M. 2014. Effect of Rht alleles on the tolerance of wheat grain set to high temperature and drought stress during booting and anthesis. *Journal of Agronomy and Crop Science,* 200**,** 36-45.

ALI, M., SADDIQUI, M. F., ULLAH, I. & BAKHT, J. 2010. Analysis of HMWGS of historical set of Pakistani bread wheat varieties using SDS-PAGE. *African Journal of Biotechnology,* 9**,** 4286-4289.

ALIZADEH, O., BARMAK, J. H. & KOUROSH, O. 2010. The effects of exogenous cytokinin application on sink size in bread wheat (Triticum aestivum). *African Journal of Agricultural Research,* 5**,** 2893-2898.

ALMESELMANI, M., DESHMUKH, P. & CHINNUSAMY, V. 2012. Effects of prolonged high temperature stress on respiration, photosynthesis and gene expression in wheat (Triticum aestivum L.) varieties differing in their thermotolerance. *Plant stress,* 6**,** 25-32.

ALVES, A. A. & SETTER, T. L. 2004. Response of cassava leaf area expansion to water deficit: cell proliferation, cell expansion and delayed development. *Annals of Botany,* 94**,** 605-613.

AMANI, I., FISCHER, R. & REYNOLDS, M. 1996. Canopy temperature depression association with yield of irrigated spring wheat cultivars in a hot climate. *Journal of Agronomy and Crop Science,* 176**,** 119-129.

ARAUS, J. L. & CAIRNS, J. E. 2014. Field high-throughput phenotyping: the new crop breeding frontier. *Trends in plant science,* 19**,** 52-61.

ARNON, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant Physiology,* 24**,** 1.

ASHRAF, M. & HAFEEZ, M. 2004. Thermotolerance of pearl millet and maize at early growth stages: growth and nutrient relations. *Biologia Plantarum,* 48**,** 81-86.

ASHRAF, M. & KARIM, F. 1991. Screening of some cultivars/lines of blackgram (Vigna mungo (L.) Hepper) for resistance to water stress. *Tropical Agriculture,* 68**,** 57-62.

ASHRAF, M. & MEHMOOD, S. 1990. Response of four Brassica species to drought stress. *Environmental and Experimental Botany,* 30**,** 93-100.

ASSENG, S., FOSTER, I. A. N. & TURNER, N. C. 2011. The impact of temperature variability on wheat yields. *Global Change Biology,* 17**,** 997-1012.

AUSTIN, R., EDRICH, J., FORD, M. & BLACKWELL, R. 1977. The fate of the dry matter, carbohydrates and 14C lost from the leaves and stems of wheat during grain filling. *Annals of Botany,* 41**,** 1309-1321.

AUSTRALIAN BUREAU OF METEOROLOGY 2014. Australian climate variability & change - Trend maps. viewed 12 October 2014,. *http://www.bom.gov.au/climate/change/index.shtml#tabs=Tracker&tracker=trend-maps*.

AUSTRALIAN BUREAU OF STATISTICS 2014. Value of Agricultural Commodities Produced, Australia, 2012-13. cat. no. 7503.0. viewed 30 May 2014, *http://www.abs.gov.au/AUSSTATS/abs@.nsf/Latestproducts/*.

BALLESTEROS, D. C., MASON, R. E., ADDISON, C. K., ACUÑA, M. A., ARGUELLO, M. N., SUBRAMANIAN, N., MILLER, R. G., SATER, H., GBUR, E. E. & MILLER, D. 2014. Tolerance of wheat to vegetative stage soil waterlogging is conditioned by both constitutive and adaptive QTL. *Euphytica***,** 1-15.

BALOTA, M., AMANI, I., REYNOLDS, M. & ACEVEDO, E. 1993. *Evaluation of membrane thermostability and canopy temperature depression as screening traits for heat tolerance in wheat*, CIMMYT.

BANOWETZ, G., AMMAR, K. & CHEN, D. 1999. Temperature effects on cytokinin accumulation and kernel mass in a dwarf wheat. *Annals of Botany,* 83**,** 303.

BARAKAT, M. N., AL-DOSS, A. A., ELSHAFEI, A. A. & MOUSTAFA, K. A. 2011. Identification of new microsatellite marker linked to the grain filling rate as indicator for heat tolerance genes in F 2 wheat population. *Australian Journal of Crop Science,* 5.

BARNABÁS, B., JÄGER, K. & FEHER, A. 2008. The effect of drought and heat stress on reproductive processes in cereals. *Plant, cell & environment,* 31**,** 11-38.

BEALES, J., TURNER, A., GRIFFITHS, S., SNAPE, J. W. & LAURIE, D. A. 2007. A pseudo-response regulator is misexpressed in the photoperiod insensitive Ppd-D1a mutant of wheat (Triticum aestivum L.). *Theoretical and Applied Genetics,* 115**,** 721-733.

BELTRANO, J., CARBONE, A., MONTALDI, E. R. & GUIAMET, J. J. 1994. Ethylene as promoter of wheat grain maturation and ear senescence. *Plant Growth Regulation,* 15**,** 107-112.

BELTRANO, J., RONCO, M. & MONTALDI, E. 1999. Drought stress syndrome in wheat is provoked by ethylene evolution imbalance and reversed by rewatering, aminoethoxyvinylglycine, or sodium benzoate. *Journal of plant growth regulation,* 18**,** 59-64.

BENNETT, D., REYNOLDS, M., MULLAN, D., IZANLOO, A., KUCHEL, H., LANGRIDGE, P. & SCHNURBUSCH, T. 2012. Detection of two major grain yield qtl in bread wheat (triticum aestivum l.) under heat, drought and high yield potential environments. *Theoretical and Applied Genetics,* 125**,** 1473-1485.

BERGER, B., DE REGT, B. & TESTER, M. 2012. High-throughput phenotyping of plant shoots. *High-Throughput Phenotyping in Plants.* Springer.

BERGER, B., PARENT, B. & TESTER, M. 2010. High-throughput shoot imaging to study drought responses. *Journal of Experimental Botany,* 61**,** 3519-3528.

BERKMAN, P. J., LAI, K., LORENC, M. T. & EDWARDS, D. 2012. Next-generation sequencing applications for wheat crop improvement. *American Journal of Botany,* 99**,** 365-371.

BERNARDIN, J. Year. Genetic modification of the wheat genome to allow growth in hot climates. *In:* Proc. 44th Australian Cereal Chemistry Conference. JF Panozzo and PG Downie, eds. RACI: Melbourne, 1994. 60-61.

BHULLAR, S. & JENNER, C. 1983. Responses to brief periods of elevated temperature in ears and grains of wheat. *Functional Plant Biology,* 10**,** 549-560.

BHULLAR, S. & JENNER, C. 1985. Differential responses to high temperatures of starch and nitrogen accumulation in the grain of four cultivars of wheat. *Functional Plant Biology,* 12**,** 363-375.

BHULLAR, S. & JENNER, C. 1986. Effects of temperature on the conversion of sucrose to starch in the developing wheat endosperm. *Functional Plant Biology,* 13**,** 605-615.

BIRD, I., CORNELIUS, M. & KEYS, A. 1977. Effects of temperature on photosynthesis by maize and wheat. *Journal of Experimental Botany,* 28**,** 519-524.

BLAKE, N., LANNING, S., MARTIN, J., DOYLE, M., SHERMAN, J., NARUOKA, Y. & TALBERT, L. 2009. Effect of variation for major growth habit genes on maturity and yield in five spring wheat populations. *Crop science,* 49**,** 1211-1220.

BLUM, A. 1997. Improving wheat grain filling under stress by stem reserve mobilisation. *Wheat: Prospects for Global Improvement.* Springer.

BLUM, A. 1998. Improving wheat grain filling under stress by stem reserve mobilisation. *Euphytica,* 100**,** 77-83.

BLUM, A. & EBERCON, A. 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop science,* 21**,** 43-47.

BLUM, A., SINMENA, B., MAYER, J., GOLAN, G. & SHPILER, L. 1994. Stem reserve mobilisation supports wheat-grain filling under heat stress. *Functional Plant Biology,* 21**,** 771-781.

BLUM, A. & SULLIVAN, C. 1997. The effect of plant size on wheat response to agents of drought stress. I. Root drying. *Functional Plant Biology,* 24**,** 35-41.

BLUM, A., SULLIVAN, C. & NGUYEN, H. 1997. The effect of plant size on wheat response to agents of drought stress. II. Water deficit, heat and ABA. *Functional Plant Biology,* 24**,** 43-48.

BLUMENTHAL, C., BARLOW, E. & WRIGLEY, C. 1993. Growth environment and wheat quality: the effect of heat stress on dough properties and gluten proteins. *Journal of Cereal Science,* 18**,** 3-21.

BLUMENTHAL, C., BATEY, I., BEKES, F., WRIGLEY, C. & BARLOW, E. 1991. Seasonal changes in wheat-grain quality associated with high temperatures during grain filling. *Australian Journal of Agricultural Research,* 42**,** 34.

BLUMENTHAL, C., BEKES, F., GRAS, P., BARLOW, E. & WRIGLEY, C. 1995. Identification of wheat genotypes tolerant to the effects of heat stress on grain quality. *Cereal chemistry,* 72**,** 539-544.

BLUMENTHAL, C., STONE, P., GRAS, P., BEKES, F., CLARKE, B., BARLOW, E., APPELS, R. & WRIGLEY, C. 1998. Heat-shock protein 70 and dough-quality changes resulting from heat stress during grain filling in wheat. *Cereal chemistry,* 75**,** 43-50.

BLUMENTHAL, C., WRIGLEY, C., BATEY, I. & BARLOW, E. 1994. The heat-shock response relevant to molecular and structural changes in wheat yield and quality. *Functional Plant Biology,* 21**,** 901-909.

BONNIN, I., ROUSSET, M., MADUR, D., SOURDILLE, P., DUPUITS, C., BRUNEL, D. & GOLDRINGER, I. 2008. FT genome A and D polymorphisms are associated with the variation of earliness components in hexaploid wheat. *Theoretical and Applied Genetics,* 116**,** 383-394.

BORRELL, A., BIDINGER, F. & SUNITHA, K. 1999. Stay-green trait associated with yield in recombinant inbred sorghum lines varying in rate of leaf senescence. *International Sorghum and Millets Newsletter,* 40**,** 31-34.

BORRELL, A. K., HAMMER, G. L. & HENZELL, R. G. 2000. Does maintaining green leaf area in sorghum improve yield under drought? II. Dry matter production and yield. *Crop science,* 40**,** 1037-1048.

BORRELL, A. K., INCOLL, L. & DALLING, M. J. 1993. The influence of the Rht1 and Rht2 alleles on the deposition and use of stem reserves in wheat. *Annals of Botany*, 71**,** 317-326.

BORRELL, A. K., MULLET, J. E., GEORGE-JAEGGLI, B., VAN OOSTEROM, E. J., HAMMER, G. L., KLEIN, P. E. & JORDAN, D. R. 2014a. Drought adaptation of stay-green sorghum is associated with canopy development, leaf anatomy, root growth, and water uptake. *Journal of Experimental Botany***,** eru232.

BORRELL, A. K., OOSTEROM, E. J., MULLET, J. E., GEORGE-JAEGGLI, B., JORDAN, D. R., KLEIN, P. E. & HAMMER, G. L. 2014b. Stay-green alleles individually enhance grain yield in sorghum under drought by modifying canopy development and water uptake patterns. *New Phytologist*.

BOTSTEIN, D., WHITE, R. L., SKOLNICK, M. & DAVIS, R. W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American journal of human genetics,* 32**,** 314.

BOYER, J. S. 1967. Leaf water potentials measured with a pressure chamber. *Plant Physiol.,* 42**,** 133-137.

BRESEGHELLO, F. & SORRELLS, M. E. 2006. Association mapping of kernel size and milling quality in wheat (Triticum aestivum L.) cultivars. *Genetics,* 172**,** 1165-1177.

BROMAN, K. W. 2010. Genetic map construction with R/qtl. *Technical Report # 214, University of Wisconsin-Madison, Department of Biostatistics & Medical Informatics*.

BROMAN, K. W., WU, H., SEN, Ś. & CHURCHILL, G. A. 2003. R/qtl: QTL mapping in experimental crosses. *Bioinformatics,* 19**,** 889-890.

BRUCKNER, P. & FROHBERG, R. 1987. Rate and duration of grain fill in spring wheat. *Crop science,* 27**,** 451-455.

BUKOVNIK, U., FU, J., BENNETT, M., PRASAD, P. V. V. & RISTIC, Z. 2009. Heat tolerance and expression of protein synthesis elongation factors, EF-Tu and EF-1 , in spring wheat. *Functional Plant Biology,* 36**,** 234-241.

BUSH, M. & EVANS, L. 1988. Growth and development in tall and dwarf isogenic lines of spring wheat. *Field Crops Research,* 18**,** 243-270.

BUTLER, D., CULLIS, B., GILMOUR, A. & GOGEL, B. 2009. ASReml-R, Reference Manual. *Technical report, Queensland Department of Primary Industries*.

BUTLER, J. D., BYRNE, P. F., MOHAMMADI, V., CHAPMAN, P. L. & HALEY, S. D. 2005. Agronomic Performance of Alleles in a Spring Wheat Population across a Range of Moisture Levels. *Crop science,* 45**,** 939-947.

CADALEN, T., BOEUF, C., BERNARD, S. & BERNARD, M. 1997. An intervarietal molecular marker map in Triticum aestivum L. Em. Thell. and comparison with a map from a wide cross. *Theoretical and Applied Genetics,* 94**,** 367-377.

CAMEJO, D., RODRÍGUEZ, P., ANGELES MORALES, M., MIGUEL DELL'AMICO, J., TORRECILLAS, A. & ALARCÓN, J. J. 2005. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. *Journal of Plant Physiology,* 162**,** 281-289.

CAMPBELL, K. G., BERGMAN, C. J., GUALBERTO, D. G., ANDERSON, J. A., GIROUX, M. J., HARELAND, G., FULCHER, R. G., SORRELLS, M. E. & FINNEY, P. L. 1999. Quantitative trait loci associated with kernel traits in a soft× hard wheat cross. *Crop science,* 39**,** 1184-1195.

CANE, K., EAGLES, H. A., LAURIE, D. A., TREVASKIS, B., VALLANCE, N., EASTWOOD, R. F., GORORO, N., KUCHEL, H. & MARTIN, P. 2013. Ppd-B1 and Ppd-D1 and their effects in southern Australian wheat. *Crop and Pasture Science,* 64**,** 100-114.

CAVANAGH, C., MORELL, M., MACKAY, I. & POWELL, W. 2008. From mutations to MAGIC: resources for gene discovery, validation and delivery in crop plants. *Current opinion in plant biology,* 11**,** 215-221.

CAVANAGH, C. R., CHAO, S., WANG, S., HUANG, B. E., STEPHEN, S., KIANI, S., FORREST, K., SAINTENAC, C., BROWN-GUEDIRA, G. L. & AKHUNOVA, A. 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,* 110**,** 8057-8062.

CEPPI, D., SALA, M., GENTINETTA, E., VERDERIO, A. & MOTTO, M. 1987. Genotype-Dependent Leaf Senescence in Maize Inheritance and effects of pollination-prevention. *Plant Physiology,* 85**,** 720-725.

CHALMERS, K. J., CAMPBELL, A. W., KRETSCHMER, J., KARAKOUSIS, A., HENSCHKE, P., PIERENS, S., HARKER, N., PALLOTTA, M., CORNISH, G. & SHARIFLOU, M. 2001. Construction of three linkage maps in bread wheat, Triticum aestivum. *Crop and Pasture Science,* 52**,** 1089-1119.

CHOULET, F., ALBERTI, A., THEIL, S., GLOVER, N., BARBE, V., DARON, J., PINGAULT, L., SOURDILLE, P., COULOUX, A. & PAUX, E. 2014. Structural and functional partitioning of bread wheat chromosome 3B. *Science,* 345**,** 1249721.

CHOWDHURY, S. & WARDLAW, I. 1978. The effect of temperature on kernel development in cereals. *Crop and Pasture Science,* 29**,** 205-223.

CHRISTOPHER, J., MANSCHADI, A., HAMMER, G. & BORRELL, A. 2008. Developmental and physiological traits associated with high yield and stay-green phenotype in wheat. *Crop and Pasture Science,* 59**,** 354-364.

CIAFFI, M., TOZZI, L., BORGHI, B., CORBELLINI, M. & LAFIANDRA, D. 1996. Effect of heat shock during grain filling on the gluten protein composition of bread wheat. *Journal of Cereal Science,* 24**,** 91-100.

CLARKE, S. M., MUR, L. A., WOOD, J. E. & SCOTT, I. M. 2004. Salicylic acid dependent signaling promotes basal thermotolerance but is not essential for acquired thermotolerance in Arabidopsis thaliana. *The Plant Journal,* 38**,** 432-447.

COLLAKU, A. & HARRISON, S. 2002. Losses in wheat due to waterlogging. *Crop science,* 42**,** 444-450.

COLLARD, B., JAHUFER, M., BROUWER, J. & PANG, E. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica,* 142**,** 169-196.

CORBELLINI, M., MAZZA, L., CIAFFI, M., LAFIANDRA, D. & BORGHI, B. 1998. Effect of heat shock during grain filling on protein composition and technological quality of wheats. *Euphytica,* 100**,** 147-154.

COSSANI, C. M. & REYNOLDS, M. P. 2012. Physiological traits for improving heat tolerance in wheat. *Plant Physiology,* 160**,** 1710-1718.

