GENETIC MAPPING AND PHYSIOLOGICAL ANALYSIS OF HEAT TOLERANCE IN WHEAT

Submitted by

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This thesis is submitted in fulfilment of the requirement for the degree

Master of Philosophy in Agriculture (MPAGRIC)

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February, 2018

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Abbreviations

Symbol	Definition
ACPFG	Australian center for plant functional genomics
AI	Auricle interval
AIToAwnEm	Days from auricle interval to awn emergence
AIToMat	Days from auricle interval to maturity
Anth	Days to anthesis
AnthToFLSen	Days from auricle interval to flag leaf senescence
AnthToFLSen	Days from anthesis to flag leaf senescence
AnthToMat	Days from anthesis to maturity
AUSC	Area under SPAD curve
AwnEm	Days to awn emergence
AwnL	Awn length at maturity
BAC	Bacterial artificial chromosome
BLAST	Basic local alignment search tool
BotGnNoSpklt>2	Grain number per spikelet at the third and above floret positions in the bottom third of the spike
BotGnNoSpklt1&2	Grain number per spikelet at floret position 1&2 in the bottom third of the spike
BPA	Bioplatforms Australia
BSA	Bovine serum albumin
CAPS	Cleaved amplified polymorphic sequence
CIM	Composite interval mapping
cM	Centimorgan
CS	Chinese spring
CSIRO	Commonwealth scientific and industrial research organisation
CulmL10	Culm length at 10 DAA
CulmLMat	Culm length at maturity
DAA	Days after anthesis
DArT	Diversity arrays technology
DAWN	Diversity among wheat genome
DaysToMat	Days to maturity
DevSpklt	Developed spikelet
DH	Double haploid
FlagL	Flag leaf length at 10 DAA
FLSen	Flag leaf senescence
FlagW	Flag leaf width at 10 DAA
G	Genotype
GBS	Genotyping by sequencing
GFD	Grain filling duration
GnNoSpk	Grain number spike^{-1}
GnNoSpklt	Grain number spikelet^{-1}
GnNoSpklt>2	Number of grains per spike at floret positions >2
GnNoSpklt1&2	Number of grains per spike at floret positions 1+2
GnWSpk	Grain weight spike^{-1}
GRDC	Grains Research and Development Corporation
GWS	Grain weight per spike
HSI	Heat susceptibility index

Abbreviations continued…

Symbol	Definition
HTISC	High-temperature-induced seedling chlorosis
IWGSC	The international wheat genome sequencing consortium
KASP	Kompetitive allele specific PCR
Kb	Kilobase
LOD	Logarithm of odds
LSD	Least significant difference
MAS	Marker assisted selection
Mb	Megabase
MidGnNoSpklt>2	Grain number per spikelet at the third and above floret positions in the middle third of the spike
MidGnNoSpklt1&2	Grain number per spikelet at floret position 1&2 in the middle third of the spike
NIL	Near isogenic line
NSW-DPI	New South Wales Department of primary industries
PBC	Pseudo black chaff
PCR	Polymerase chain reaction
PedL10 DAA	Peduncle length at 10 DAA
PedL10ToMat	Peduncle length between 10 DAA and at maturity
PedLMat	Peduncle length at maturity
PropSpltUndDev	Proportion of under development spikelet per spike
QTL	Quantitative trait locus
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphisms
RIL	Recombinant inbred line
SGW	Single grain weight
ShootW	Shoot weight
ShW	Shoot weight
SingGW	Single grain weight
SNP	Single nucleotide polymorphism
SowToAI	Days from sow to target auricle interval
SowToAnth	Days from sow to target anthesis
SPAD	Soil plant analysis development
SPAD10 DAA	Chlorophyll content at 10 DAA
SPAD13 DAA	Chlorophyll content at 13 DAA
SPAD27 DAA	Chlorophyll content at 27 DAA
SpkLMat	Spike length
SSR	Simple sequence repeat
T	Treatment
TopGnNoSpklt>2	Grain number per spikelet at the third and above floret positions in the top third of the spike
TopGnNoSpklt1&2	Grain number per spikelet at floret position 1&2 in the top third of the spike
TotSpltSpk	Total spikelet per spike
TPA	The plant accelerator
UnderdevSpklt	Under developed spikelet
UnderdevSpklt	Number of basal under-developed spikelets per spike
WSC	Water soluble carbohydrate

Abstract

High temperature induced loss of wheat production is a global phenomenon and posing a threat to food security. This study is focused on the genetic mapping of heat tolerance traits of wheat to understand the genetics and underlying mechanism of heat tolerance at reproductive stages.

A total of six varieties of varying heat tolerance for floret fertility and grain filling were tested for the effects of heat applied at 5 cm auricle interval (AI) and 10 days after anthesis (DAA) tiller stages in the presence and absence of shallow standing water to investigate the potential effects of standing water on the responses to this heat treatment protocol (Chapter 2). Heat reduced grain number, chlorophyll content and single grain weight in heat susceptible varieties but not in the tolerant genotypes. No additional effect of standing water was observed therefore keeping plant pots in standing water could be considered as safe watering method for this heat tolerance screening protocol.

In Chapter 3, 34 homozygous NILs from nine families contrasting for two grain filling and chlorophyll heat tolerance QTLs, *QHsgw.aww-3B* and *QChlr13.aww-6B*, were phenotyped to study effects of the QTLs on heat tolerance. Heat treatment reduced single grain weight in three lines containing the Drysdale (intolerance) allele at the 3B locus, relative to their corresponding tolerant sibling lines, while no significant effects of the QTL were found in the remaining lines. Shoot weight and culm length at maturity, and anthesis date, remained unaffected for both QTL alleles after heat treatment. Lines carrying the Drysdale allele at *QHsgw.aww-3B* locus showed a small amount of chlorophyll loss just after heat treatment but the loss increased by two weeks after heat treatment, and the loss was greater than in the lines carrying the tolerance allele from Waagan. Homozygous NIL pairs from the WW30674 family showed contrasting phenotypes for all the key traits and had also resulted in recombination in the 3B locus region, allowing the locus to be delimited further.

In Chapter 4, further mapping was undertaken to further delimit the *QHsgw.aww-3B* locus on the tip of the short arm of chromosome 3B. New markers that were further distal, or targeting the large gap in the map between positions 3.2 and 34.6 cM, were designed using available genetic maps and genome sequence information. Twelve new markers were developed, of which two were positioned distal of the distal-most markers from the previous map, four were

mapped 1.5 cM proximal of the previous most distal marker, and two of which were generated in the upper part of the gap region.