CROSSA, J., BURGUENO, J., DREISIGACKER, S., VARGAS, M., HERRERA-FOESSEL, S. A., LILLEMO, M., SINGH, R. P., TRETHOWAN, R., WARBURTON, M. & FRANCO, J. 2007. Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. *Genetics,* 177**,** 1889-1913.

CUKADAR-OLMEDO, B. & MILLER, J. F. 1997. Inheritance of the stay green trait in sunflower. *Crop science,* 37**,** 150-153.

CULLIS, B. R., SMITH, A. B. & COOMBES, N. E. 2006. On the design of early generation variety trials with correlated data. *Journal of agricultural, biological, and environmental statistics,* 11**,** 381-393.

DARROCH, B. & BAKER, R. 1990. Grain filling in three spring wheat genotypes: statistical analysis. *Crop science,* 30**,** 525-529.

DAWSON, I. & WARDLAW, I. 1989. The tolerance of wheat to high temperatures during reproductive growth. III. Booting to anthesis. *Crop and Pasture Science,* 40**,** 965-980.

DE VRIES, F., BRUNSTING, A. & VAN LAAR, H. 1974. Products, requirements and efficiency of biosynthesis a quantitative approach. *Journal of Theoretical Biology,* 45**,** 339-377.

DERKX, A. P., ORFORD, S., GRIFFITHS, S., FOULKES, M. J. & HAWKESFORD, M. J. 2012. Identification of Differentially Senescing Mutants of Wheat and Impacts on Yield, Biomass and Nitrogen PartitioningF. *Journal of integrative plant biology,* 54**,** 555-566.

DÍAZ, A., ZIKHALI, M., TURNER, A. S., ISAAC, P. & LAURIE, D. A. 2012. Copy number variation affecting the Photoperiod-B1 and Vernalization-A1 genes is associated with altered flowering time in wheat (Triticum aestivum). *Plos One,* 7**,** e33234.

DISTELFELD, A., AVNI, R. & FISCHER, A. M. 2014. Senescence, nutrient remobilization, and yield in wheat and barley. *Journal of Experimental Botany***,** ert477.

DORION, S., LALONDE, S. & SAINI, H. S. 1996. Induction of male sterility in wheat by meiotic-stage water deficit is preceded by a decline in invertase activity and changes in carbohydrate metabolism in anthers. *Plant Physiology,* 111**,** 137-145.

DOWLER, K. 2004. Scorcher slashes 'bin buster'. *Stock J. viewed 7th July 2011,* http://sj.farmonline.com.au/news/state/grains-and-cropping/general/scorcher-slashes-bin-buster/37144.aspx.

DRECCER, M. F., WOCKNER, K. B., PALTA, J. A., MCINTYRE, C. L., BORGOGNONE, M. G., BOURGAULT, M., REYNOLDS, M. & MIRALLES, D. J. 2014. More fertile florets and grains per spike can be achieved at higher temperature in wheat lines with high spike biomass and sugar content at booting. *Functional Plant Biology,* 41**,** 482-495.

EAGLES, H. A., CANE, K., KUCHEL, H., HOLLAMBY, G. J., VALLANCE, N., EASTWOOD, R. F., GORORO, N. & MARTIN, P. 2010. Photoperiod and vernalization gene effects in southern Australian wheat. *Crop and Pasture Science,* 61**,** 721-730.

EDWARDS, M. D., STUBER, C. W. & WENDEL, J. F. 1987. Molecular-Marker-Facilitated Investigations of Quantitative-Trait Loci in Maize. I. Numbers, Genomic Distribution and Types of Gene Action. *Genetics,* 116**,** 113-125.

EHDAIE, B., ALLOUSH, G., MADORE, M. & WAINES, J. 2006. Genotypic variation for stem reserves and mobilization in wheat. *Crop science,* 46**,** 2093-2103.

EHDAIE, B., ALLOUSH, G. & WAINES, J. 2008. Genotypic variation in linear rate of grain growth and contribution of stem reserves to grain yield in wheat. *Field Crops Research,* 106**,** 34-43.

ELLIS, M. H., BONNETT, D. G. & REBETZKE, G. J. 2007. A 192bp allele at the Xgwm261 locus is not always associated with the Rht8 dwarfing gene in wheat (Triticum aestivum L.). *Euphytica,* 157**,** 209-214.

EMEBIRI, L., OLIVER, J., MRVA, K. & MARES, D. 2010. Association mapping of late maturity α-amylase (LMA) activity in a collection of synthetic hexaploid wheat. *Molecular Breeding,* 26**,** 39-49.

ENDO, M., TSUCHIYA, T., HAMADA, K., KAWAMURA, S., YANO, K., OHSHIMA, M., HIGASHITANI, A., WATANABE, M. & KAWAGISHI-KOBAYASHI, M. 2009. High temperatures cause male

sterility in rice plants with transcriptional alterations during pollen development. *Plant and Cell Physiology,* 50**,** 1911.
ESSEMINE, J., GOVINDACHARY, S., AMMAR, S., BOUZID, S. & CARPENTIER, R. 2012. Enhanced sensitivity of the photosynthetic apparatus to heat stress in digalactosyl-diacylglycerol deficient Arabidopsis. *Environmental and Experimental Botany,* 80**,** 16-26.
EVANS, L. T. 1975. *Crop physiology: some case histories,* CUP Archive.
FAOSTAT 2012. Food and Agricultural commodities production. *viewed 30 September 2013,* http://faostat.fao.org/.
FARIS, J., LADDOMADA, B. & GILL, B. 1998. Molecular mapping of segregation distortion loci in Aegilops tauschii. *Genetics,* 149**,** 319-327.
FAROOQ, M., BRAMLEY, H., PALTA, J. A. & SIDDIQUE, K. H. M. 2011. Heat Stress in Wheat during Reproductive and Grain-Filling Phases. *Critical Reviews in Plant Sciences,* 30**,** 491-507.
FISCHER, R. 1985a. Number of kernels in wheat crops and the influence of solar radiation and temperature. *Journal of Agricultural Science,* 105**,** 447-461.
FISCHER, R. 1985b. Physiological limitations to producing wheat in semitropical and tropical environments and possible selection criteria. *Wheats for more tropical environments. A proceedings of the international symposium, September 24-28, 1984 Mexico DF***,** 209-230.
FISCHER, R. & MAURER, R. 1978. Drought resistance in spring wheat cultivars. I. Grain yield responses. *Crop and Pasture Science,* 29**,** 897-912.
FISCHER, R. A. & MAURER, R. 1976. Crop temperature modification and yield potential in a dwarf spring wheat. *Crop science,* 16**,** 855-859.
FLINT-GARCIA, S. A., THORNSBERRY, J. M. & IV, B. 2003. Structure of Linkage Disequilibrium in Plants*. *Annual Review of Plant Biology,* 54**,** 357-374.
FOKAR, M., NGUYEN, H. T. & BLUM, A. 1998. Heat tolerance in spring wheat. I. Estimating cellular thermotolerance and its heritability. *Euphytica,* 104**,** 1-8.
FOULKES, M., SCOTT, R. & SYLVESTER-BRADLEY, R. 2002. The ability of wheat cultivars to withstand drought in UK conditions: formation of grain yield. *The Journal of Agricultural Science,* 138**,** 153-169.
FRANCKI, M. G., WALKER, E., CRAWFORD, A. C., BROUGHTON, S., OHM, H. W., BARCLAY, I., WILSON, R. E. & MCLEAN, R. 2009. Comparison of genetic and cytogenetic maps of hexaploid wheat (Triticum aestivum L.) using SSR and DArT markers. *Molecular Genetics and Genomics,* 281**,** 181-191.
FREDERICK, J. R. & CAMBERATO, J. J. 1995. Water and nitrogen effects on winter wheat in the southeastern Coastal Plain: I. Grain yield and kernel traits. *Agronomy Journal,* 87**,** 521-526.
FU, D., SZŰCS, P., YAN, L., HELGUERA, M., SKINNER, J. S., VON ZITZEWITZ, J., HAYES, P. M. & DUBCOVSKY, J. 2005. Large deletions within the first intron in VRN-1 are associated with spring growth habit in barley and wheat. *Molecular Genetics and Genomics,* 273**,** 54-65.
FURBANK, R. T. & TESTER, M. 2011. Phenomics–technologies to relieve the phenotyping bottleneck. *Trends in plant science,* 16**,** 635-644.
GALE, M. D., YOUSSEFIAN, S. & RUSSELL, G. 1985. Dwarfing genes in wheat. *Progress in plant breeding-1***,** 1-35.
GEBEYEHOU, G., KNOTT, D. & BAKER, R. 1982. Rate and duration of grain filling in durum wheat cultivars. *Crop science,* 22**,** 337-340.
GEORGIEVA, K. 1999. Some mechanisms of damage and acclimation of the photosynthetic apparatus due to high temperature. *Bulg. J. Plant Physiol,* 25**,** 89-99.
GOLZARIAN, M. R., FRICK, R. A., RAJENDRAN, K., BERGER, B., ROY, S., TESTER, M. & LUN, D. S. 2011. Accurate inference of shoot biomass from high-throughput images of cereal plants. *Plant Methods,* 7**,** 1-11.
GORNY, A. G. & GARCZYNSKI, S. 2002. Genotypic and nutrition-dependent variation in water use efficiency and photosynthetic activity of leaves in winter wheat (Triticum aestivum L.). *Journal of applied genetics,* 43**,** 145-160.
GRAYBOSCH, R., PETERSON, C., BAENZIGER, P. & SHELTON, D. 1995. Environmental modification of hard red winter wheat flour protein composition. *Journal of Cereal Science,* 22**,** 45-51.
GROOS, C., ROBERT, N., BERVAS, E. & CHARMET, G. 2003. Genetic analysis of grain protein-content, grain yield and thousand-kernel weight in bread wheat. *Theoretical and Applied Genetics,* 106**,** 1032-1040.
GUPTA, P., MIR, R., MOHAN, A. & KUMAR, J. 2008. Wheat genomics: present status and future prospects. *International journal of plant genomics,* 2008**,** 896451_1-896451_36.
GUPTA, P. K., RUSTGI, S. & KULWAL, P. L. 2005. Linkage disequilibrium and association studies in higher plants: present status and future prospects. *Plant Molecular Biology,* 57**,** 461-485.
GUTIÉRREZ-RODRIGUEZ, M., REYNOLDS, M. & LARQUE-SAAVEDRA, A. 2000. Photosynthesis of wheat in a warm, irrigated environment: II. Traits associated with genetic gains in yield. *Field Crops Research,* 66**,** 51-62.
HACKETT, C. & BROADFOOT, L. 2003. Effects of genotyping errors, missing values and segregation distortion in molecular marker data on the construction of linkage maps. *Heredity,* 90**,** 33-38.

HANOCQ, E., LAPERCHE, A., JAMINON, O., LAINÉ, A.-L. & LE GOUIS, J. 2007. Most significant genome regions involved in the control of earliness traits in bread wheat, as revealed by QTL meta-analysis. *Theoretical and Applied Genetics,* 114**,** 569-584.

HANSEN, J., SATO, M. & RUEDY, R. 2012. Perception of climate change. *Proceedings of the National Academy of Sciences,* 109**,** E2415-E2423.

HAQUE, M. S., KJAER, K. H., ROSENQVIST, E., SHARMA, D. K. & OTTOSEN, C. O. 2014. Heat stress and recovery of photosystem II efficiency in wheat (Triticum aestivum L.) cultivars acclimated to different growth temperatures. *Environmental and Experimental Botany,* 99**,** 1-8.

HAWKER, J. & JENNER, C. 1993. High temperature affects the activity of enzymes in the committed pathway of starch synthesis in developing wheat endosperm. *Functional Plant Biology,* 20**,** 197-209.

HAYES, J. E., PALLOTTA, M., BAUMANN, U., BERGER, B., LANGRIDGE, P. & SUTTON, T. 2013. Germanium as a tool to dissect boron toxicity effects in barley and wheat. *Functional Plant Biology,* 40**,** 618-627.

HAYS, D. B., DO, J. H., MASON, R. E., MORGAN, G. & FINLAYSON, S. A. 2007. Heat stress induced ethylene production in developing wheat grains induces kernel abortion and increased maturation in a susceptible cultivar. *Plant Science,* 172**,** 1113-1123.

HIREL, B., LE GOUIS, J., NEY, B. & GALLAIS, A. 2007. The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *Journal of Experimental Botany,* 58**,** 2369-2387.

HISCOX, J. T. & ISRAELSTAM, G. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany,* 57**,** 1332-1334.

HONG, S.-W., LEE, U. & VIERLING, E. 2003. Arabidopsis hot mutants define multiple functions required for acclimation to high temperatures. *Plant Physiology,* 132**,** 757-767.

HONSDORF, N., MARCH, T. J., BERGER, B., TESTER, M. & PILLEN, K. 2014. High-Throughput Phenotyping to Detect Drought Tolerance QTL in Wild Barley Introgression Lines. *Plos One,* 9**,** e97047.

HOSOI, F. & OMASA, K. 2009. Estimating vertical plant area density profile and growth parameters of a wheat canopy at different growth stages using three-dimensional portable lidar imaging. *ISPRS Journal of Photogrammetry and Remote Sensing,* 64**,** 151-158.

HOSSAIN, A., SEARS, R., COX, T. S. & PAULSEN, G. 1990. Desiccation tolerance and its relationship to assimilate partitioning in winter wheat. *Crop science,* 30**,** 622-627.

HUANG, B., JOHNSON, J. W., NESMITH, D. S. & BRIDGES, D. C. 1994. Root and shoot growth of wheat genotypes in response to hypoxia and subsequent resumption of aeration. *Crop science,* 34**,** 1538-1544.

HUANG, B. E., GEORGE, A. W., FORREST, K. L., KILIAN, A., HAYDEN, M. J., MORELL, M. K. & CAVANAGH, C. R. 2012. A multiparent advanced generation inter-cross population for genetic analysis in wheat. *Plant Biotechnology Journal,* 10**,** 826-839.

HUNT, L., VAN DER POORTEN, G. & PARARAJASINGHAM, S. 1991. Postanthesis temperature effects on duration and rate of grain filling in some winter and spring wheats. *Canadian Journal of Plant Science,* 71**,** 609-617.

HURKMAN, W. J., MCCUE, K. F., ALTENBACH, S. B., KORN, A., TANAKA, C. K., KOTHARI, K. M., JOHNSON, E. L., BECHTEL, D. B., WILSON, J. D. & ANDERSON, O. D. 2003. Effect of temperature on expression of genes encoding enzymes for starch biosynthesis in developing wheat endosperm. *Plant Science,* 164**,** 873-881.

IPCC 2007. Intergovernmental Panel on Climate Change fourth assessment report: Climate change 2007. Synthesis Report. *World Meteorological Organization, Geneva, Switzerland.*

IRMAK, S., NAEEM, H. A., LOOKHART, G. L. & 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.

JENNER, C. 1991a. Effects of exposure of wheat ears to high temperature on dry matter accumulation and carbohydrate metabolism in the grain of two cultivars. I. Immediate responses. *Functional Plant Biology,* 18**,** 165-177.

JENNER, C. 1991b. Effects of exposure of wheat ears to high temperature on dry matter accumulation and carbohydrate metabolism in the grain of two cultivars. II. Carry-over effects. *Functional Plant Biology,* 18**,** 179-190.

JENNER, C. 1994. Starch synthesis in the kernel of wheat under high temperature conditions. *Functional Plant Biology,* 21**,** 791-806.

JENNER, C. & HAWKER, J. 1993. Sink strength: soluble starch synthase as a measure of sink strength in wheat endosperm. *Plant, cell & environment,* 16**,** 1023-1024.

JOSHI, A., KUMARI, M., SINGH, V., REDDY, C., KUMAR, S., RANE, J. & CHAND, R. 2007. Stay green trait: variation, inheritance and its association with spot blotch resistance in spring wheat (Triticum aestivum L.). *Euphytica,* 153**,** 59-71.

KAMMHOLZ, S. J., CAMPBELL, A. W., SUTHERLAND, M. W., HOLLAMBY, G., MARTIN, P., EASTWOOD, R., BARCLAY, I., WILSON, R., BRENNAN, P. S. & SHEPPARD, J. 2001.

Establishment and characterisation of wheat genetic mapping populations. *Crop and Pasture Science,* 52**,** 1079-1088.
KANE, N. A., AGHARBAOUI, Z., DIALLO, A. O., ADAM, H., TOMINAGA, Y., OUELLET, F. & SARHAN, F. 2007. TaVRT2 represses transcription of the wheat vernalization gene TaVRN1. *The Plant Journal,* 51**,** 670-680.
KANE, N. A., DANYLUK, J., TARDIF, G., OUELLET, F., LALIBERTÉ, J.-F., LIMIN, A. E., FOWLER, D. B. & SARHAN, F. 2005. TaVRT-2, a member of the StMADS-11 clade of flowering repressors, is regulated by vernalization and photoperiod in wheat. *Plant Physiology,* 138**,** 2354-2363.
KAO, C.-H., ZENG, Z.-B. & TEASDALE, R. D. 1999. Multiple interval mapping for quantitative trait loci. *Genetics,* 152**,** 1203-1216.
KEELING, P., BACON, P. & HOLT, D. 1993. Elevated temperature reduces starch deposition in wheat endosperm by reducing the activity of soluble starch synthase. *Planta,* 191**,** 342-348.
KEELING, P., BANISADR, R., BARONE, L., WASSERMAN, B. & SINGLETARY, G. 1994. Effect of temperature on enzymes in the pathway of starch biosynthesis in developing wheat and maize grain. *Functional Plant Biology,* 21**,** 807-827.
KEELING, P. L. & MYERS, A. M. 2010. Biochemistry and genetics of starch synthesis. *Annual Review of Food Science and Technology,* 1**,** 271-303.
KHAN, N. A. 2006. *Ethylene action in plants,* Springer.
KICHEY, T., HIREL, B., HEUMEZ, E., DUBOIS, F. & LE GOUIS, J. 2007. In winter wheat (Triticum aestivum L.), post-anthesis nitrogen uptake and remobilisation to the grain correlates with agronomic traits and nitrogen physiological markers. *Field Crops Research,* 102**,** 22-32.
KIPP, S., MISTELE, B. & SCHMIDHALTER, U. 2014. Identification of stay-green and early senescence phenotypes in high-yielding winter wheat, and their relationship to grain yield and grain protein concentration using high-throughput phenotyping techniques. *Functional Plant Biology,* 41**,** 227-235.
KORZUN, V., RÖDER, M., GANAL, M., WORLAND, A. & LAW, C. 1998. Genetic analysis of the dwarfing gene (Rht8) in wheat. Part I. Molecular mapping of Rht8 on the short arm of chromosome 2D of bread wheat (Triticum aestivum L.). *Theoretical and Applied Genetics,* 96**,** 1104-1109.
KOSAMBI, D. 1943. The estimation of map distances from recombination values. *Annals of Eugenics,* 12**,** 172-175.
KUCHEL, H., HOLLAMBY, G., LANGRIDGE, P., WILLIAMS, K. & JEFFERIES, S. P. 2006. Identification of genetic loci associated with ear-emergence in bread wheat. *Theoretical and Applied Genetics,* 113**,** 1103-1112.
KUCHEL, H., WILLIAMS, K., LANGRIDGE, P., EAGLES, H. A. & JEFFERIES, S. P. 2007. Genetic dissection of grain yield in bread wheat. II. QTL-by-environment interaction. *TAG Theoretical and Applied Genetics,* 115**,** 1015-1027.
KUMAR, S., GILL, B. S. & FARIS, J. D. 2007. Identification and characterization of segregation distortion loci along chromosome 5B in tetraploid wheat. *Molecular Genetics and Genomics,* 278**,** 187-196.
KUMAR, S., KUMARI, P., KUMAR, U., GROVER, M., SINGH, A. K., SINGH, R. & SENGAR, R. 2013. Molecular approaches for designing heat tolerant wheat. *Journal of Plant Biochemistry and Biotechnology***,** 1-13.
KUMAR, U., JOSHI, A. K., KUMARI, M., PALIWAL, R., KUMAR, S. & RÖDER, M. S. 2010. Identification of QTLs for stay green trait in wheat (Triticum aestivum L.) in the 'Chirya 3'×'Sonalika'population. *Euphytica,* 174**,** 437-445.
KUMARI, M., PUDAKE, R., SINGH, V. & JOSHI, A. K. 2013. Association of staygreen trait with canopy temperature depression and yield traits under terminal heat stress in wheat (Triticum aestivum L.). *Euphytica,* 190**,** 87-97.
KUMARI, M., SINGH, V., TRIPATHI, R. & JOSHI, A. 2007. Variation for staygreen trait and its association with canopy temperature depression and yield traits under terminal heat stress in wheat. *Wheat Production in Stressed Environments.* Springer.
LANDER, E. S. & BOTSTEIN, D. 1989. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics,* 121**,** 185-99.
LANGRIDGE, P., LAGUDAH, E. S., HOLTON, T., APPELS, R., SHARP, P. & CHALMERS, K. J. 2001. Trends in genetic and genome analyses in wheat: a review. *Crop and Pasture Science,* 52**,** 1043-1077.
LAW, C. & WORLAND, A. 1985. An effect of temperature on the fertility of wheats containing dwarfing genes Rht1, Rht2 and Rht3. *Annual Report of the Plant Breeding Institute 1984***,** 69-71.
LAW, C. N., SNAPE, J. W. & A. J. WORLAND 1981. Reduced Fertility of Wheat Associated With Rht3. *Plant Breeding Institute Annual Report 1980. Plant Breeding Institute, Cambridge.***,** 72–73.
LÊ, S., JOSSE, J. & HUSSON, F. 2008. FactoMineR: An R package for multivariate analysis. *Journal of statistical software,* 25**,** 1-18.
LEE, T., JANG, C., KIM, J., SEONG, R., KIM, I., KIM, D. & SEO, Y. 2007. Expressed sequence tags from wheat roots under hypoxia. *Russian journal of plant physiology,* 54**,** 659-668.
LI, Z., MOUILLE, G., KOSAR-HASHEMI, B., RAHMAN, S., CLARKE, B., GALE, K. R., APPELS, R. & MORELL, M. K. 2000. The structure and expression of the wheat starch synthase III gene. Motifs in the expressed gene define the lineage of the starch synthase III gene family. *Plant Physiology,* 123**,** 613.

LIEW, M., PRYOR, R., PALAIS, R., MEADOWS, C., ERALI, M., LYON, E. & WITTWER, C. 2004. Genotyping of single-nucleotide polymorphisms by high-resolution melting of small amplicons. *Clinical Chemistry,* 50**,** 1156-1164.

LOPES, M. S. & REYNOLDS, M. P. 2012. Stay-green in spring wheat can be determined by spectral reflectance measurements (normalized difference vegetation index) independently from phenology. *Journal of Experimental Botany,* 63**,** 3789-3798.

LOSS, S., KIRBY, E., SIDDIQUE, K. & PERRY, M. 1989. Grain growth and development of old and modern Australian wheats. *Field Crops Research,* 21**,** 131-146.

MACCAFERRI, M., SANGUINETI, M. C., CORNETI, S., ORTEGA, J. L. A., SALEM, M. B., BORT, J., DEAMBROGIO, E., DEL MORAL, L. F. G., DEMONTIS, A. & EL-AHMED, A. 2008. Quantitative trait loci for grain yield and adaptation of durum wheat (Triticum durum Desf.) across a wide range of water availability. *Genetics,* 178**,** 489-511.

MALIK, A. I., COLMER, T. D., LAMBERS, H. & SCHORTEMEYER, M. 2001. Changes in physiological and morphological traits of roots and shoots of wheat in response to different depths of waterlogging. *Functional Plant Biology,* 28**,** 1121-1131.

MANLY, K. F., CUDMORE, J. R. H. & MEER, J. M. 2001. Map Manager QTX, cross-platform software for genetic mapping. *Mammalian Genome,* 12**,** 930-932.

MANTOVANI, P., MACCAFERRI, M., SANGUINETI, M. C., TUBEROSA, R., CATIZONE, I., WENZL, P., THOMSON, B., CARLING, J., HUTTNER, E. & DEAMBROGIO, E. 2008. An integrated DArT-SSR linkage map of durum wheat. *Molecular Breeding,* 22**,** 629-648.

MAPHOSA, L., COLLINS, N., TAYLOR, J. & MATHER, D. 2014. Post-anthesis heat and a Gpc-B1 introgression have similar but non-additive effects in bread wheat. *Functional Plant Biology*.

MARTÍNEZ-BALLESTA, M. C., LÓPEZ-PÉREZ, L., MURIES, B., MUÑOZ-AZCARATE, O. & CARVAJAL, M. 2009. Climate change and plant water balance: the role of aquaporins–a review. *Climate Change, Intercropping, Pest Control and Beneficial Microorganisms.* Springer.

MASON, R. E., HAYS, D. B., MONDAL, S., IBRAHIM, A. M. & BASNET, B. R. 2013. QTL for yield, yield components and canopy temperature depression in wheat under late sown field conditions. *Euphytica,* 194**,** 243-259.

MASON, R. E., MONDAL, S., BEECHER, F. W. & HAYS, D. B. 2011. Genetic loci linking improved heat tolerance in wheat (Triticum aestivum L.) to lower leaf and spike temperatures under controlled conditions. *Euphytica,* 180**,** 181-194.

MASON, R. E., MONDAL, S., BEECHER, F. W., PACHECO, A., JAMPALA, B., IBRAHIM, A. M. H. & HAYS, D. B. 2010. QTL associated with heat susceptibility index in wheat (Triticum aestivum L.) under short-term reproductive stage heat stress. *Euphytica,* 174**,** 423-436.

MATTHEWS, P., MCCAFFERY, D. & JENKINS, L. 2013. Winter crop variety sowing guide 2013. *Department of Primary Industries, NSW.*

MATTHEWS, P., MCCAFFERY, D. & JENKINS, L. 2015. Winter crop variety sowing guide 2015. *Department of Primary Industries, NSW.*

MAYER, K. F., ROGERS, J., DOLEŽEL, J., POZNIAK, C., EVERSOLE, K., FEUILLET, C., GILL, B., FRIEBE, B., LUKASZEWSKI, A. J. & SOURDILLE, P. 2014. A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. *Science,* 345**,** 1251788.

MCDONALD, G., SUTTON, B. & ELLISON, F. 1983. The effects of time of sowing on the grain yield of irrigated wheat in Namoi Valley, New South Wales. *Aust. J. Agric. Res,* 34**,** 224–229.

MCMULLEN, M. D., KRESOVICH, S., VILLEDA, H. S., BRADBURY, P., LI, H., SUN, Q., FLINT-GARCIA, S., THORNSBERRY, J., ACHARYA, C. & BOTTOMS, C. 2009. Genetic properties of the maize nested association mapping population. *Science,* 325**,** 737-740.

MINOLTA 1989. Chlorophyll meter SPAD-502. *Instruction manual. Minolta Co., Ltd., Radiometric Instruments Operations. Osaka, Japan.*

MOFFATT, J., SEARS, R. & PAULSEN, G. 1990. Wheat high temperature tolerance during reproductive growth. I. Evaluation by chlorophyll fluorescence. *Crop science,* 30**,** 881-885.

MOHAMMADI, V., MODARRESI, M. & BYRNE, P. 2008a. Detection of QTLs for heat tolerance in wheat measured by grain filling duration. *In Proc:11th International Wheat Genetics Symposium. Brisbane. Australia***,** 962.

MOHAMMADI, V., ZALI, A. & BIHAMTA, M. 2008b. Mapping QTLs for heat tolerance in wheat. *Journal of Agricultural Science and Technology,* 10**,** 261-267.

MORALES, D., RODRÍGUEZ, P., DELL'AMICO, J., NICOLAS, E., TORRECILLAS, A. & SÁNCHEZ-BLANCO, M. J. 2003. High-temperature preconditioning and thermal shock imposition affects water relations, gas exchange and root hydraulic conductivity in tomato. *Biologia Plantarum,* 47**,** 203-208.

MUFTI, M. U. 2005. *Screening wheat seedlings for heat and drought tolerance by in vivo chlorophyll fluorescence.* MSc thesis, The University of Adelaide, Adelaide.

MUNNS, R. & TESTER, M. 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.,* 59**,** 651-681.

NARUOKA, Y., SHERMAN, J., LANNING, S., BLAKE, N., MARTIN, J. & TALBERT, L. 2012. Genetic analysis of green leaf duration in spring wheat. *Crop science,* 52**,** 99-109.

NELSON, J. 1997. QGENE: software for marker-based genomic analysis and breeding. *Molecular Breeding,* 3**,** 239-245.
NELSON, J. C., ANDREESCU, C., BRESEGHELLO, F., FINNEY, P. L., GUALBERTO, D. G., BERGMAN, C. J., PEÑA, R. J., PERRETANT, M. R., LEROY, P. & QUALSET, C. O. 2006. Quantitative trait locus analysis of wheat quality traits. *Euphytica,* 149**,** 145-159.
NEUMANN, K., KOBILJSKI, B., DENČIĆ, S., VARSHNEY, R. & BÖRNER, A. 2011. Genome-wide association mapping: a case study in bread wheat (Triticum aestivum L.). *Molecular Breeding,* 27**,** 37-58.
NICOLAS, M. E., GLEADOW, R. M. & DALLING, M. J. 1984. Effects of drought and high temperature on grain growth in wheat. *Functional Plant Biology,* 11**,** 553-566.
OAKEY, H., VERBYLA, A., PITCHFORD, W., CULLIS, B. & KUCHEL, H. 2006. Joint modeling of additive and non-additive genetic line effects in single field trials. *Theoretical and Applied Genetics,* 113**,** 809-819.
ORTIZ, R., SAYRE, K. D., GOVAERTS, B., GUPTA, R., SUBBARAO, G., BAN, T., HODSON, D., DIXON, J. M. & IVÁN, O. M. 2008. Climate change: Can wheat beat the heat? *Agriculture, Ecosystems & Environment,* 126**,** 46-58.
PAILLARD, S., SCHNURBUSCH, T., WINZELER, M., MESSMER, M., SOURDILLE, P., ABDERHALDEN, O., KELLER, B. & SCHACHERMAYR, G. 2003. An integrative genetic linkage map of winter wheat (Triticum aestivum L.). *Theoretical and Applied Genetics,* 107**,** 1235-1242.
PALIWAL, R., RÖDER, M. S., KUMAR, U., SRIVASTAVA, J. & JOSHI, A. K. 2012. QTL mapping of terminal heat tolerance in hexaploid wheat (T. aestivum L.). *Theoretical and Applied Genetics,* 125**,** 561-575.
PALLOTTA, M. A., GRAHAM, R. D., LANGRIDGE, P., SPARROW, D. & BARKER, S. J. 2000. RFLP mapping of manganese efficiency in barley. *Theoretical and Applied Genetics,* 101**,** 1100-1108.
PARENT, B. & TARDIEU, F. 2012. Temperature responses of developmental processes have not been affected by breeding in different ecological areas for 17 crop species. *New Phytologist,* 194**,** 760-774.
PARENT, B., TURC, O., GIBON, Y., STITT, M. & TARDIEU, F. 2010. Modelling temperature-compensated physiological rates, based on the co-ordination of responses to temperature of developmental processes. *Journal of Experimental Botany,* 61**,** 2057.
PASSIOURA, J. B. 2006. Viewpoint: The perils of pot experiments. *Functional Plant Biology,* 33**,** 1075-1079.
PATNAIK, D. & KHURANA, P. 2001. Wheat biotechnology: A minireview. *Electronic Journal of Biotechnology,* 4**,** 7-8.
PAYNE, P. I. 1987. Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. *Annual Review of Plant Physiology,* 38**,** 141-153.
PAYNE, R. W. 2009. GenStat. *Wiley Interdisciplinary Reviews: Computational Statistics,* 1**,** 255-258.
PECK, A. W. & MCDONALD, G. K. 2010. Adequate zinc nutrition alleviates the adverse effects of heat stress in bread wheat. *Plant and soil,* 337**,** 355-374.
PELEG, Z., SARANGA, Y., SUPRUNOVA, T., RONIN, Y., RÖDER, M. S., KILIAN, A., KOROL, A. B. & FAHIMA, T. 2008. High-density genetic map of durum wheat× wild emmer wheat based on SSR and DArT markers. *Theoretical and Applied Genetics,* 117**,** 103-115.
PENG, J., KOROL, A. B., FAHIMA, T., RÖDER, M. S., RONIN, Y. I., LI, Y. C. & NEVO, E. 2000. Molecular genetic maps in wild emmer wheat, Triticum dicoccoides: genome-wide coverage, massive negative interference, and putative quasi-linkage. *Genome Research,* 10**,** 1509-1531.
PENGELLY, J. J., SIRAULT, X. R., TAZOE, Y., EVANS, J. R., FURBANK, R. T. & VON CAEMMERER, S. 2010. Growth of the C4 dicot Flaveria bidentis: photosynthetic acclimation to low light through shifts in leaf anatomy and biochemistry. *Journal of Experimental Botany,* 61**,** 4109-4122.
PERCIVAL, J. 1921. The wheat plant: a monograph. Duckworth, London.
PHILLIPS, D. A., PIERCE, R. O., EDIE, S. A., FOSTER, K. A. & KNOWLES, P. F. 1984. Delayed leaf senescence in soybean. *Crop science,* 24**,** 518-522.
PINTO, R. S., REYNOLDS, M. P., MATHEWS, K. L., MCINTYRE, C. L., OLIVARES-VILLEGAS, J.-J. & CHAPMAN, S. C. 2010. Heat and drought adaptive QTL in a wheat population designed to minimize confounding agronomic effects. *Theoretical and Applied Genetics,* 121**,** 1001-1021.
PITTOCK, A. B. 2003. *Climate change: an Australian guide to the science and potential impacts*, Australian Greenhouse Office Canberra.
POLAND, J. A., BROWN, P. J., SORRELLS, M. E. & JANNINK, J.-L. 2012. Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. *Plos One,* 7**,** e32253.
PRASAD, P., STAGGENBORG, S. & RISTIC, Z. 2008. Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. *Response of crops to limited water: Understanding and modeling water stress effects on plant growth processes***,** 301-355.
PRATT, H. K. & GOESCHL, J. 1969. Physiological roles of ethylene in plants. *Annual Review of Plant Physiology,* 20**,** 541-584.

QIN, D., WU, H., PENG, H., YAO, Y., NI, Z., LI, Z., ZHOU, C. & SUN, Q. 2008. Heat stress-responsive transcriptome analysis in heat susceptible and tolerant wheat (Triticum aestivum L.) by using Wheat Genome Array. *BMC genomics,* 9**,** 432.

QUARRIE, S., STEED, A., CALESTANI, C., SEMIKHODSKII, A., LEBRETON, C., CHINOY, C., STEELE, N., PLJEVLJAKUSIĆ, D., WATERMAN, E. & WEYEN, J. 2005. A high-density genetic map of hexaploid wheat (Triticum aestivum L.) from the cross Chinese Spring× SQ1 and its use to compare QTLs for grain yield across a range of environments. *Theoretical and Applied Genetics,* 110**,** 865-880.