In Chapter 5, the stem rust resistance gene *Sr2*, and genes *NRT2.5* and *GoGat* involved in nitrogen utilization, were tested as candidates for the *QHsgw.aww-3B* heat tolerance effect. The *csSr2* semi-diagnostic marker for *Sr2*, the pseudo black chaff pleotropic effect of *Sr2*, and the *Lr27* leaf rust resistance locus tightly linked gene to *Sr2* were scored in 144 Drysdale x Waagan DH lines. All of these loci were found to be tightly linked to the heat tolerance effects, hence the *Sr2* stem rust resistance gene (or the gene encoding PBC, if different from that conditioning rust resistance), was considered to be a good candidate for the gene controlling the heat tolerance effect. Marker assays designed for *NRT2.5* and *GoGat* failed to show polymorphism. A panel of 101 hexaploid wheat genotypes, for which there were grain filling and chlorophyll heat tolerance data available, were scored for *csSr2*, to further test the link between *Sr2* and the 3B heat tolerance locus. On average, genotypes carrying the null *csSr2* marker allele (associated with rust susceptibility at *Sr2*) appeared more tolerant to the effects of heat on final grain size than those carrying either the second marker allele associated with rust-susceptibility (Marquis allele), or the resistance-associated marker allele (CS (Hope 3B) allele). Therefore, selection of *Sr2* stem rust resistance in breeding might come at a cost of enhanced heat susceptibility, and if not selecting for *Sr2*, the particular rust-susceptibility allele that is present may influence heat tolerance. If they are not the same gene then they could be separated through breeding.

In Chapter 6, a population of 250 Young x Reeves DH lines, with parents previously shown to contrast for heat tolerance of grain filling and floret fertility, were used to identify heat tolerance QTL. Plants were heat treated at the 6 cm AI and 10 DAA stages to target the effects on grain number and grain size, respectively. No grain size heat tolerance QTL were detected. Two floret fertility heat tolerance QTL were detected, on chromosomes 2B and 6A.

In Chapter 7, 21 Australian hexaploid wheat varieties were screened for heat tolerance applied at 6 cm AI and 10 DAA to identify tolerant varieties for the farmers and breeders. Baxter and EGA Gregory were classified as tolerant for both effects.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

I 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 Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Mohammed Mizanur Rahman

Signature Date: 10/07/2018

Acknowledgements

I would like to express my gratitude to the University of Adelaide for providing me with an Australian Postgraduate Award (APA) scholarship, enrolment and providing research facilities. I also extend my gratefulness to ACPFG for temporary top up scholarship and GRDC for providing operational cost of my research.

My sincere gratitude goes to my principal supervisor Dr. Nicholas Collins for providing all the support to finish this MPhil program. I am indebted to him for continuous guidance with utmost patience, and encouragement. I find him wise, humble, and hardworking. I am in shortage of words to thank him for everything. Having a supervisor like him was truly a privilege for me.

I would like also to thank my co-supervisor Dr. Ute Baumann and independent advisor Dr. Tim March for their great guidance and support. Also Nathan Watson-Haigh for guidance on accessing wheat genome sequence data.

I am grateful to Dr. Julian Taylor and Sabela Munoz-Santa for helping me with experimental deign and some statistical analysis.

I sincerely acknowledge the technical support received from TPA staff Robin Hosking, Richard Norrish, Paul Jenkins and Lidia Mischis for providing good greenhouse and growth chamber facilities, pest management and climate log data.

Thanks and huge appreciation goes to Iman for his brilliant technical support and inspiration. Thanks to Million for his help to solve statistical problems, being a good friend and extending support on different occasions.

I express my heartfelt gratefulness to my family members for supporting and encouraging me during this one of the hardest time I have ever passed through.

My all praises go to almighty Allah for giving me patience and strength to overcome all the hurdles.

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 supervising whole research work, data analysis, interpretation, and suggestions on thesis text.

Dr. Ute Baumann and Nathan Watson-Haigh for their constructive suggestions for further mapping (Chapter 5).

Mr. Iman Lohraseb for his help with the experiment presented in Chapter 6. He helped in data collection, DNA extraction and fertility scoring.

Dr. Julian Taylor and Sabela Munoz-Santa for providing experimental designs, statistical analysis for Chapters 2, 6 and 7. Dr. Julian Taylor also constructed the Young x Reeves map.

Chapter 1 : Literature review and aims

1.1 Wheat is an important cereal crop

Wheat is one of the three most dominant cultivated cereal crops (Gustafson et al. 2009; Shewry, P 2009; Zohary & Hopf 2000) with a global annual production of 749.46 million tonnes(FAOSTATa). Since its domestication around 10,000 years ago (Dubcovsky & Dvorak 2007) cultivation of wheat spread over 100 countries (Shewry, P 2009), including 200 million hectares of farmland (Ortiz et al. 2008) in the northern and southern hemisphere and the highlands of tropics and sub-tropics (Feldman M 1995; Reiner L). World wheat production is 95% hexaploid bread wheat (*Triticum aestivum* L.) and 5% durum wheat (*Triticum durum*) (Peng, Sun & Nevo 2011; Shewry, P 2009). Wheat plays a crucial role in the global agricultural economy and food security as one of the world's most important staple crops. It is a staple food for over 35% of the world's population (Paux et al. 2008), accounts for 20% of human consumption of calories (FAOSTATb) and is an important source of protein, vitamins, carbohydrates and minerals. Demand for wheat as human food is expected to grow by 1.6% per annum, and as animal feed by 2.6% per annum, in developing countries, until 2020. The global average wheat yield will have to increase during the coming 25 years from 2.6 to 3.5 tonnes/ha to meet the demand of the projected increases in population (Ortiz et al. 2008).

1.2 Effect of heat stress on wheat production

Like in other cereals, wheat production is affected by biotic and abiotic stresses and heat is one of the major abiotic stresses in wheat. Temperatures above the optimum for growth are deleterious, causing injury or irreversible damage, which is generally called 'heat stress' (Wahid et al. 2007). Heat stress is a function of the magnitude and rate of temperature increase, as well as the duration of exposure to the raised temperature (Wahid et al. 2007). The capability of crop plants to survive and produce economically viable grain yield under heat stress is heat tolerance (Wahid et al. 2007).

In the last three decades global wheat production has fallen 5.5%, an amount equal to the annual wheat production in France (33 MT). Due to heat there was a 15% decline in wheat production in Russia alone during 1980-2008 (Lobell, Sibley & Ortiz-Monasterio 2012). Even a brief period of heat stress (>35 °C) affects wheat yield and grain quality (Graybosch et al. 1995; Mason et al. 2010; Rane & Nagarajan 2004; Wardlaw & Wrigley 1994). Asseng, Foster and Turner (2011) showed that there are on average 1-5 days with >34 °C during grain filling at

locations across the Australian wheat belt. Their modelling suggested a yield reduction by 5% for each such day, because of a dramatic acceleration of leaf senescence proportionate to the number of such shocks (Asseng, Foster & Turner 2011). In the Mediterranean region, the USA (Graybosch et al. 1995; Mason et al. 2010), India (Rane & Nagarajan 2004) and Australia, heat stress at grain filling reduces yield significantly. Annual average yield losses of 10- 15% (~AUD 300-400 M) due to heat were estimated for Australia and the USA (Wardlaw & Wrigley 1994). Analysing heat events across the Australian wheat belt based on 50 years of historical records it was found that winter temperature is increasing during pre-flowering which causes serious damage to yield, therefore warmer winters would shorten the wheat season by up to 6 weeks (Zheng et al. 2012).

Under moderate temperature stress during grain filling (25–32 °C), wheat grain yield declines by 3-4% for each 1 °C rise in average temperature above 15 °C under both controlled conditions (Wardlaw, Dawson & Munibi 1989b; Wardlaw et al. 1989a) and field conditions (Dhadhwal 1989; Wiegand & Cuellar 1980). This phenomenon affects about 9 million hectares of wheat grown in tropical and subtropical areas which experience temperatures above 17 °C even in the coolest month of the growing season (Ortiz et al. 2008). The Indo-Gangetic Plains contribute 15% of global wheat production but by 2050 about 51% this area is predicted to be reclassified as a heat-stressed as a result of climate change (Ortiz et al. 2008).

Global warming is characterized by shifts in weather patterns with increases in the frequency and magnitude of extreme weather events. Increasing temperature and incidence of drought are posing serious threats to food security (Lobell, Sibley & Ortiz-Monasterio 2012). The global average temperature of both land and sea increased 0.85 from 1880 to 2012 and is predicted to increase a further by 1.5 to < 2 °C by the end of this century (Pachauri et al. 2014). It is projected that Southern Australia and Western Australia will experience an increase of 2.2 to 2.5 °C, and 10% decreased rainfall, by 2070 (Cai & Cowan 2008). Future climates will also be characterized by greater variability in temperature and increased frequencies of hot days (Pittock 2003). Therefore, a major concern arises for the long-term productivity and sustainability of cropping systems under future climate conditions (Anwar et al. 2013; Challinor et al. 2014; Rodriguez, Cox & Power 2014; Stokes & Howden 2010). Major wheat-producing regions show a trend of increasing growing season temperatures (Alexander et al. 2006; Gaffen & Ross 1998; Hennessy & Flagship 2008). Therefore to adapt crop varieties to

the future climate, it is necessary to understand how crops respond to elevated temperatures and how tolerance to heat can be improved (Halford 2009).

Burgeoning global population and global warming are putting ever greater pressure on wheat farmers to increase yields. With the global population projected to exceed 9 billion by 2050 (Roberts 2011) researchers, breeders and growers are facing the challenge of increasing world food production by about 79% to meet future demands (Tweeten & Thompson 2008).

1.3 Wheat genetics

Wheat yields have increased significantly in the last century, due to genetic improvements and better management practises (Semenov et al. 2012). Wheat genetics and molecular breeding is complex due to a highly redundant, large (~17,000 Mb) and complex genome compared to other model plants e.g. Rice is 400 Mb and *Arabidopsis thaliana* is 125 Mb (Choulet, Alberti, Theil, Glover, Barbe, Daron, Pingault, Sourdille, Couloux & Paux 2014; Initiative 2000; Martínez-Pérez et al. 1999). The bread wheat genome is a segmental allohexaploid (2n=6x=42, AABBDD) which is a product of two separate hybridisation events involving three progenitors (Choulet, Alberti, Theil, Glover, Barbe, Daron, Pingault, Sourdille, Couloux & Paux 2014). The genome is subdivided into 3 homoeologous groups of chromosomes, the A, B, and D genomes, each comprised of 7 pairs of chromosomes (AABBDD) (Salamini et al. 2002). The genome of tetraploid wheat (also called hard or durum wheat) is 11,000 Mb (Rombauts 2015) and contains two sets of genomes, the A and B (2n=4x=28, AABB) (Choulet, Alberti, Theil, Glover, Barbe, Daron, Pingault, Sourdille, Couloux & Paux 2014).

So far, wheat genetic studies have improved our understanding of genetic mechanisms for disease resistance (Lagudah et al. 2009) but the molecular basis of heat adaptation is poorly understood and no heat tolerance gene has been cloned from wheat (Cossani & Reynolds 2012).

1.4 Molecular markers

Molecular markers (dominant and codominant) are frequently used to identify/categorize individuals on the basis of sequence variations in the genome. A number of different types of polymorphic DNA molecular markers are used in wheat genetic studies including amplified fragment length polymorphisms (AFLPs) (Vos, P et al. 1995); random amplified polymorphic DNA (RAPD) (Williams et al. 1990); restriction fragment length polymorphisms (RFLPs)

(Botstein et al. 1980); simple sequence repeats (SSRs or microsatellites) (Jarne & Lagoda 1996); diversity arrays technology (DArT) (Akbari et al. 2006) and single nucleotide polymorphisms (SNPs) (Rafalski 2002) markers. Advances in high throughput and low cost DNA sequencing and SNP detection technologies e.g. GBS (Elshire et al. 2011), invader assay (Mein et al. 2000), Illumina GoldenGate (Fan, J-B et al. 2003) and DArTSeq (http://www.diversityarrays.com) has revealed a large number of Single Nucleotide Polymorphisms (SNPs) in wheat which are relatively evenly distributed throughout the genome and abundant in number (Berkman et al. 2012). The International Wheat SNP working group has contributed to SNP discovery in wheat and has constructed a marker array containing almost 9,000 features (Cavanagh et al. 2013), and a subsequent version containing 90,000 features (Wang et al. 2014), scorable using the iSelect technology. The Functional genomics group at the University of Bristol, UK (http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB /indexNEW.php) also generated a publicly available SNP marker database and arrays e.g., Axiom 820K and 35K SNP Arrays, KASP probes, TaqMan for wheat research. Platforms such as these can now be used for cost-effective QTL discovery, to produce high-resolution maps and facilitate positional cloning of genes of interest.