R DEVELOPMENT CORE TEAM 2014. R: A Language and Environment for Statistical Computing. *Vienna, Austria: R Foundation for Statistical Computing. ISBN 3-900051-07-0*.

RADFORD, B. J. 1987. Effect of constant and fluctuating temperature regimes and seed source on the coleoptile length of tall and semidwarf wheats. *Australian Journal of Experimental Agriculture,* 27**,** 113-117.

RAHMAN, M. & WILSON, J. 1978. Determination of spikelet number in wheat. III. Effect of varying temperature on ear development. *Aust. J. Agric. Res,* 29**,** 459-467.

RAHMAN, M., WILSON, J. & AITKEN, Y. 1977. Determination of spikelet number in wheat. II. Effect of varying light level on ear development. *Australian Journal of Agricultural Research,* 28**,** 575-581.

RAHMAN, M. A., CHIKUSHI, J., YOSHIDA, S. & KARIM, A. 2009. Growth and yield components of wheat genotypes exposed to high temperature stress under control environment. *Bangladesh Journal of Agricultural Research,* 34**,** 360-372.

RAJENDRAN, K., TESTER, M. & ROY, S. J. 2009. Quantifying the three main components of salinity tolerance in cereals. *Plant, cell & environment,* 32**,** 237-249.

RAMPINO, P., SPANO, G., PATALEO, S., MITA, G., NAPIER, J. A., DI FONZO, N., SHEWRY, P. R. & PERROTTA, C. 2006. Molecular analysis of a durum wheat 'stay green'mutant: expression pattern of photosynthesis-related genes. *Journal of Cereal Science,* 43**,** 160-168.

RANDALL, P. & MOSS, H. 1990. Some effects of temperature regime during grain filling on wheat quality. *Australian Journal of Agricultural Research,* 41**,** 603-617.

RANE, J. & NAGARAJAN, S. 2004. High temperature index—for field evaluation of heat tolerance in wheat varieties. *Agricultural Systems,* 79**,** 243-255.

REBETZKE, G., CONDON, A., RICHARDS, R. & FARQUHAR, G. 2002. Selection for reduced carbon isotope discrimination increases aerial biomass and grain yield of rainfed bread wheat. *Crop science,* 42**,** 739-745.

REVELLE, W. 2011. Psych: Procedures for psychological, psychometric, and personality research. *R package version,* 1019.

REYNOLDS, M., BALOTA, M., DELGADO, M., AMANI, I. & FISCHER, R. 1994. Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. *Functional Plant Biology,* 21**,** 717-730.

REYNOLDS, M., DELGADO B, M., GUTIÉRREZ-RODRíGUEZ, M. & LARQUÉ-SAAVEDRA, A. 2000. Photosynthesis of wheat in a warm, irrigated environment: I: Genetic diversity and crop productivity. *Field Crops Research,* 66**,** 37-50.

REYNOLDS, M., NAGARAJAN, S., RAZZAQUE, M. & AGEEB, O. 2001. Heat tolerance. *Application of physiology in wheat breeding***,** 124-135.

REYNOLDS, M., PASK, A. & MULLAN, D. 2012. *Physiological breeding I: Interdisciplinary approaches to improve crop adaptation*, CIMMYT.

REYNOLDS, M., SINGH, R., IBRAHIM, A., AGEEB, O., LARQUÉ-SAAVEDRA, A. & QUICK, J. 1998. Evaluating physiological traits to complement empirical selection for wheat in warm environments. *Euphytica,* 100**,** 85-94.

RICHARDS, R. A. 2006. Physiological traits used in the breeding of new cultivars for water-scarce environments. *Agricultural Water Management,* 80**,** 197-211.

RICHARDSON, A. D., DUIGAN, S. P. & BERLYN, G. P. 2002. An evaluation of noninvasive methods to estimate foliar chlorophyll content. *New Phytologist,* 153**,** 185-194.

RISTIC, Z., BUKOVNIK, U., PRASAD, P. & WEST, M. 2008. A model for prediction of heat stability of photosynthetic membranes. *Crop science,* 48**,** 1513-1522.

RISTIC, Z., BUKOVNIK, U. & PRASAD, P. V. 2007. Correlation between heat stability of thylakoid membranes and loss of chlorophyll in winter wheat under heat stress. *Crop science,* 47**,** 2067-2073.

RÖDER, M. S., KORZUN, V., WENDEHAKE, K., PLASCHKE, J., TIXIER, M.-H., LEROY, P. & GANAL, M. W. 1998. A microsatellite map of wheat. *Genetics,* 149**,** 2007-2023.

ROGOWSKY, P., GUIDET, F., LANGRIDGE, P., SHEPHERD, K. & KOEBNER, R. 1991. Isolation and characterization of wheat-rye recombinants involving chromosome arm 1DS of wheat. *Theoretical and Applied Genetics,* 82**,** 537-544.

ROSIELLE, A. & HAMBLIN, J. 1981. Theoretical aspects of selection for yield in stress and non-stress environment. *Crop science,* 21**,** 943-946.

ROSYARA, U., KHADKA, K., SUBEDI, S., SHARMA, R. & DUVEILLER, E. 2009. Field resistance to spot blotch is not associated with undesirable physio-morphological traits in three spring wheat populations. *Journal of Plant Pathology,* 91**,** 113-122.

ROSYARA, U. R., SUBEDI, S., DUVEILLER, E. & SHARMA, R. C. 2010a. The effect of spot blotch and heat stress on variation of canopy temperature depression, chlorophyll fluorescence and chlorophyll content of hexaploid wheat genotypes. *Euphytica,* 174**,** 377-390.

ROSYARA, U. R., SUBEDI, S., DUVEILLER, E. & SHARMA, R. C. 2010b. Photochemical Efficiency and SPAD value as indirect selection criteria for combined selection of spot blotch and terminal heat stress in wheat. *Journal of Phytopathology,* 158**,** 813-821.

RUUSKA, S. A., REBETZKE, G. J., VAN HERWAARDEN, A. F., RICHARDS, R. A., FETTELL, N. A., TABE, L. & JENKINS, C. L. 2006. Genotypic variation in water-soluble carbohydrate accumulation in wheat. *Functional Plant Biology,* 33**,** 799-809.

SAADALLA, M., QUICK, J. & SHANAHAN, J. 1990a. Heat tolerance in winter wheat: II. Membrane thermostability and field performance. *Crop science,* 30**,** 1248-1251.

SAADALLA, M., SHANAHAN, J. & QUICK, J. 1990b. Heat tolerance in winter wheat: I. Hardening and genetic effects on membrane thermostability. *Crop science,* 30**,** 1243-1247.

SAINI, H. & ASPINALL, D. 1982a. Abnormal sporogenesis in wheat (Triticum aestivum L.) induced by short periods of high temperature. *Annals of Botany,* 49**,** 835.

SAINI, H. & ASPINALL, D. 1982b. Abnormal sporogenesis in wheat (Triticum aestivum L.) induced by short periods of high temperature. *Annals of Botany,* 49**,** 835-846.

SAINI, H., SEDGLEY, M. & ASPINALL, D. 1983. Effect of Heat Stress During Floral Development on Pollen Tube Growth and Ovary Anatomy in Wheat (Triticum aestivum L.). *Functional Plant Biology,* 10**,** 137-144.

SAINI, H., SEDGLEY, M. & ASPINALL, D. 1984. Development anatomy in wheat of male sterility induced by heat stress, water deficit or abscisic acid. *Functional Plant Biology,* 11**,** 243-253.

SAVCHENKO, G., KLYUCHAREVA, E., ABRAMCHIK, L. & SERDYUCHENKO, E. 2002. Effect of periodic heat shock on the inner membrane system of etioplasts. *Russian journal of plant physiology,* 49**,** 349-359.

SCHALLER, G. E. 2012. Ethylene and the regulation of plant development. *BMC biology,* 10**,** 9.

SCHAPENDONK, A., XU, H., VAN DER PUTTEN, P. & SPIERTZ, J. 2007. Heat-shock effects on photosynthesis and sink-source dynamics in wheat (Triticum aestivum L.). *NJAS-Wageningen Journal of Life Sciences,* 55**,** 37-54.

SCHREIBER, U., BILGER, W. & NEUBAUER, C. 1995. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. *Ecophysiology of photosynthesis.* Springer.

SEMAGN, K., BJØRNSTAD, Å., SKINNES, H., MARØY, A. G., TARKEGNE, Y. & WILLIAM, M. 2006. Distribution of DArT, AFLP, and SSR markers in a genetic linkage map of a doubled-haploid hexaploid wheat population. *Genome,* 49**,** 545-555.

SEMAGN, K., BJØRNSTAD, Å. & XU, Y. 2010. The genetic dissection of quantitative traits in crops. *Electronic Journal of Biotechnology,* 13**,** 16-17.

SENTHIL-KUMAR, M., KUMAR, G., SRIKANTHBABU, V. & UDAYAKUMAR, M. 2007. Assessment of variability in acquired thermotolerance: potential option to study genotypic response and the relevance of stress genes. *Journal of Plant Physiology,* 164**,** 111-125.

SHAH, N. & PAULSEN, G. 2003. Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. *Plant and soil,* 257**,** 219-226.

SHAKIBA, M., EHDAIE, B., MADORE, M. & WAINES, J. 1996. Contribution of internode reserves to grain yield in a tall and semidwarf spring wheat [Triticum aestivum]. *Journal of Genetics & Breeding (Italy)*.

SHARMA, D. K. 2013. Heat tolerance in wheat: linking physiological phenotyping to quantitative genetics. *PhD thesis, University of Copenhagen, Denmark.*

SHARMA, D. K., ANDERSEN, S. B., OTTOSEN, C.-O. & ROSENQVIST, E. 2012. Phenotyping of wheat cultivars for heat tolerance using chlorophyll a fluorescence. *Functional Plant Biology,* 39**,** 936-947.

SHEWRY, P. R., POPINEAU, Y., LAFIANDRA, D. & BELTON, P. 2000. Wheat glutenin subunits and dough elasticity: findings of the EUROWHEAT project. *Trends in Food Science & Technology,* 11**,** 433-441.

SHINDO, C., TSUJIMOTO, H. & SASAKUMA, T. 2003. Segregation analysis of heading traits in hexaploid wheat utilizing recombinant inbred lines. *Heredity,* 90**,** 56-63.

SHPILER, L. & BLUM, A. 1986. Differential reaction of wheat cultivars to hot environments. *Euphytica,* 35**,** 483-492.

SILVA, S. A., DE CARVALHO, F. I., DA R. CAETANO, V., DE OLIVEIRA, A. C., DE COIMBRA, J. L., DE VASCONCELLOS, N. J. S. & LORENCETTI, C. 2001. Genetic basis of stay-green trait in bread wheat. *Journal of New Seeds,* 2**,** 55-68.

SINGH, K., GHAI, M., GARG, M., CHHUNEJA, P., KAUR, P., SCHNURBUSCH, T., KELLER, B. & DHALIWAL, H. 2007. An integrated molecular linkage map of diploid wheat based on a Triticum boeoticum× T. monococcum RIL population. *Theoretical and Applied Genetics,* 115**,** 301-312.

SLAFER, G. A. & RAWSON, H. M. 1994. Sensitivity of wheat phasic development to major environmental-factors-a reexamination of some assumptions made by physiologists and modelers. *Australian Journal of Plant Physiology,* 21**,** 393-426.

SNAPE, J., BUTTERWORTH, K., WHITECHURCH, E. & WORLAND, A. 2001. Waiting for fine times: genetics of flowering time in wheat. *Euphytica,* 119**,** 185-190.

SOFIELD, I., EVANS, L., COOK, M. & WARDLAW, I. F. 1977. Factors influencing the rate and duration of grain filling in wheat. *Functional Plant Biology,* 4**,** 785-797.
SOTO-CERDA, B. J. & CLOUTIER, S. 2012. Association mapping in plant genomes. *Genetic Diversity in Plants***,** 29-54.
SPANO, G., DI FONZO, N., PERROTTA, C., PLATANI, C., RONGA, G., LAWLOR, D., NAPIER, J. & SHEWRY, P. 2003. Physiological characterization of 'stay green'mutants in durum wheat. *Journal of Experimental Botany,* 54**,** 1415-1420.
STONE, P. & NICOLAS, M. 1994. Wheat cultivars vary widely in their responses of grain yield and quality to short periods of post-anthesis heat stress. *Functional Plant Biology,* 21**,** 887-900.
STONE, P. & NICOLAS, M. 1995a. 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. 1995b. A survey of the effects of high temperature during grain filling on yield and quality of 75 wheat cultivars. *Australian Journal of Agricultural Research,* 46**,** 475-492.
STONE, P. & NICOLAS, M. 1996. Effect of timing of heat stress during grain filling on two wheat varieties differing in heat tolerance. II. Fractional protein accumulation. *Functional Plant Biology,* 23**,** 739-749.
TALUKDER, A., MCDONALD, G. K. & GILL, G. S. 2013. Effect of short-term heat stress prior to flowering and at early grain set on the utilization of water-soluble carbohydrate by wheat genotypes. *Field Crops Research,* 147**,** 1-11.
TARDIEU, F., REYMOND, M., HAMARD, P., GRANIER, C. & MULLER, B. 2000. Spatial distributions of expansion rate, cell division rate and cell size in maize leaves: a synthesis of the effects of soil water status, evaporative demand and temperature. *Journal of Experimental Botany,* 51**,** 1505-1514.
TASHIRO, T. & WARDLAW, I. 1990a. The response to high temperature shock and humidity changes prior to and during the early stages of grain development in wheat. *Functional Plant Biology,* 17**,** 551-561.
TASHIRO, T. & WARDLAW, I. F. 1989. A comparison of the effect of high temperature on grain development in wheat and rice. *Annals of Botany,* 64**,** 59-65.
TASHIRO, T. & WARDLAW, I. F. 1990b. The effect of high temperature at different stages of ripening on grain set, grain weight and grain dimensions in the semi-dwarf wheat 'Banks'. *Annals of Botany,* 65**,** 51-61.
TAYLOR, J. 2013. Statistics for the Australian Grains Industry Technical Report Series A vignette for the wgaim R package.
TAYLOR, J., DIFFEY, S. & TAYLOR, M. J. 2012. Package 'wgaim'.
TEWARI, A. K. & TRIPATHY, B. C. 1998. Temperature-stress-induced impairment of chlorophyll biosynthetic reactions in cucumber and wheat. *Plant Physiology,* 117**,** 851-858.
TEWOLDE, H., FERNANDEZ, C. & ERICKSON, C. 2006. Wheat Cultivars Adapted to Post-Heading High Temperature Stress. *Journal of Agronomy and Crop Science,* 192**,** 111-120.
THOMAS, H. & HOWARTH, C. J. 2000. Five ways to stay green. *Journal of Experimental Botany,* 51**,** 329-337.
THOMAS, H. & OUGHAM, H. 2014. The stay-green trait. *Journal of Experimental Botany***,** eru037.
TIWARI, C., WALLWORK, H., KUMAR, U., DHARI, R., ARUN, B., MISHRA, V., REYNOLDS, M. & JOSHI, A. K. 2013. Molecular mapping of high temperature tolerance in bread wheat adapted to the Eastern Gangetic Plain region of India. *Field Crops Research,* 154**,** 201-210.
TODD, G. W. 1982. Photosynthesis and respiration of vegetative and reproductive parts of wheat and barley plants in response to increasing temperature. *Proc. Okla. Acad. Sci,* 62**,** 57-62.
TUINSTRA, M., EJETA, G. & GOLDSBROUGH, P. 1998. Evaluation of near-isogenic sorghum lines contrasting for QTL markers associated with drought tolerance. *Crop science,* 38**,** 835-842.
TURNER, N. C. 1988. Measurement of plant water status by the pressure chamber technique. *Irrigation Science,* 9**,** 289.
UDDLING, J., GELANG-ALFREDSSON, J., PIIKKI, K. & PLEIJEL, H. 2007. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. *Photosynthesis Research,* 91**,** 37-46.
UHLEN, A., HAFSKJOLD, R., KALHOVD, A. H., SAHLSTRÖM, S., LONGVA, Å. & MAGNUS, E. 1998. Effects of cultivar and temperature during grain filling on wheat protein content, composition, and dough mixing properties. *Cereal chemistry,* 75**,** 460-465.
VADEZ, V., DESHPANDE, S., KHOLOVA, J., RAMU, P. & HASH, C. T. 2013. Molecular Breeding for Stay-Green: Progress and Challenges in Sorghum. *Translational Genomics for Crop Breeding: Volume 2-Improvement for Abiotic Stress, Quality and Yield Improvement***,** 125.
VADEZ, V., DESHPANDE, S. P., KHOLOVA, J., HAMMER, G. L., BORRELL, A. K., TALWAR, H. S. & HASH, C. T. 2011. Stay-green quantitative trait loci's effects on water extraction, transpiration efficiency and seed yield depend on recipient parent background. *Functional Plant Biology,* 38**,** 553-566.
VAN HERWAARDEN, A., ANGUS, J., RICHARDS, R. & FARQUHAR, G. 1998a. 'Haying-off', the negative grain yield response of dryland wheat to nitrogen fertiliser. II. Carbohydrate and protein dynamics. *Australian Journal of Agricultural Research,* 49**,** 1083-1093.

VAN HERWAARDEN, A., FARQUHAR, G., ANGUS, J., RICHARDS, R. & HOWE, G. 1998b. Haying, the negative grain yield response of dryland wheat to nitrogen fertiliser: I. Biomass, grain yield, and water use.

VERBYLA, A., CULLIS, B. & THOMPSON, R. 2007. The analysis of QTL by simultaneous use of the full linkage map. *Theoretical and Applied Genetics,* 116**,** 95-111.