1.5 Near Isogenic Lines for evaluating QTL effects

Near Isogenic Lines (NILs), a pair of lines differing only/mainly for a chromosome region containing a specific/targeted locus (e.g., QTL region). Contrasting parents are crossed to obtain F_1 individuals. F_1 lines with the target trait are selected for back crossing with the standard line (the recurrent parent). This process is repeated for multiple generations, each time donor allele is selected in the progenies and back crossed with the recurrent parent, to give a NIL pair (the resulting line, together with the recurrent parent) NILs. RILs heterozygous for a target region could be identified by molecular marker assay and selected as NILs for that target region. NILs offer excellent opportunity to study in detail the effects of the allele/s of interest, including validation of QTL effects in the field, using only a pair of lines (as opposed to the whole original QTL mapping population).

NILs can also be useful to delimit the target locus for a specific trait. To fine map a QTL, recombinant inbred lines (RILs) which are heterozygous at that particular locus can be identified using markers, selfed, and progeny of the two homozygous types (containing contrasting alleles) marker-selected. The progeny of these individuals can then be

phenotyped. Different pairs of RIL-derived NILs may result from different recombination points at the borders of the contrasting chromosome segments, as identified using markers, and phenotyping of the lines would thus help to further delimit the QTL.

1.6 Fine mapping

Fine mapping of a QTL is required to find markers close enough to the QTL to be useful to breeders for marker assisted selection and may lead eventually to the identification of the underlying gene. Alleles of the causal gene can also be used to identify other superior alleles in wheat germplasm. Gene cloning helps reveal the molecular function of the gene, identify homologues and allows trans-genic experiments. It also can also enable design of diagnostic markers, based on DNA sequence within the gene that are always present with the desirable allele, and which are thereby very useful for marker assisted selection (MAS) during breeding (Ogbonnaya et al. 2001).

1.7 Impact of heat on different aspects of wheat

Deleterious effects of heat stress at different developmental stages of wheat are discussed as follows:

1.7.1 Floret death

The wheat spikelet is an indeterminate structure, but as a part of normal development only a proportion of the florets that begin development remain green by anthesis and hence are competent to produce a grain (notwithstanding infertility of individual floret organs caused by stress). Floret abortion begins at booting and ends at about heading or anthesis stage (Kirby 1988; Langer & Hanif 1973; Siddique, Kirby & Perry 1989). Half of the total initial florets within a spikelet are not fertilized due insufficient development, and the florets initiated after the terminal spikelet is initiated do not set grain (Whingwiri & Stern 1982). Floret death occurs during the period of maximal stem and peduncle growth (Siddique, Kirby & Perry 1989) due to competition between the ear and the stem for resources (Kirby 1988). To my knowledge, there have been no reports addressing whether heat could affect grain number by influencing this phenomenon of whole-floret death.

1.7.2 Grain filling (grain size)

Grain filling is sensitive to elevated temperature (Ferris et al. 1998). Heat stress accelerates grain filling rate, hasten senescence (Wardlaw & Wrigley 1994) and physiological maturity

after anthesis (Shpiler & Blum 1986; Warrington, Dunstone & Green 1977) and shortens grain filling duration (Dias & Lidon 2009).

1.7.3 Grain set (number of grains per spikelet)

Heat stress due to moderately high temperatures (>20 °C) between spike initiation and anthesis can substantially reduce grain number per spike (Fischer, RA 1976; Warrington, Dunstone & Green 1977). Grain number per spike decreased by 4% for every 1 °C (from 15–22 °C) increase in the 30 days before anthesis (Fischer, R 1985). Wheat plants exposed to 30 °C for 3 consecutive days or for 3 days to day/night temperatures of 30/20 °C when pollen mother cells were dividing, markedly reduced grain set and therefore grain yield (Saini & Aspinall 1982). Wheat grain set is affected by high temperature in various ways and at different stages. Some of the effects of heat stress are discussed below:

1.7.3.1 Floret sterility due to floret organ failure

Heat, at or around meiosis, has been reported to lead to failed fertilization (sterility), due to failure of individual floret organs. Complete sterility was observed when wheat plants were grown at high temperature (35 °C) during ear emergence and onward (Owen 1971; Saini & Aspinall 1982). Saini and colleagues showed that heat stress can affect both male and female organs in wheat (Saini & Aspinall 1982; Saini, Sedgley & Aspinall 1983). Adverse effects of heat stress on pollen tube growth has also been reported (Saini, Sedgley & Aspinall 1983).

Approximately 60% reduction in pollen viability was observed in cotton after a 5 hour incubation at 39°C (Burke 2007), and reduced pollen viability under heat stress has been linked to altered carbohydrate metabolism and starch deficiency in other cereals like sorghum (Jain et al. 2007; Prasad, PV & Djanaguiraman 2011). Rice plants showed complete spikelet sterility after heat treatment at 39 °C/30 °C for 2-4 days at the microspore stage (Endo et al. 2009).

Although less researched, female reproductive organs are also affected heat stress. Heat stress at meiosis has been reported to result in abnormal ovary development and accelerated stigma and ovule development, which may contribute to reduced pollen tube growth and seed set (Barnabás, Jäger & Fehér 2008).

1.7.3.2 Early grain abortion

Heat stress within the first three days after pollination can lead to early abortion of grain growth (Saini & Aspinall 1982; Saini, Sedgley & Aspinall 1983; Tashiro & Wardlaw 1990b; Wardlaw et al. 1989a). Free nuclei in the developing endosperm multiply in the three days after pollination and heat treatment at this stage may result in abnormal nuclear division, which might explain the appearance of abortive or shrunken grains in heat-treated plants (Tashiro & Wardlaw 1990b).

1.7.4 Photosynthesis, stay green and senescence

The wheat photosynthetic apparatus is affected by heat both functionally and structurally (Baker 1991; Sharkey 2005). Electron transport activity and fluorescence of chloroplasts decreases due to an increase in peroxidation of thylakoid lipids in heat treated leaves (Mishra & Singhal 1992). Thylakoid membranes and photosystem II (PS II) are very sensitive to high temperatures and their destruction under high temperatures can limit photosynthesis (Ristic, Bukovnik & Prasad 2007). Degradation of chlorophyll a and b, separation of light harvesting complex II from PS II (Schreiber & Berry 1977), dissociation of oxygen evolving complex (OEC) from PS II, reduction of photosynthetic pigments, reduction in RuBisCO activity, and other changes in photosynthesis machinery due to heat stress, reduce photosynthesis rate (Wahid et al. 2007).

Photosynthesis is related to the stay-green trait. Stay-green is the ability of plants to delay senescence and maintain green leaf area during the reproductive stage. Senescence reduces chlorophyll which in turn affects photosynthesis and photo-assimilate supply. Heat stress accelerates loss of chlorophyll, hence stay-green can also represent the reduction of this stress induced effect.

Stem reserves and current photosynthesis contribute to grain growth. Under optimum conditions, stem reserves contribute less to grain growth compared to the current photosynthesis, while under heat stress conditions when current photosynthesis is impaired it tends to contribute proportionately more to grain growth, depending on the genotype (Blum 1998; Yang et al. 2002). These studies suggest that maintaining photosynthate supply to the developing grain, either through maintaining access to high levels of stem reserves or sugars from current photosynthesis, may play a role in tolerance of grain filling under heat-stress conditions.

Heat stress hastens the senescence-related metabolic changes in wheat (Al-Khatib & Paulsen 1999; Paulsen 1994) by inhibiting chlorophyll biosynthesis (Tewari & Tripathy 1998) and accelerating the breakdown of thylakoid components (Harding, Guikema & Paulsen 1990). 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 (Distelfeld, Avni & Fischer 2014). Positive associations have been reported between stay-green and grain yield in wheat (Kumari, Goyal & Jain 2013; Lopes & Reynolds 2012; Reynolds et al. 1994; Reynolds et al. 1998).

Stay-green sorghum genotypes under drought stress at grain filling stage exhibit increased xylem pressure potential, delayed loss of photosynthetic competence, modification of canopy development, leaf anatomy, root growth, water uptake, and enhanced nitrogen uptake (Borrell et al. 2014a; Borrell et al. 2014b; Tuinstra, Ejeta & Goldsbrough 1998; Vadez et al. 2013). Delayed senescence is positively correlated with high water use efficiency during grain filling (Gorny & Garczynski 2002) and a root architecture that allows water to be extracted from deep in the soil profile post-anthesis under field conditions (Christopher et al. 2008; Kirby 1988).

Silva et al. (2001) reported control of stay-green by a single locus, showing high heritability and partial dominance in crosses of four contrasting genotypes of bread wheat. Joshi et al. (2007) found stay-green to be controlled by around 4 additive genes. Vijayalakshmi et al. (2010) observed polygenic inheritance of stay-green in recombinant inbred line (RIL) populations under field and controlled environment under high temperature conditions. A stay green durum wheat mutant showed increased leaf area and grain filling rate (Spano et al. 2003). 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).

Positive correlations were found between stay green and heat tolerance for grain weight under late season heat and drought conditions (Distelfeld, Avni & Fischer 2014; Naruoka, Y. et al. 2012). However, stay-green may have negative impact on yield under regular conditions (Derkx et al. 2012; Kichey et al. 2007; Kipp, Mistele & Schmidhalter 2014; Naruoka, Y et al. 2012) because it might hamper remobilization of assimilate reserves to grains, resulting in more of the storage carbohydrates remaining in the straw (Yang et al. 2002). 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, Brunsting & Van Laar 1974; Hirel et al. 2007; Kipp, Mistele & Schmidhalter 2014).

1.7.5 Dough quality

Wheat proteins gliadins and glutenins are associated with dough extensibility and elasticity respectively. Glutenins link via disulphide bonds to form high molecular weight polymers which confer the elasticity to dough (Ali et al. 2010; Shewry, PR et al. 2000). A shorter grain filling period as a result of heat stress lead to reduced disulphide bound formation due to shortening of the disulphide bond formation process (Blumenthal, C et al. 1994). Extensibility and elasticity are important factors in bread baking performance because of their contribution to the dough strength and ability of dough to rise and maintain its shape as it is baked. Heat stress (>35 °C) for 3 days during grain-filling can reduce dough strength (highest resistance to dough mixing) by 50% (Blumenthal, C et al. 1995), leading to a loss of quality for bread making (Blumenthal, CS et al. 1991; Blumenthal, C et al. 1995; Corbellini et al. 1998). Exposure to 32 °C for 1-4 days during the grain-filling period can damage wheat quality by altering starch and protein composition (Wardlaw & Wrigley 1994).

Stone and Nicolas (1994) studied grain yield and quality in response to short periods of high temperature in five wheat cultivars and the gliadin: glutenin ratio was found to be altered in the range -9 to +18% depending on variety. Proteomic analysis of grains from heat treated plants showed that under heat stress expression of several gliadins were increased but not glutenins.

1.7.6 General mechanisms of heat tolerance and damage

Some general mechanisms of heat tolerance and damage are discussed below:

1.7.6.1 Cell function

Plants react to changes in ambient temperature through changes in metabolism, membrane fluidity, protein conformation and assembly of the cytoskeleton (Ruelland & Zachowski 2010). 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 are involved in responses of plant cells to heat (Qin et al.

2008). Heat has been documented to negatively affect cellular function in several ways. High temperature alters membrane fluidity (Alfonso et al. 2001; Sangwan et al. 2002) and enzyme function through denaturation (Kampinga et al. 1995; Vierling 1991). Heat stress induced membrane and protein damage can result in elevated concentrations of reactive oxygen species (ROS) that in turn create oxidative stress which can be harmful to plant tissues (Almeselmani, Deshmukh & Sairam 2009; Mittler 2002; Sairam, Srivastava & Saxena 2000). Hence detoxification of ROS by enzymatic and non-enzymatic antioxidant systems (Noctor & Foyer 1998) are important for protecting plants against heat stress. The activities of antioxidants (e.g. superoxide dismutase and catalase) increase when heat stress (34/22 °C) is applied during the reproductive phase (Zhao et al. 2007). Heat stress can also induce programmed cell death (Swidzinski, Sweetlove & Leaver 2002; Vacca et al. 2004) and activate expression of heat shock proteins (HSPs) as a protective mechanism (Blumenthal, C et al. 1994).

1.7.6.2 Hormone signalling

Plant growth and development is regulated by hormones (Santner & Estelle 2009). Ethylene is a hormone known to regulate growth and development and to trigger senescence and maturation in wheat (Beltrano, Jose et al. 1994; Khan 2006; Pratt & Goeschl 1969; Schaller 2012). Increased ethylene production in response to heat is associated with short grain filling period, decreased 1000 kernel weight and accelerated maturity (Beltrano, J, Ronco & Montaldi 1999).

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, since endogenous application of an ethylene receptor inhibitor reduced heat stress induced kernel abortion kernel weight reductions in an otherwise susceptible wheat genotype (Hays et al. 2007). Enhanced production of ethylene in the wheat spike has also been found during or after recovery from water stress (Beltrano, José, Montaldi & Carbone 1997; Beltrano, J, Ronco & Montaldi 1999; Morgan et al. 1990; Narayana, Lalonde & Saini 1991). Ethylene is also known to reduce root and embryo growth (Wilkinson, Sally & Davies 2010). In soybean, heat stress increased ethylene production rate which triggered premature leaf senescence (Djanaguiraman & Prasad 2010).