VETTAKKORUMAKANKAV, N. N., FALK, D., SAXENA, P. & FLETCHER, R. A. 1999. A crucial role for gibberellins in stress protection of plants. *Plant and Cell Physiology,* 40**,** 542.

VIJAYALAKSHMI, K., FRITZ, A. K., PAULSEN, G. M., BAI, G., PANDRAVADA, S. & GILL, B. S. 2010. Modeling and mapping QTL for senescence-related traits in winter wheat under high temperature. *Molecular Breeding,* 26**,** 163-175.

VOORRIPS, R. 2002. MapChart: software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity,* 93**,** 77-78.

VOS, P., HOGERS, R., BLEEKER, M., REIJANS, M., VAN DE LEE, T., HORNES, M., FRITERS, A., POT, J., PALEMAN, J. & KUIPER, M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research,* 23**,** 4407-4414.

WAHID, A. 2007. Physiological implications of metabolite biosynthesis for net assimilation and heat-stress tolerance of sugarcane (Saccharum officinarum) sprouts. *Journal of plant Research,* 120**,** 219-228.

WAHID, A. & CLOSE, T. 2007. Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves. *Biologia Plantarum,* 51**,** 104-109.

WAHID, A., GELANI, S., ASHRAF, M. & FOOLAD, M. 2007. Heat tolerance in plants: an overview. *Environmental and Experimental Botany,* 61**,** 199-223.

WAHID, A. & GHAZANFAR, A. 2006. Possible involvement of some secondary metabolites in salt tolerance of sugarcane. *Journal of Plant Physiology,* 163**,** 723-730.

WANG, R., HAI, L., ZHANG, X., YOU, G., YAN, C. & XIAO, S. 2009. QTL mapping for grain filling rate and yield-related traits in RILs of the Chinese winter wheat population Heshangmai× Yu8679. *Theoretical and Applied Genetics,* 118**,** 313-325.

WANG, S., BASTEN, C. J. & ZENG, Z.-B. 2012b. Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC *http://statgen.ncsu.edu/qtlcart/WQTLCart.htm*.

WANG, S., WONG, D., FORREST, K., ALLEN, A., CHAO, S., HUANG, B. E., MACCAFERRI, M., SALVI, S., MILNER, S. G. & CATTIVELLI, L. 2014. Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. *Plant Biotechnology Journal*.

WANG, X., CAI, J., LIU, F., JIN, M., YU, H., JIANG, D., WOLLENWEBER, B., DAI, T. & CAO, W. 2012. Pre-anthesis high temperature acclimation alleviates the negative effects of post-anthesis heat stress on stem stored carbohydrates remobilization and grain starch accumulation in wheat. *Journal of Cereal Science,* 55**,** 331-336.

WARDLAW, I., DAWSON, I. & MUNIBI, P. 1989a. The tolerance of wheat to high temperatures during reproductive growth. 2. Grain development. *Australian Journal of Agricultural Research,* 40**,** 15-24.

WARDLAW, I., DAWSON, I., MUNIBI, P. & FEWSTER, R. 1989b. The tolerance of wheat to high temperatures during reproductive growth. I. Survey procedures and general response patterns. *Crop and Pasture Science,* 40**,** 1-13.

WARDLAW, I. & MONCUR, L. 1995. The response of wheat to high temperature following anthesis. I. The rate and duration of kernel filling. *Functional Plant Biology,* 22**,** 391-397.

WARDLAW, I. & PORTER, H. 1967. The redistribution of stem sugars in wheat during grain development. *Australian Journal of Biological Sciences,* 20**,** 309-318.

WARDLAW, I., SOFIELD, I. & CARTWRIGHT, P. 1980. Factors limiting the rate of dry matter accumulation in the grain of wheat grown at high temperature. *Functional Plant Biology,* 7**,** 387-400.

WARDLAW, I. & WRIGLEY, C. 1994. Heat tolerance in temperate cereals: an overview. *Functional Plant Biology,* 21**,** 695-703.

WARDLAW, I. F., LARROQUE, O., BLUMENTHAL, C. & WRIGLEY, C. W. 2002. Contrasting effects of chronic heat stress and heat shock on kernel weight and flour quality in wheat. *Functional Plant Biology,* 29**,** 25-34.

WARDLAW, I. F. & WILLENBRINK, J. 1994. Carbohydrate storage and mobilisation by the culm of wheat between heading and grain maturity: the relation to sucrose synthase and sucrose-phosphate synthase. *Functional Plant Biology,* 21**,** 255-271.

WEISS, U. & BIBER, P. 2011. Plant detection and mapping for agricultural robots using a 3D LIDAR sensor. *Robotics and autonomous systems,* 59**,** 265-273.

WHITE, J. W., ANDRADE-SANCHEZ, P., GORE, M. A., BRONSON, K. F., COFFELT, T. A., CONLEY, M. M., FELDMANN, K. A., FRENCH, A. N., HEUN, J. T. & HUNSAKER, D. J. 2012. Field-based phenomics for plant genetics research. *Field Crops Research,* 133**,** 101-112.

WHITING, D. 2004. *Wheat varieties in Australia, 1968-2001,* D. Whiting.

WILLIAMS, J. G., KUBELIK, A. R., LIVAK, K. J., RAFALSKI, J. A. & TINGEY, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research,* 18**,** 6531-6535.

WITTWER, C. T., REED, G. H., GUNDRY, C. N., VANDERSTEEN, J. G. & PRYOR, R. J. 2003. High-resolution genotyping by amplicon melting analysis using LCGreen. *Clinical Chemistry,* 49**,** 853-860.

WOLLENWEBER, B., PORTER, J. R. & SCHELLBERG, J. 2003. Lack of interaction between extreme high-temperature events at vegetative and reproductive growth stages in wheat. *Journal of Agronomy and Crop Science,* 189**,** 142-150.

WORLAND, A., KORZUN, V., RÖDER, M., GANAL, M. & LAW, C. 1998. Genetic analysis of the dwarfing gene Rht8 in wheat. Part II. The distribution and adaptive significance of allelic variants at the Rht8 locus of wheat as revealed by microsatellite screening. *Theoretical and Applied Genetics,* 96**,** 1110-1120.

WRIGLEY, C. 2007. Mitigating the Damaging Effects of Growth and Storage Conditions on Grain Quality. *Wheat Production in Stressed Environments***,** 425-439.

XU, Y., ZHU, L., XIAO, J., HUANG, N. & MCCOUCH, S. 1997. Chromosomal regions associated with segregation distortion of molecular markers in F2, backcross, doubled haploid, and recombinant inbred populations in rice (Oryza sativa L.). *Molecular and General Genetics MGG,* 253**,** 535-545.

YAN, L., FU, D., LI, C., BLECHL, A., TRANQUILLI, G., BONAFEDE, M., SANCHEZ, A., VALARIK, M., YASUDA, S. & DUBCOVSKY, J. 2006. The wheat and barley vernalization gene VRN3 is an orthologue of FT. *Proceedings of the National Academy of Sciences,* 103**,** 19581-19586.

YANG, J., HU, C., HU, H., YU, R., XIA, Z., YE, X. & ZHU, J. 2008. QTLNetwork: mapping and visualizing genetic architecture of complex traits in experimental populations. *Bioinformatics,* 24**,** 721-723.

YANG, J., SEARS, R., GILL, B. & PAULSEN, G. 2002a. Genotypic differences in utilization of assimilate sources during maturation of wheat under chronic heat and heat shock stresses. *Euphytica,* 125**,** 179-188.

YANG, J., SEARS, R., GILL, B. & PAULSEN, G. 2002b. Quantitative and molecular characterization of heat tolerance in hexaploid wheat. *Euphytica,* 126**,** 275-282.

YANG, J. & ZHANG, J. 2006. Grain filling of cereals under soil drying. *New Phytologist,* 169**,** 223-236.

YANG, J., ZHANG, J., HUANG, Z., ZHU, Q. & WANG, L. 2000. Remobilization of carbon reserves is improved by controlled soil-drying during grain filling of wheat. *Crop science,* 40**,** 1645-1655.

YEMM, E. & WILLIS, A. 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochemical Journal,* 57**,** 508.

YU, J., HOLLAND, J. B., MCMULLEN, M. D. & BUCKLER, E. S. 2008. Genetic design and statistical power of nested association mapping in maize. *Genetics,* 178**,** 539-551.

ZADOKS, J. C., CHANG, T. T. & KONZAK, C. F. 1974. A decimal code for the growth stages of cereals. *Weed research,* 14**,** 415-421.

ZAHEDI, M. & JENNER, C. F. 2003. Analysis of effects in wheat of high temperature on grain filling attributes estimated from mathematical models of grain filling. *The Journal of Agricultural Science,* 141**,** 203-212.

ZAHEDI, M., SHARMA, R. & JENNER, C. F. 2003. Effects of high temperature on grain growth and on the metabolites and enzymes in the starch-synthesis pathway in the grains of two wheat cultivars differing in their responses to temperature. *Functional Plant Biology,* 30**,** 291-300.

ZEEMAN, S. C., KOSSMANN, J. & SMITH, A. M. 2010. Starch: its metabolism, evolution, and biotechnological modification in plants. *Annual Review of Plant Biology,* 61**,** 209-234.

ZENG, Z. B. 1994. Precision mapping of quantitative trait loci. *Genetics,* 136**,** 1457-68.

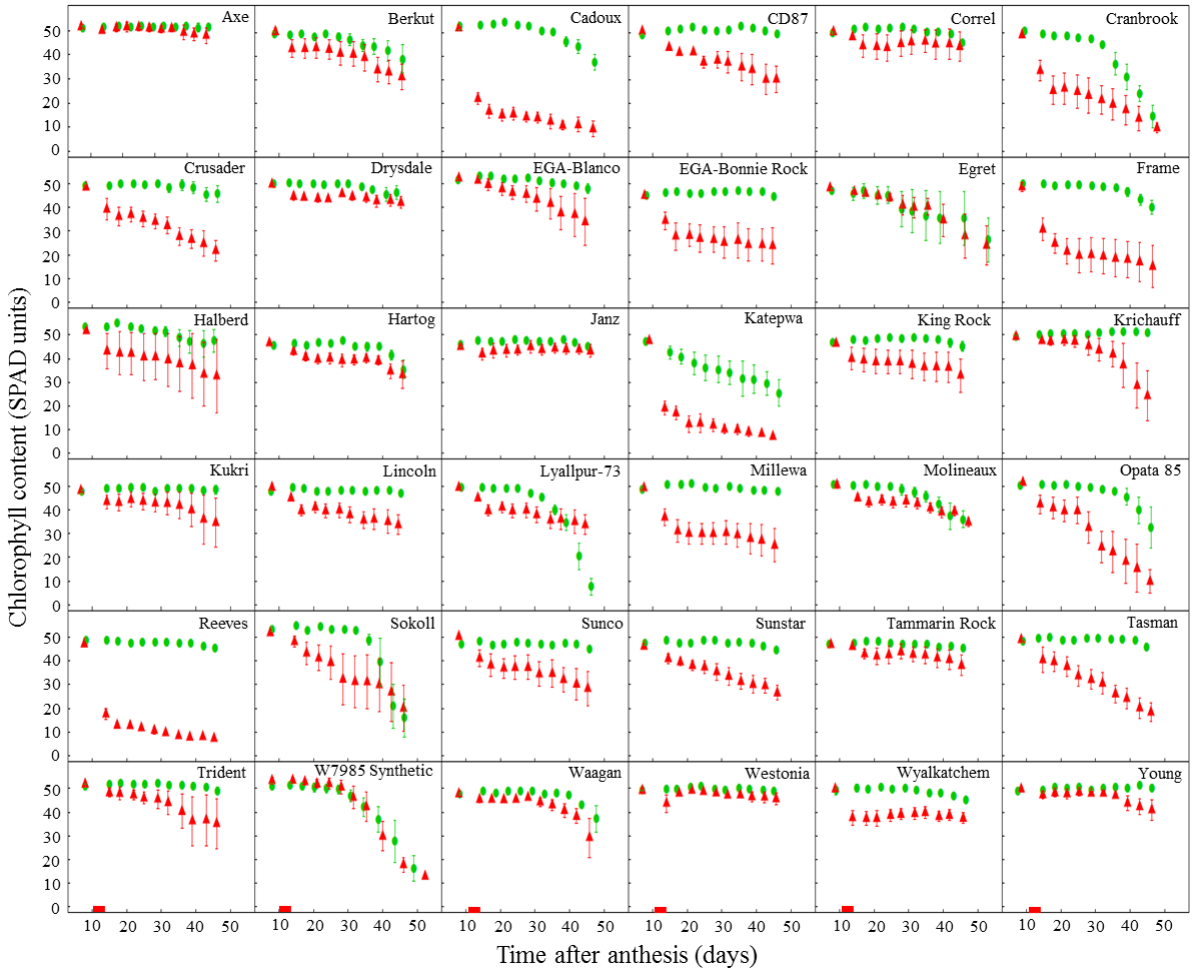
ZHENG, B., CHENU, K., FERNANDA DRECCER, M. & CHAPMAN, S. C. 2012. Breeding for the future: what are the potential impacts of future frost and heat events on sowing and flowering time requirements for Australian bread wheat (Triticum aestivium) varieties? *Global Change Biology,* 18**,** 2899-2914.

ZHU, C., GORE, M., BUCKLER, E. S. & YU, J. 2008. Status and prospects of association mapping in plants. *The plant genome,* 1**,** 5-20.

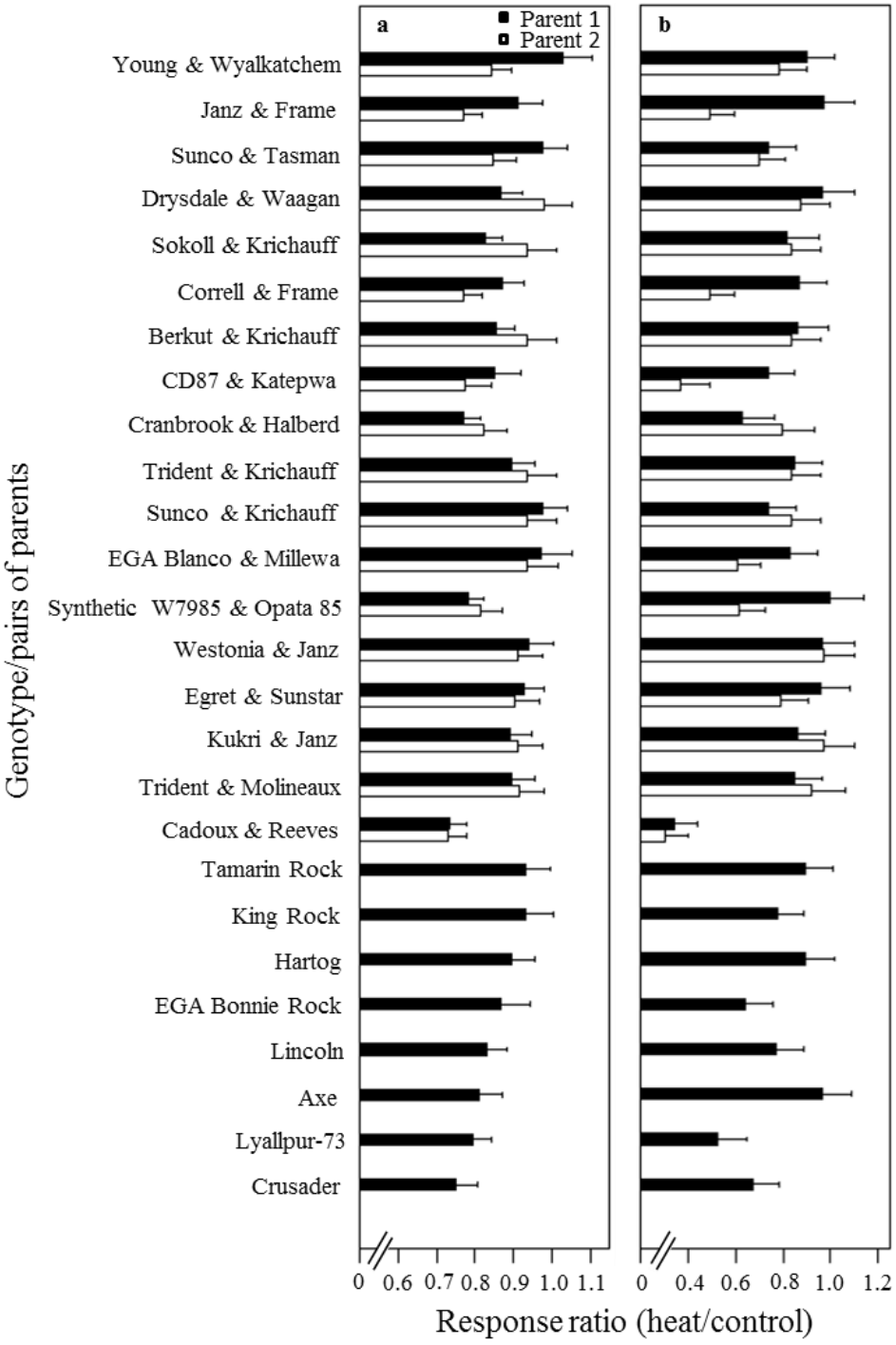
Appendices

Appendix 1 List of available mapping populations for the screened genotypes in this study (Identified by the Australian Winter Cereals Pre-Breeding Alliance, and from the literature).