Application of gibberellic acid to seeds of a heat tolerant barley led to loss of tolerance due to loss of membrane stability and physiological damage to the photosynthetic apparatus (Vettakkorumakankav et al. 1999). Exogenous application of cytokinin at the ear emergence stage increased the number of grain endosperm cells, and increased grain weight and grain filling duration under normal temperatures (Alizadeh, Haghighi & Ordookhani 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, Ammar & Chen 1999). This suggests that cytokinins might have a role in the heat stress response.

Root-shoot signalling involving abscisic acid (ABA) under water deficit conditions (Wilkinson, S & Davies 2002). Drought stress at the reproductive stage causes pollen sterility and grain loss in wheat due to ABA accumulation in spikes in drought-sensitive varieties (Ji et al. 2011).

1.7.6.3 Stem water soluble carbohydrate

Positive associations have been observed between stem water soluble carbohydrate content and floret fertility and grain number in wheat under high temperature conditions. High Water Soluble Carbohydrate (WSC) lines were found to have more grains per spike associated with more (~10 more) fertile florets per spike at anthesis and a higher glucose content and biomass spike at booting. At booting, high WSC lines showed higher rates of ^{13}C fixation and higher levels of expression of genes involved in photosynthesis, sucrose transport and lower expression of genes involved in sucrose degradation, compared with Low WSC lines (Dreccer et al. 2014). The ability to set grain under heat might therefore be related to carbon availability, suggesting an area for further in depth study.

1.7.6.4 Water relations

Increased evapotranspiration under high temperatures can increase plant water stress if the soil moisture and hydraulic conductivity of the soil or plant cannot keep up with the evaporative demand and leads to a critically low water potential of leaves and grains (Wahid et al. 2007). Elevated temperature tends to increase hydraulic conductivity of membranes and plant tissues due to increased aquaporin activity, membrane fluidity and permeability (Martínez-Ballesta et al. 2009). Increasing hydraulic conductivity may also be beneficial if water supply is not limiting as it would allow stomata to stay open for longer. Transpiration also serves to cool the plant tissues, thereby alleviating heat stress.

Drought and waterlogging represent opposite ends of the spectrum of water supply. At least for waterlogging, the effect of combining with heat stress has not been widely investigated. Excess rain or irrigation may cause waterlogging and under this condition roots cannot respire due to a shortage of oxygen (hypoxia). Waterlogging can reduce root and vegetative biomass (Huang et al. 1994a; Lee et al. 2007; Malik et al. 2001), photosynthesis (Huang et al. 1994b) and induce leaf chlorosis (Huang et al. 1994b; Lee et al. 2007). In cotton 10% (Bange, Milroy & Thongbai 2004) to 40% (Hodgson 1982) yield loss could be attributed by waterlogging (Collaku & Harrison 2002). Oxygen already has a lower diffusion rate in water than in air (Christianson et al. 2010) and oxygen solubility in water decreases further with increasing temperature.

Low oxygen levels cause rapid changes in gene transcription, protein synthesis/degradation, and cellular metabolism (Bailey-Serres & Voesenek 2008). Waterlogging causes the accumulation of ethylene in the soil which can ultimately impede root growth and function thereby adversely affecting shoot growth (Arshad & Frankenberger Jr 1990; Smith & Russell 1969). It also caused a substantial reduction in grain yield and grain protein content and reduced processing quality of the wheat grain (Fan, X et al. 2004). Zheng, CF et al. (2009) reported that waterlogging decreased protein and starch content in the grains of the two wheat cultivars, Yangmai 12 and Huaimai 17. In heat effect studies researchers put their plant pots (e.g. 8 × 8 cm, 18 cm depth) in trays with shallow water during heat treatment in the growth chamber to minimize the drought effect and to maintain optimum humidity. This arose a possibility of short term waterlogging to the plants leading to potential interference with the observation under heat. Waterlogging combined with heat stress might have deleterious effect on wheat physiology and so far our knowledge goes it has never been studied.

1.8 Aim of the thesis

High temperature for short period at reproductive stage adversely affects both floret fertility and grain filling in wheat. Therefore, understanding of heat tolerance mechanism at these stages in wheat is crucial for further development and enhance wheat production. Present work is focused on the unveiling of underlying genetic mechanism for heat tolerance in wheat.

Standing water creates anoxic condition in soil and considered to be deleterious to wheat production. When heat combines, these two stresses together might cause more damage to wheat plants. In wheat heat researches under greenhouse condition researchers places their

plant pots in trays with shallow water to minimize drought stress and maintain soil moisture during heat treatment. It raises a possibility of getting confounding results from heat research due to short term shallow water logging. Study of the effects of both stresses separately and/or together in varieties of varying heat tolerance for grain number and grain size would allow us to have better understanding of the tolerance mechanism against these stresses under controlled condition. It would also shed light on the reliability of the vastly used soil moisture controlling practice to avoid drought stress in heat researches.

RIL-NILs are generated from recombinant inbred lines (RIL) carrying different recombination around the introgressed region of target QTLs. Marker scoring of the target QTL region helps to identify and select RIL-NILs with desirable recombination. RIL-NILs carrying contrasting introgressed region for two grain filling and chlorophyll heat tolerance QTLs, QTL11 (*QHsgw.aww-3B*) and QTL27 (*QChlr13.aww-6B*) identified by in a Drysdale x Waagan DH population were good candidates for phenotyping to reveal contributions of different chromosome segments in the QTL regions under certain culture condition. It also would aid further narrowing down the segment through further mapping using new markers around this region.

Further mapping of aforementioned grain filling heat tolerance QTL, *QHsgw.aww-3B* (also associated with staygreen) could include development of new markers to narrow down the region. It could lead to identification and cloning of the gene/s controlling the trait and development of a diagnostic marker to enable breeders to select for heat tolerance. Around *QHsgw.aww-3B*, six genetically non redundant loci were identified with a big gap on the map which needs to be filled by additional markers to help delimit the QTL more accurately.

Some genes known in that vicinity of *QHsgw.aww-3B* influence chlorophyll content or stability, namely, the stem rust resistance gene *Sr2* showing heat induced seedling chlorosis as a likely pleotropic effect, and nitrogen use efficiency genes. These genes could be considered as candidates for the gene controlling the *QHsgw.aww-3B* QTL effects. It would be worthwhile to investigate these genes as candidates for *QHsgw.aww-3B* using available resources.

Wheat cultivars Young and Reeves are known to contrast well for tolerance to both the grain filling and grain number effects of heat. A Young x Reeves DH population that is available

would therefore be suitable for mapping new heat tolerance QTL and addressing whether the two types of tolerance are genetically related.

The main objective of this research was to have understanding of the genetic and physiological basis of heat tolerance in wheat. Therefore, a number of experiments were conducted with the following objectives:

1. To investigate the effect of short term standing water during heat treatment on grain size, floret fertility and chlorophyll responses.
2. To evaluate effect of 3B and 6B QTLs for grain filling and chlorophyll heat tolerance in near isogenic lines under controlled conditions.
3. Further mapping of the 3B QTL for grain filling to narrow down the locus and to explore the genes potentially associated with the QTL.
4. To identify QTL associated with floret fertility and grain filling in one mapping population to test for genetic overlap between heat tolerance at these two reproductive stages.
5. To explore genetic variation for heat tolerance for grain filling and floret fertility in a set of Australian elite hexaploid wheat varieties, to provide recommendations on choice of varieties for breeding and cultivation.

Chapter 2: Effect of standing water during heat treatment on grain size, floret fertility and chlorophyll responses

2.1 Introduction

Waterlogging refers to the condition when soil contains excessive moisture resulting in low availability of dissolved oxygen around the root zone of a plant. Excess rain or over watering may cause waterlogging and under this condition roots cannot respire due to a shortage of oxygen (hypoxia). Wheat is susceptible to waterlogging stress and up to 44% yield loss (Collaku & Harrison 2002) and 21% shoot weight loss (Thomson et al. 1992) has been attributed to waterlogging.

Low oxygen availability to the plants may cause rapid changes in gene transcription, protein synthesis/degradation, and cellular metabolism (Bailey-Serres & Voesenek 2008). Waterlogging and oxygen deficiency contribute to decreased root hydraulic conductance due to inhibition of plasma-membrane aquaporins (Bramley & Tyerman 2010). Waterlogging causes the accumulation of ethylene in the soil which can ultimately impede root growth and function thereby adversely affecting shoot growth (Arshad & Frankenberger Jr 1990; Smith & Russell 1969). It may cause a substantial reduction in grain yield, grain protein content and grain processing quality in wheat (Fan, X et al. 2004; Zheng, Chunfang et al. 2009). Waterlogging even for a short period of time can negatively affect plant growth (Malik et al. 2002). Greenhouse experiments also show that long-term waterlogging of soil can induce early leaf senescence after anthesis in wheat (Nishida, Ida & Tanaka 1993) and in barley (Ishikawa, Takeuchi & Shiroishi 1953). Reduction in grain weight, and mobilization of stem water soluble carbohydrate (Araki et al. 2012) was observed in wheat due to waterlogging at stem elongation and anthesis stages.

High temperature at the reproductive stage is one of the major yield reducing environmental factors in wheat production (Stone et al. 1995; Wardlaw, Sofield & Cartwright 1980). Diffusion rate of oxygen is lower in water than in air (Wilke & Chang 1955) and it decreases further with increasing temperature (Mysels 1955). Hence, yield reductions in wheat due to waterlogging in colder areas are less severe compared to the more temperate and tropical areas of the world (Samad et al. 2001). Wheat plants under combined stresses of waterlogging and heat might experience more deleterious effects due to increased hypoxia.

In Western Turkey with a typical Mediterranean climate, waterlogging is one of the major yield reducing environmental factors for wheat (Giorgi & Lionello 2008; Sayre et al. 1994; Yavas, Unay

& Aydin 2012). Western Japan experiences waterlogging due to heavy precipitation during the wheat growing season especially after the heading stage which might trigger abnormal early ripening in wheat (Araki et al. 2012). In India, increases in winter rainfall (Dash et al. 2007; Francia et al. 2005) and temperature due to climate change are evident (Gupta et al. 2010; Ortiz et al. 2008), therefore combined heat and short term waterlogging stress is likely to be increasing. In Western Turkey, waterlogging combined with heat stress at tillering and jointing stage has been found to significantly reduce plant height, shoot biomass and single plant yield in eight wheat varieties (Yavas, Unay & Aydin 2012). Waterlogging effects are well studied at the vegetative stage in wheat (Musgrave 1994; Sharma & Swarup 1988) but not at the reproductive stage or in combination with short term heat stress.

Waterlogging and heat stress reduce wheat yield, therefore tolerant varieties for these stresses would benefit farmers. Study of wheat responses to these stresses may reveal novel tolerance mechanisms/genes that are specific to the combined stresses, hence should reveal whether tolerance to a third type of stress (the two stresses combined) is needed.

Increased temperatures increase the evaporative demand on plants and hence leaf transpiration rates. Accordingly, in various studies of heat responses at reproductive stages in wheat (Pradhan et al. 2012; Prasad, PVV et al. 2008; Randall & Moss 1990; Shirdelmoghanloo, Taylor, et al. 2016; Stone & Nicolas 1994; Tashiro & Wardlaw 1990b) researchers placed their plant pots into trays with shallow water (~2 cm depth) during the heat treatment to minimize drought stress. This is a convenient method for maintaining soil moisture during heat treatment but it also raises the concern that measures of heat tolerance obtained from those experiments might be confounded by (or even completely derived from) waterlogging effects. Therefore, an experiment was conducted to test the impact of this watering method on wheat in heat tolerance experiments (in non-heated controls or heat-treated plants). This exercise was expected to (a) help determine if there were any tolerance mechanisms specific to the combined stresses, and (b) indicate if this method of watering was appropriate for screens intended to assess tolerance to heat stress only.

2.2 Materials and Methods

2.2.1 Plant materials

Six wheat genotypes were used in this experiment. Four varieties: Westonia, Drysdale, Waagan, Kukri, and two breeding lines: HTWYT_12 (from CIMMYT) and RAC875 (from the University of

Adelaide breeding program). These genotypes previously showed contrasting levels of tolerance to the floret sterility and grain filling effects of heat stress (Erena 2015; Shirdelmoghanloo 2011; Lohraseb and Collins 2014; unpublished) (Table 2.1).

Table 2.1 Fertility and grain filling heat tolerance of six genotypes.

Genotype	Fertility tolerance index	Grain filling tolerance index
Drysdale	0	0
HTWYT_12	3	0
Kukri	1	1
RAC875	2	2
Waagan	3	4
Westonia	0	4

* Subjective classification from 0 to 4, with 4 representing most tolerant.