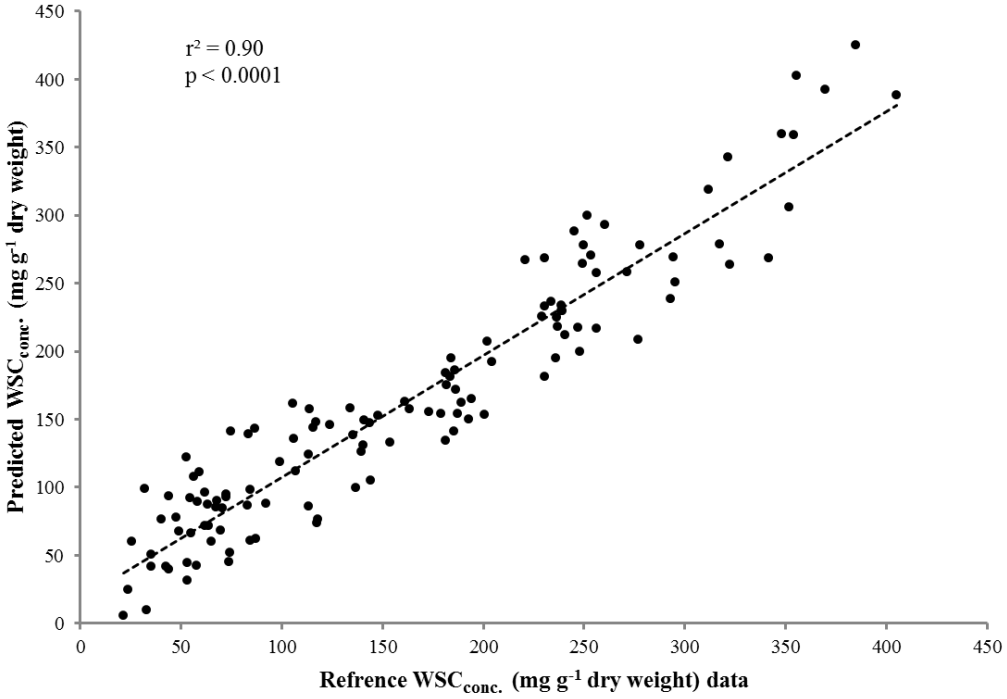
Pairs of parents	
AUS1408/Cascades	Morocco 426/Janz
Avocet/Cappelle Desprez	PI624933-1 /Wyalkatchem
Avocet /Cook	PI625123-3 /Calingiri
Batavia /Ernie	PI625983-1 /Wyalkatchem
Berkut/Krichauff	PI626580-4/Correll
Cadoux/Reeves	Rosella/Matong
CD87/Katepwa	Seri/Hartog
Chara/WW2449	Seri M82/Babax
CM18/Vigour18	Sokoll/Krichauff
Cook/Avocet S	Spica /Maringa (Rht1)
Correll/Frame	Spica /Maringa (Rht2)
CPI133814 /Janz	SUN325B/QT7475
CPI133872 /Janz	Sunco/Batavia
CPI33842 /Janz	Sunco/Tasman
CPI33859 /Janz	Sunco/QT7475
Cranbrook/Halberd	Sunco/SUN325B
Currawong/CD87	Sunco /Krichauff
Diamondbird/Janz	Sunco /Indis
EGA Blanco/Millewa	Synthetic W7985/Opata 85
Egret/Sunstar	Tammin/Excalibur
Excalibur/Kukri	Trident/Krichauff
Gladius/Drysdale	Trident/Molineaux
Hereward/Avocet S	Drysdale/Waagan
Iraq 43/Janz	Westonia/Janz
Janz/Frame	Whistler/WW1842
Janz/AUS1408	Young/Wyalkatchem
Janz/AUS1490	
Krichauff/Roblin	
Kukri/Janz	
Kukri/RAC875	



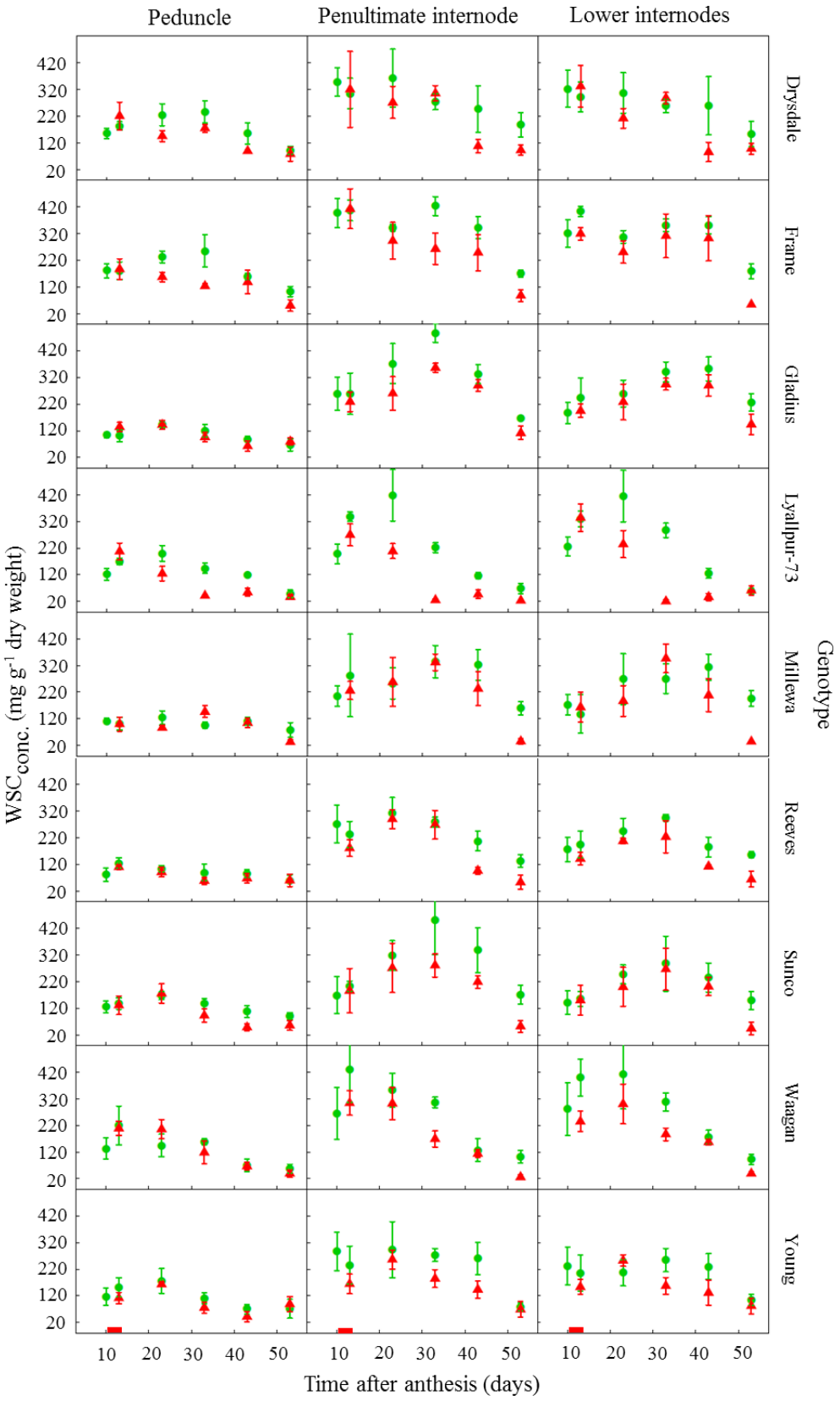
Appendix 3. 1 Relative chlorophyll content of flag leaves (means ± S.E.) in control (green circles) and heat-treated plants (red triangles), before the period of brief heat treatment (represented by red horizontal bar on x axes) and thereafter, in 36 bread wheat genotypes.



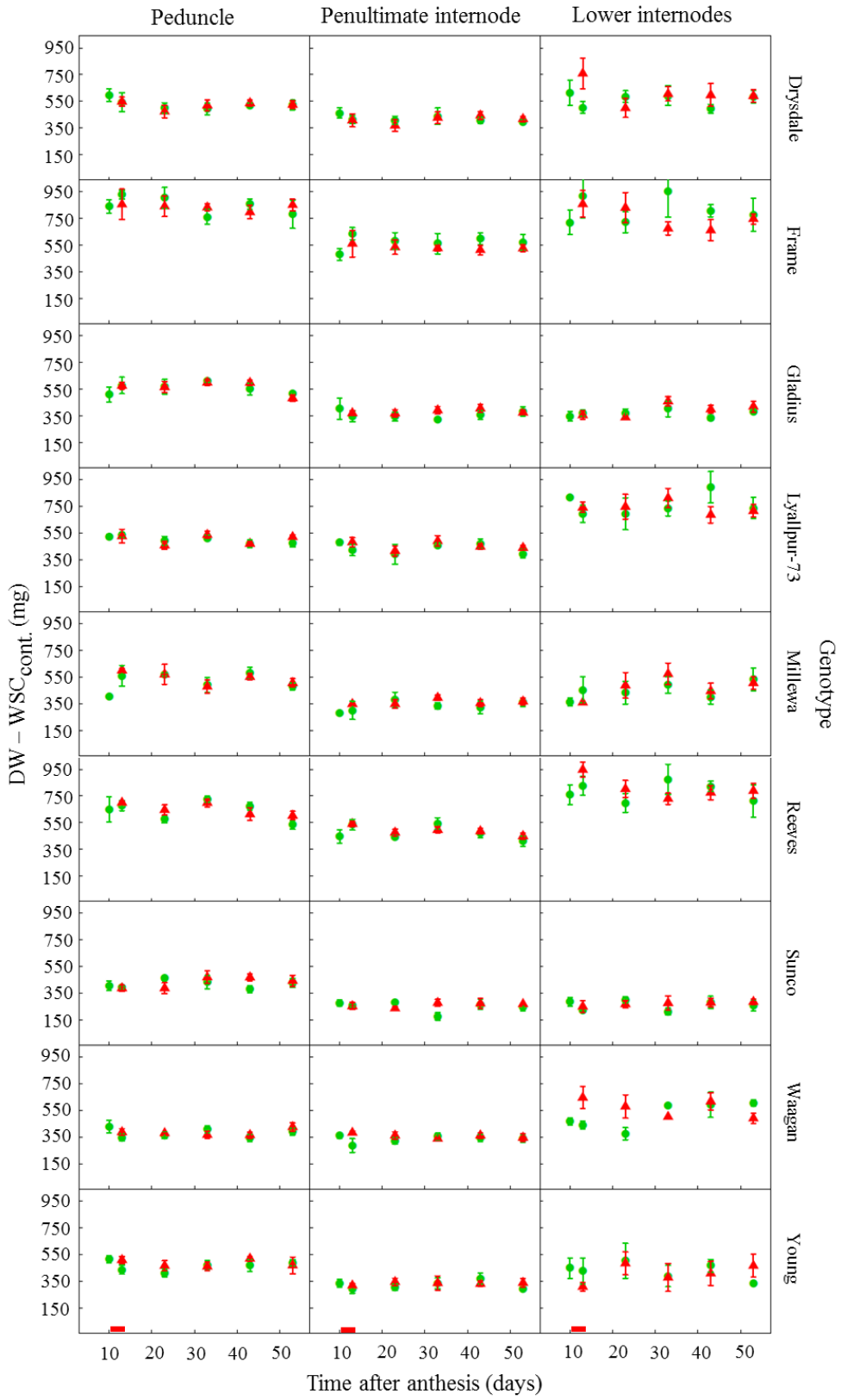
Appendix 3. 2 Response ratio (+ S.E.) of single grain weight (SGW, a) and area under SPAD curve (AUSC, b) of studied genotypes. Genotypes forming pairs of mapping parents are neighboured and sorted from pairs with the highest contrast to the ones with the lowest contrast for SGW (the order of parents/genotypes for AUSC was kept similar to SGW). Genotypes without pairs are listed at far end of the graphs.



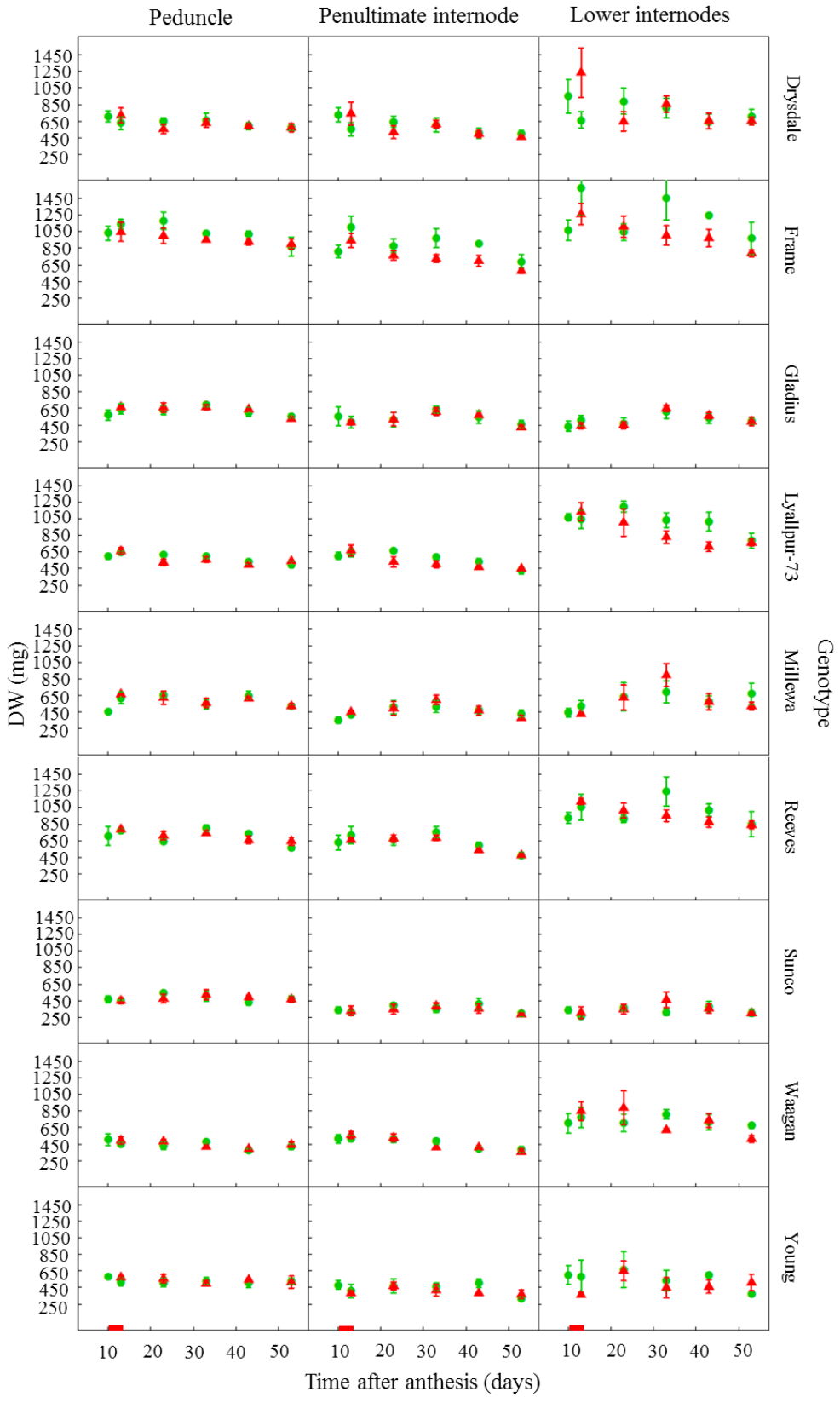
Appendix 4. 1 Water soluble carbohydrate concentration ($WSC_{conc.}$, mg g^{-1} dry weight) in a chosen reference set of 125 wheat stem samples determined using anthrone method, plotted against WSC content of the samples predicted using attenuated total reflectance midinfrared spectroscopy. Dashed line represents the theoretical regression line.



Appendix 4. 2 Time courses of water soluble carbohydrates concentration ($WSC_{conc.}$ mg g^{-1} dry weight) of peduncle, penultimate and lower internodes of the main stem from control (green circles) and heat-treated plants (red triangles) of 9 bread wheat genotypes (mean ± S.E.). The red bar on the x axis represents the period of brief heat treatment.



Appendix 4. 3 Time courses of subtracted stem dry weight from water soluble carbohydrates content ($WSC_{cont.}$) (DW – $WSC_{cont.}$, mg) of peduncle, penultimate and lower internodes of the main stem from control (green circles) and heat-treated plants (red triangles) of 9 bread wheat genotypes (mean ± S.E.). The red bar on the x axis represents the period of brief heat treatment.

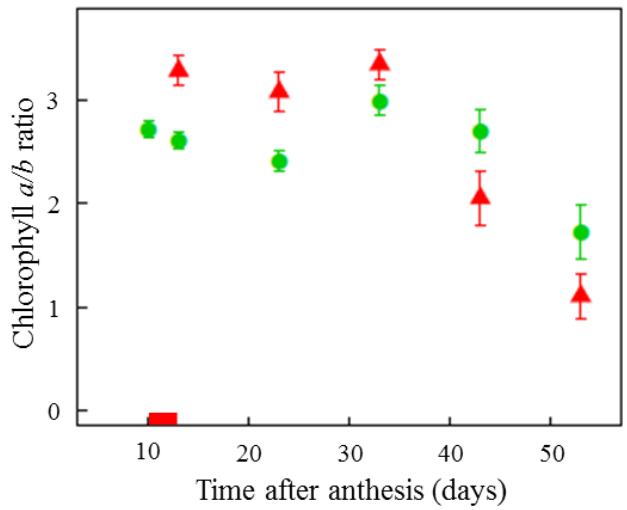


Appendix 4. 4 Time courses of stem dry weight (DW, mg) of peduncle, penultimate and lower internodes of the main stem from control (green circles) and heat-treated plants (red triangles) of 9 bread wheat genotypes (mean ± S.E.). The red bar on the x axis represents the period of brief heat treatment.

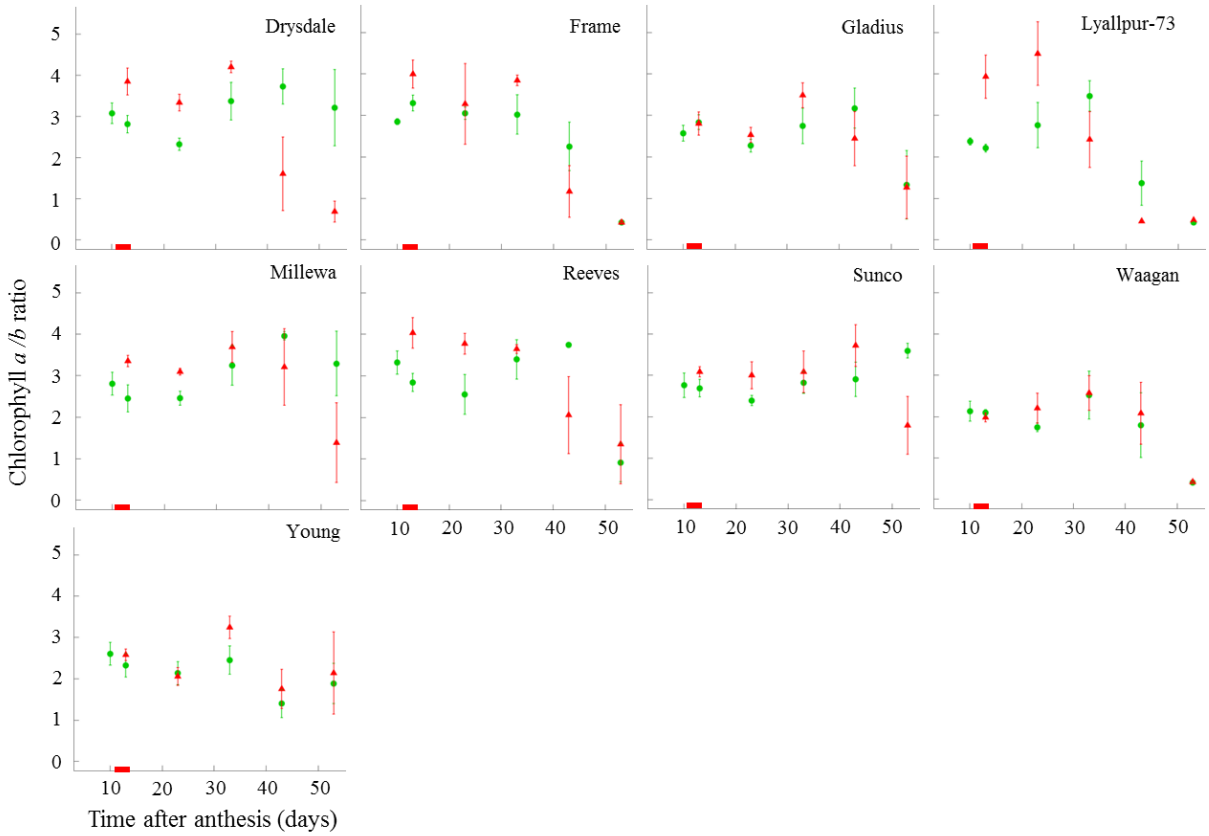
Appendix 4. 5 Association between trait potentials (value under control conditions) and response ratios of traits (Mean trait value$_{\text{Heat treatment}}$ / Mean trait value$_{\text{Control}}$). Trait potentials and response ratios are listed on horizontal and vertical axes, respectively. FGW, final grain weight; GFD, grain-filling duration; TIP, time to inflection point; MGR, maximum growth rate; SGR, sustained grain growth rate; TotChl$_{av.}$, total chlorophyll content averaged over all time points; Chl$a_{av.}$ and Chlb_{av}, chlorophyll a and b content averaged over all time points; WSC$_{max}$, maximum water soluble carbohydrate content; WSC$_{min}$, minimum water soluble carbohydrate content; WSC$_{cont.av.}$, water soluble carbohydrate content averaged over all harvest times; MWSC, mobilized WSC; WSCME, WSC mobilization efficiency; DW$_{av.}$, stem dry weight averaged over all harvest times.

Stem segment										Peduncle						Penultimate internode						Lower internodes					
	Trait	FGW	GFD	TIP	MGR	SGR	TotChl$_{av.}$	Chl$a_{av.}$	Chl$b_{av.}$	WSC$_{max}$	WSC$_{min}$	WSC$_{cont.av.}$	MWSC	WSCME	DW$_{av.}$	WSC$_{max}$	WSC$_{min}$	WSC$_{cont.av.}$	MWSC	WSCME	DW$_{av.}$	WSC$_{max}$	WSC$_{min}$	WSC$_{cont.av.}$	MWSC	WSCME	DW$_{av.}$
	FGW	-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	-0.50	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	0.30	-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*	-0.50	-0.73*	-0.76*	-0.49	-0.75*	-0.70*	-0.60	-0.72*	-0.63	0.10	-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	-0.20	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	-0.50	0.53	0.20	-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	-0.60	0.22	-0.50	-0.75*	-0.85**	-0.63
	TotChl$_{av.}$	-0.78*	-0.15	-0.30	-0.71*	-0.74*	0.85**	0.76*	0.85**	-0.59	-0.38	-0.60	-0.63	-0.45	-0.63	-0.46	-0.34	-0.49	-0.43	0.04	-0.69*	-0.78*	-0.40	-0.70*	-0.74*	-0.34	-0.82**
	Chl$a_{av.}$	-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**
	Chl$b_{av.}$	-0.73*	-0.21	-0.31	-0.62	-0.65	0.76*	0.65	0.80*	-0.50	-0.32	-0.51	-0.54	-0.37	-0.58	-0.34	-0.29	-0.40	-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*	-0.60	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	-0.40
	WSC$_{min}$	0.37	0.30	0.35	0.26	0.21	0.11	0.12	0.08	-0.25	-0.33	-0.24	-0.20	0.22	-0.02	-0.08	-0.13	-0.04	-0.04	0.19	0.10	-0.04	-0.03	-0.07	-0.03	0.17	0.12
	WSC$_{cont.av.}$	-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	-0.40	-0.24	-0.45	-0.40	-0.08	-0.54	-0.59	-0.14	-0.53	-0.61	-0.40	-0.56
	MWSC	-0.73*	-0.17	-0.42	-0.70*	-0.69*	0.41	0.37	0.40	-0.53	-0.40	-0.52	-0.56	-0.40	-0.68*	-0.62	-0.40	-0.69*	-0.61	-0.17	-0.74*	-0.58	-0.44	-0.58	-0.51	-0.32	-0.62
	WSCME	-0.73*	-0.20	-0.44	-0.71*	-0.67*	0.34	0.35	0.27	-0.20	0.04	-0.18	-0.29	-0.59	-0.32	-0.38	-0.13	-0.37	-0.43	-0.30	-0.50	-0.44	-0.17	-0.40	-0.44	-0.43	-0.51
	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*	0.10	-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	-0.20	-0.09	-0.34	0.05	0.18	0.10	-0.01	-0.31	0.23	-0.08	0.37	0.10	-0.26	-0.65	0.19	0.08	0.63	0.17	-0.12	-0.49	0.22
	WSC$_{min}$	-0.08	0.22	0.24	-0.16	-0.22	0.58	0.51	0.59	-0.07	0.04	-0.05	-0.10	-0.15	-0.01	-0.12	-0.26	-0.03	-0.04	0.39	-0.06	-0.24	-0.27	-0.18	-0.18	0.20	-0.17
	WSC$_{cont.av.}$	-0.46	0.20	-0.03	-0.61	-0.57	0.17	0.30	-0.06	-0.17	0.09	-0.13	-0.27	-0.55	-0.03	-0.17	0.44	-0.03	-0.40	-0.83**	-0.11	-0.32	0.54	-0.19	-0.54	-0.78*	-0.26
	MWSC	-0.27	0.06	-0.14	-0.37	-0.31	-0.22	-0.08	-0.40	0.00	0.16	0.05	-0.07	-0.38	0.13	-0.12	0.40	0.02	-0.32	-0.77*	0.07	0.00	0.59	0.07	-0.20	-0.60	0.09
	WSCME	-0.21	0.09	-0.12	-0.33	-0.25	-0.27	-0.10	-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	-0.60	-0.03
	DW$_{av.}$	-0.52	0.15	-0.10	-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	0.30	0.03	-0.75*	-0.72*	0.34	0.40	0.20	-0.31	-0.11	-0.27	-0.37	-0.47	-0.42	-0.44	0.07	-0.39	-0.58	-0.59	-0.48	-0.50	-0.02	-0.41	-0.56	-0.53	-0.51
	WSC$_{min}$	0.05	0.17	0.23	0.01	-0.06	0.45	0.33	0.56	-0.24	-0.30	-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
	WSC$_{cont.av.}$	-0.21	0.61	0.38	-0.52	-0.49	0.20	0.35	-0.07	-0.10	0.10	-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	-0.50
	MWSC	-0.63	0.00	-0.30	-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	-0.50	-0.74*	-0.35
	WSCME	-0.60	-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	-0.20	-0.40	-0.69*	-0.25	-0.31	0.36	-0.27	-0.47	-0.81**	-0.30
	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

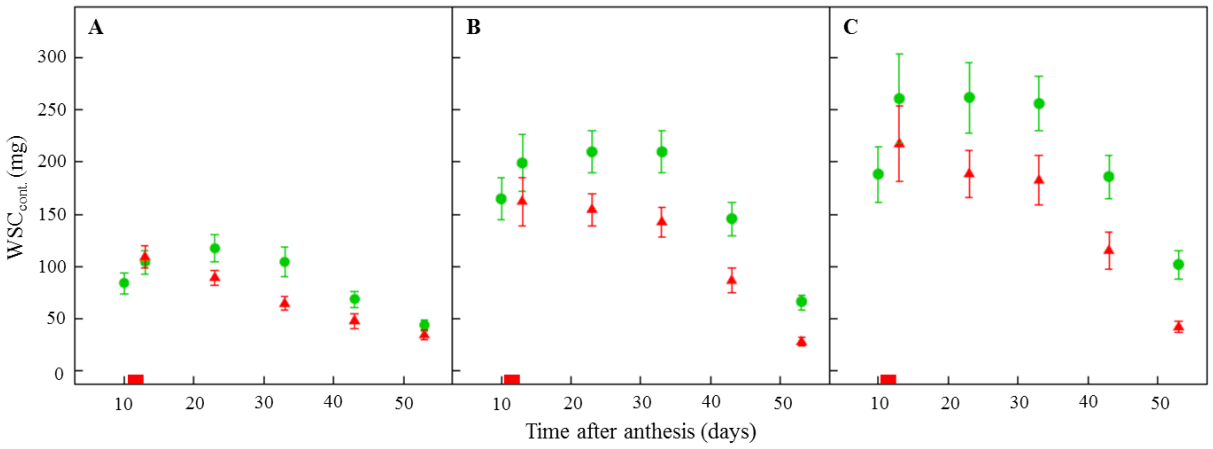
Values are Pearson correlation coefficients, with significance levels indicated by asterisks: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.



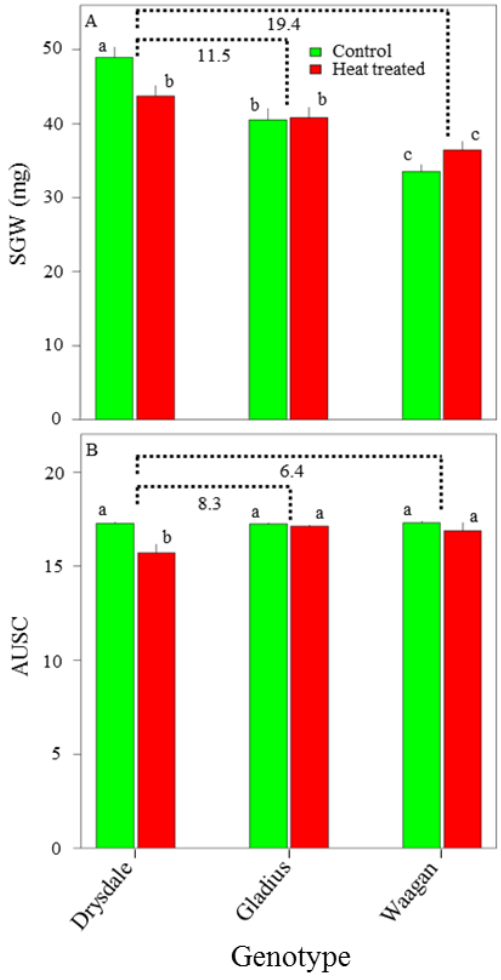
Appendix 4. 6 Time courses of flag leaf chlorophyll *a/b* ratio of all genotypes in control (green circles) and heat-treated plants (red triangles) (mean ± S.E.). The red bar on the x axis represents the period of brief heat treatment.



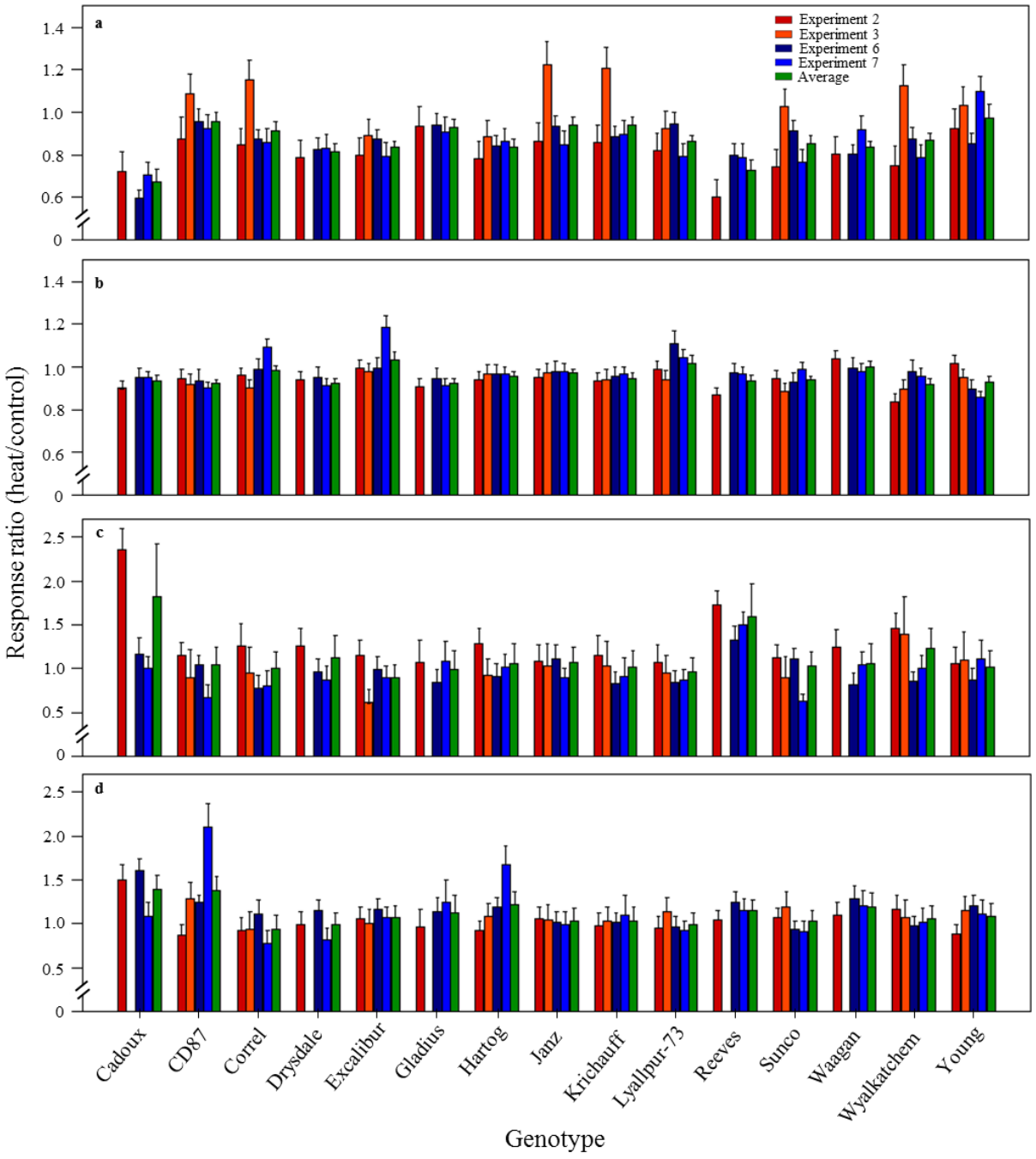
Appendix 4. 7 Time courses of flag leaf chlorophyll *a/b* ratio of control (green circles) and heat-treated plants (red triangles) of 9 bread wheat genotypes (mean ± S.E.). The red bar on the x axis represents the period of brief heat treatment.



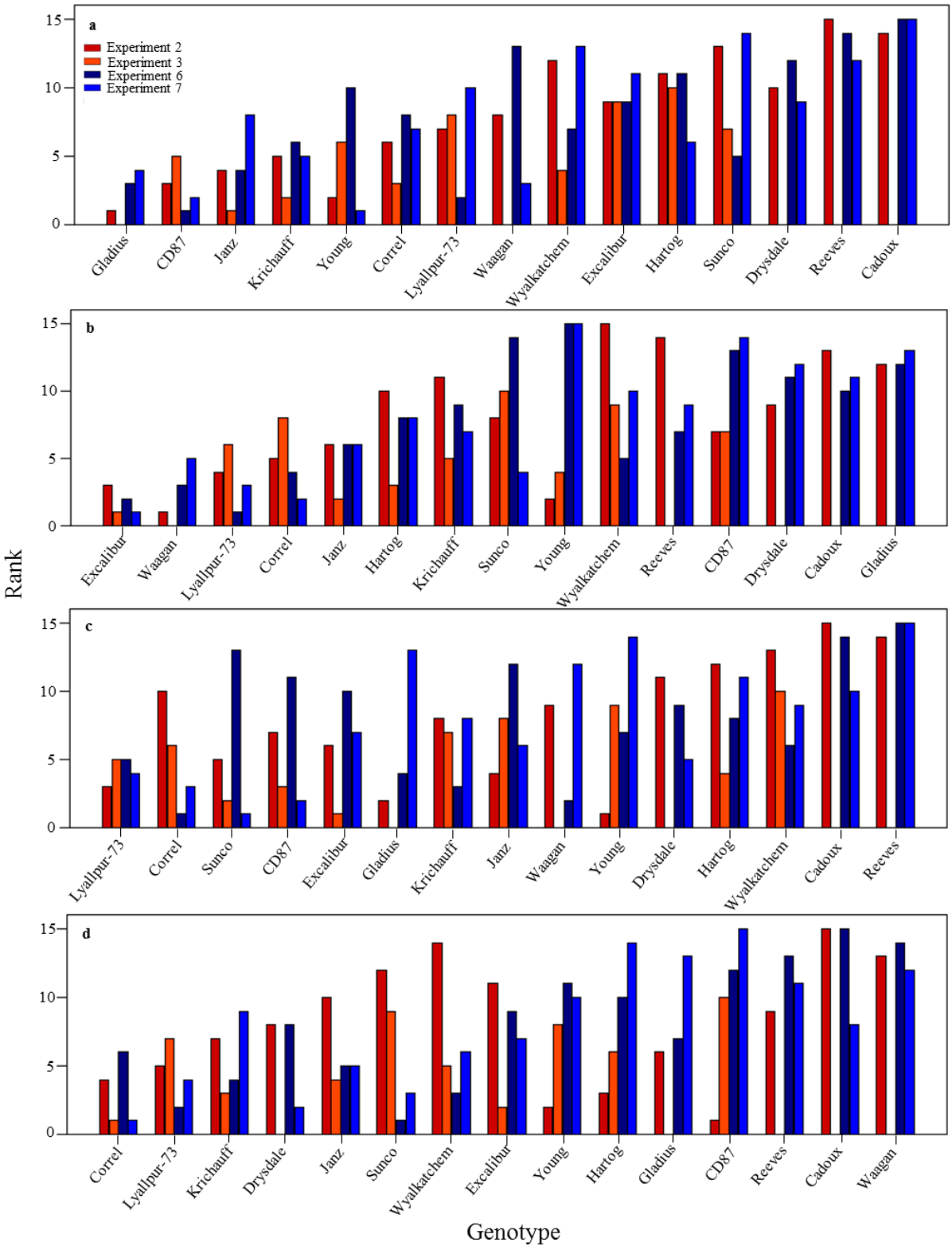
Appendix 4. 8 Time courses of water soluble carbohydrates content ($WSC_{cont.}$, mg) in peduncle (A), penultimate internode (B) and lower internodes (C) of control (green circles) and heat-treated plants (red triangles) averaged across all genotypes within each time point (mean ± S.E.). The red bar on the x axis represents the period of brief heat treatment.



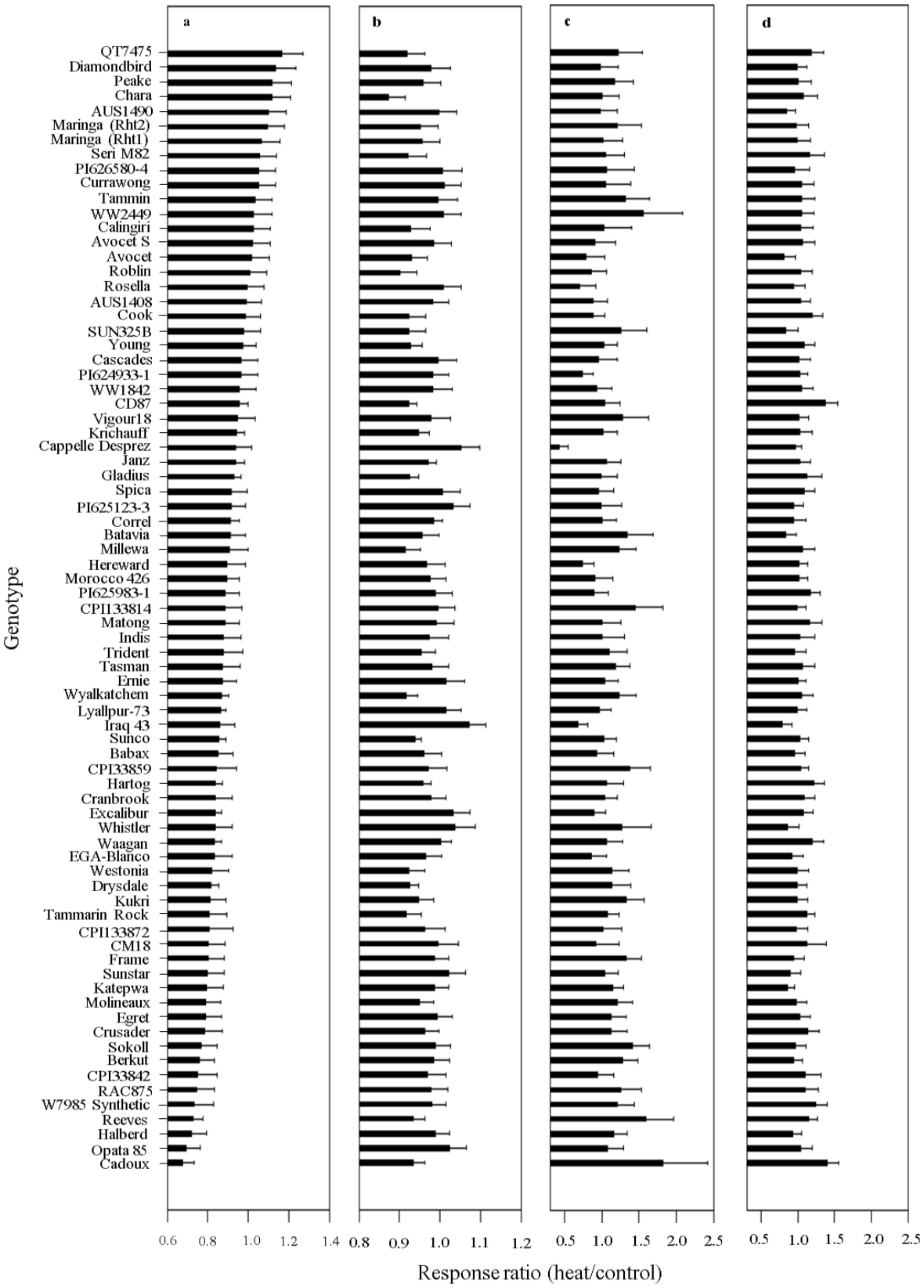
Appendix 6. 1 Single grain weight (SGW, A) and area under SPAD curve (AUSC, B) in control and heat-treated plants. AUSC measured on normalized SPAD readings at 10, 13, and 27 DAA, which appeared to be informative according to the results presented in Chapter 3. Numbers under the dashed lines indicate contrast percentage between pairs of parents (Gladius and Drysdale, and Drysdale and Waagan) for heat response of the corresponding trait. Bars indicate mean + S.E. (n=12 per genotype/treatment). Means with the same letter were not significantly different at $p > 0.05$ (LSD test).



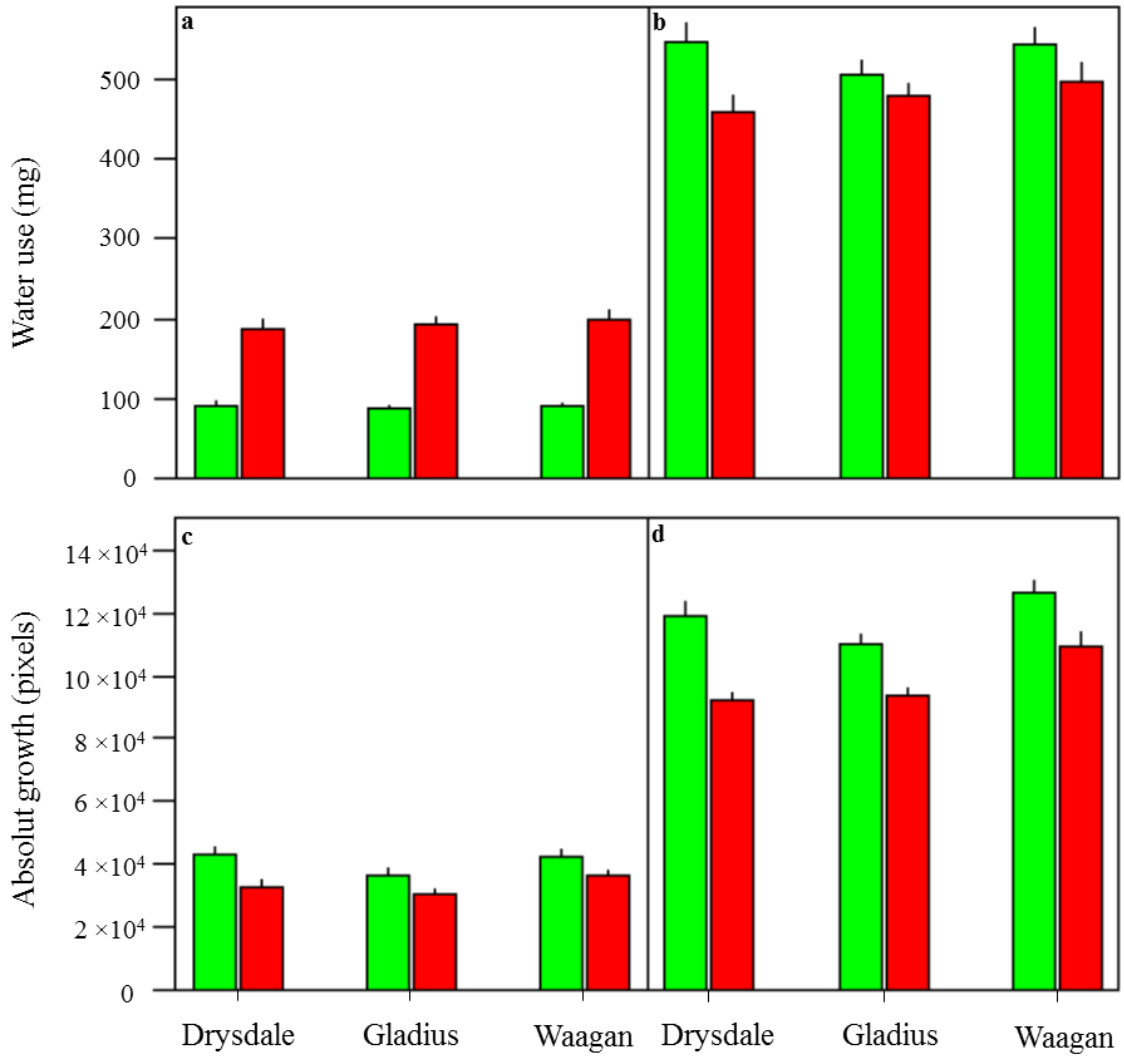
Appendix 7. 1 Response ratio (+ S.E.) of relative growth rate during treatment (from 25 to 28 days after sowing; a), relative growth rate from 28 to 39 days after sowing (b), and proportion of senescent area at 28 (directly after treatment, c) and 39 days after sowing (d), of genotypes common between Experiments 2, 3, 6, and 7. Cadoux, Drysdale, Gladius, Reeves, and Waagan were not assayed in Experiment 3.



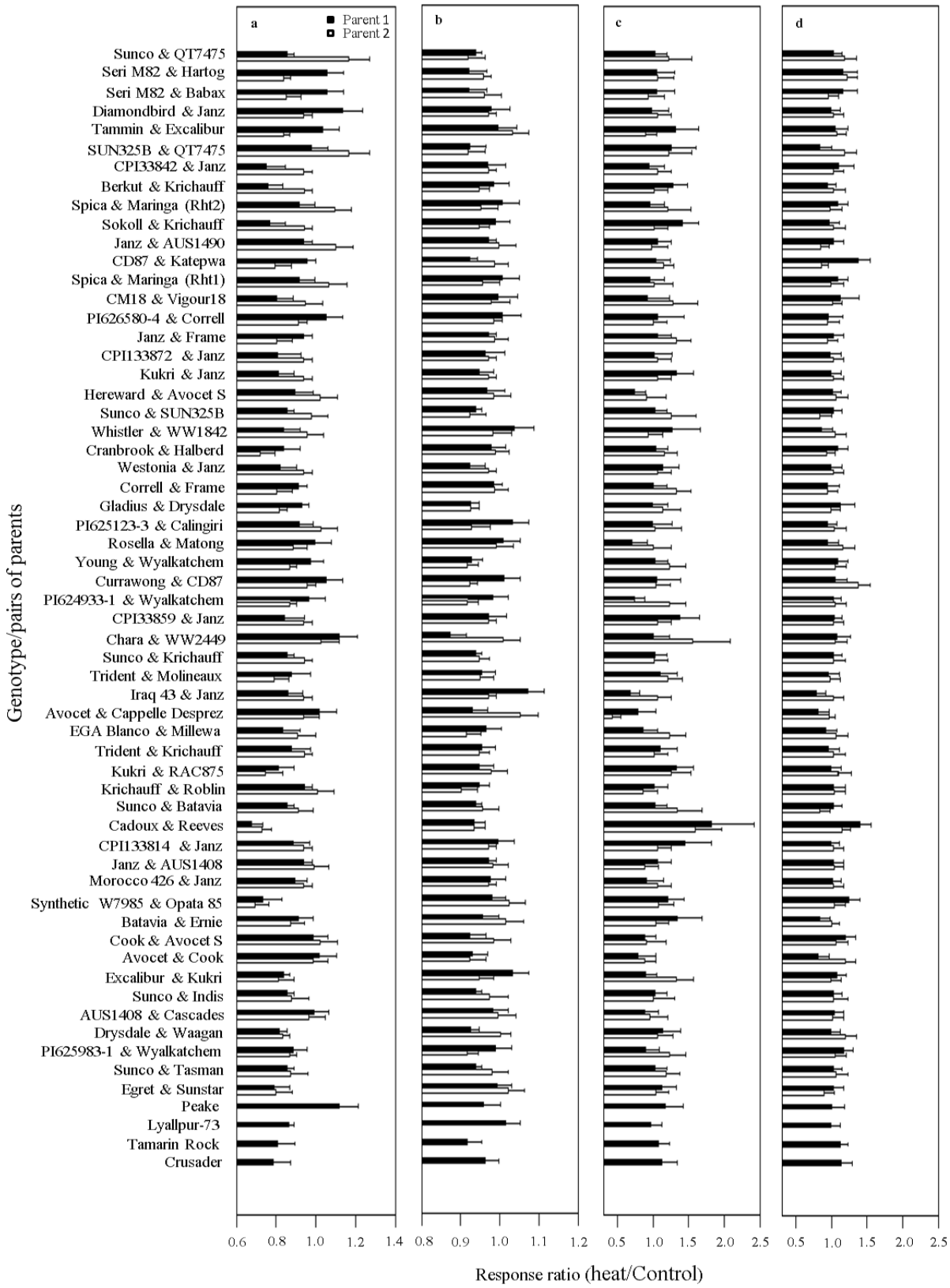
Appendix 7. 2 Rank of the genotypes common between Experiments 2, 3, 6, and 7 for response ratios of relative growth rate during treatment (from 25 to 28 days after sowing, RGRDT; a), relative growth rate from 28 to 39 days after sowing (RGRAT; b), proportion of senescent area at 28 (PSA28DAS; c) and 39 (PSA39DAS; d) days after sowing. Cadoux, Drysdale, Gladius, Reeves and Waagan were not assayed in Experiment 3. Higher rank (smaller number) indicates genotypes with greatest tolerance. Genotypes were ordered by average tolerance rank across experiments for each trait.



Appendix 7. 3 Response ratio (+ S.E.) of relative growth rate during treatment (from 25 to 28 days after sowing, RGRDT; a), relative growth rate from 28 to 39 days after sowing (RGRAT; b), proportion of senescent area at 28 (PSA28DAS; c) and 39 days after sowing (PSA39DAS; d) of 77 genotypes. Where genotypes were used in multiple experiments, the mean across experiments were used. Genotypes were sorted from those showing the greatest positive response (increase) to the ones showing the greatest negative response (decrease) for RGRDT.



Appendix 7. 4 Water use (i.e., water transpired by plants; mg, a and b) and absolute growth (pixels, c and d) of 3 wheat varieties from 25 to 28 (during treatment; a and c, respectively) and 28 to 34 (after treatment; b and d, respectively) days after sowing. Bars indicate mean + S.E.



Appendix 7. 5 Response ratio (+ S.E.) of relative growth rate during treatment (from 25 to 28 days after sowing, RGRDT; a), relative growth rate from 28 to 39 days after sowing (RGRAT; b), proportion of senescent area at 28 (PSA28DAS; c) and 39 (PSA39DAS; d) days after sowing. Mapping parents used to make a population are paired, and pairs are ordered by highest to lowest contrast for RGRDT. Genotypes assayed in the absence of a corresponding second parent are listed at the end of the graphs.