2.2.2 Plant growth conditions

Two seeds per pot were sown on the 13th March, 2015, then following germination, the plants were thinned to one per pot. Plastic pots (8 × 8 cm, 18 cm depth) contained a steam-sterilized mixture of coco peat: Waikerie sand (2:1; pH 6.0-6.5) containing the following added nutrients (mg/L): agricultural lime: 561, dolomite lime: 202, hydrated lime: 131, iron phosphate: 505, iron chelate: 33.7, gypsum: 202, super phosphate: 202, trace elements (Scotts Micromax): 202, calcium nitrate: 505 and slow-release fertilizer pellets (Mini Osmocote): 2022.

Two naturally lit greenhouse rooms 29 and 30 and a growth chamber WI.5 (BDW120, Conviron Asia Pacific Pty Ltd. Melbourne, Australia), located in The Plant Accelerator (TPA, The University of Adelaide, Waite Campus, Adelaide), were used in this experiment.

Greenhouse conditions were recorded every 30 minutes using a data logger. Measured greenhouse conditions during the experiment are shown in Table 2.2. Greenhouse rooms were equipped with evaporative cooling systems which are capable of cooling ~10°C relative to the outside temperature therefore actual temperature on some days were higher than the set maximum temperature due to high outside temperature (Table 2.2).

Growth chamber conditions were similarly logged. Temperature was maintained very close to the programmed regime (not shown): maximum (37°C) and minimum (27°C) temperature of the growth chamber was held for 8 and 10 hours respectively each day with 3-hour transition periods used either side to linearly ramp the temperature up and down. Lighting (a mixture of

metal halide and tungsten incandescent) was set at 630 µM $m^{-2}s^{-1}$ at spike height for 10 hours each day, with 2-hour transition steps either side at 460 µM $m^{-2}s^{-1}$. Humidity was not controlled and averaged 51.5% relative humidity during heat treatments.

For the control watering regime (normal watering) pots were placed in plastic tubs containing drain holes (12 pots to a tub) to prevent the tall pots from falling over, and plants were watered from the top often enough to avoid any drought stress. For the standing-water treatment, plants were placed in tubs lacking drain holes and containing ~2 cm depth of water.

Table 2.2 Measured temperatures (°C) and relative humidity in greenhouse rooms. Set maximum-day/minimum-night temperatures were 20/16 °C.

	Month	Daily average	Average daily min.	Average daily max.	Days >30°C	Average % relative humidity
Room 30	Mar	22.1	15.8	31.7	5	62.8
	Apr	19.2	14.6	28.0	0	70.0
	May	18.1	14.3	25.9	0	77.3
	Jun	18.0	14.2	24.5	0	73.0
	Jul	18.3	14.6	22.8	0	66.2
	Aug	19.5	14.9	34.8	9	60.5
	Sept	17.5	15.1	21.8	0	62.3
Room 29	Mar	21.7	17.6	29.5	0	56.3
	Apr	21.0	17.3	25.8	0	53.0
	May	19.3	14.6	26.0	0	60.1

2.2.3 Treatments

Watering/temperature treatments were applied at two different growth stages to study their effect on grain size and grain number. Booting stage (around meiosis) is sensitive to heat effects on floret fertility (Saini & Aspinall 1982), so plants were moved for treatment when the distance between the base of the main tiller flag leaf blade and the blade of the next leaf down (auricle interval, AI) was 5 cm. Grain filling in wheat is sensitive to elevated temperature ~5 days before anthesis to 10-15 days after anthesis (DAA) (Savin et al. 1999; Wardlaw & Willenbrink 1994; Wardlaw & Wrigley 1994) therefore early grain filling (10 days after anthesis) was used to target effects on grain size.

All plants were initially grown in greenhouse room 30. When the plants reached the targeted developmental stage, they were moved to other locations for the 3-day treatments (growth chamber for heat or greenhouse room 29 for treatments not involving heat, including control) and then returned to greenhouse room 30 to reach maturity. Treatments without heat were

done in another greenhouse room, mainly as there was insufficient space in room 30 for the additional trays. Room 29 was adjacent to room 30 and had the same temperature settings.

Seven treatments viz. T1, T2, T3, T4, T5, T6 and T7 (Table 2.3) combining heat or no heat with standing water or drained conditions, were applied. One treatment (no heat and free-drainage; T1) was intended to serve as a 'universal' control for treatments applied at either 5 cm AI or 10DAA. Plants for T1 were also moved to greenhouse room 29 at a randomly selected stage (5 cm AI), to subject these plants to movement, in case movement itself had any effect.

Table 2.3 Experimental treatments.

Treatment name	Heat treatment	Standing water treatment	Developmental stage at which treatment applied	For treatment plants moved to	Target traits
T1	No	No	Moved when AI 5 cm	Room 29	Grain number and size
T2	No	Yes	Booting, 5cm AI	Room 29	Grain number
T3	No	Yes	Grain filling, 10 DAA	Room 29	Grain size
T4	Yes	Yes	Booting, 5cm AI	Growth chamber	Grain number
T5	Yes	Yes	Grain filling, 10 DAA	Growth chamber	Grain size
T6	Yes	No	Booting, 5cm AI	Growth chamber	Grain number
T7	Yes	No	Grain filling, 10 DAA	Growth chamber	Grain size

2.2.4 Experimental design

Three hundred and thirty-six plants were used in this experiment. The experiment employed a split plot layout with 8 blocks (replications) and treatments were randomly allocated to main plots and varieties were randomly allocated to subplots. Table 2.4 presents a summary of the design. Pots remained in the same block when moved to the room 29 or the heat chamber.

Table 2.4 Main features of the greenhouse 30, greenhouse 29 and growth chamber designs.

Place	Layout	Plots	Rows	Columns	Number of treatments	Genotypes	Blocks
Greenhouse 30	Split plot	336	28	12	7	6	8
Greenhouse 29	Split plot	96	8	12	2	6	8
Growth chamber	Split plot	96	16	12	2	6	8

2.2.5 Data collection

A total of 15 traits were measured, with most being scored for only particular treatments (Table 2.5). Except for grain number per spike, scoring of all grain and spikelet number traits and chlorophyll traits were unintentionally omitted for the T1 treatment. Chlorophyll related traits

were measured during development while the other traits were measured at maturity. The primary tiller of each plant was tagged with various colored tape after reaching the target stage to identify which tiller was to be scored afterwards and to confirm treatment status (whether it had been treated or not). All traits were measured from the primary tiller only and these were as follows:

Chlorophyll content

Flag leaf relative chlorophyll content was measured using a portable SPAD chlorophyll meter (SPAD-502; Minolta Co. Ltd., Japan) just before heat treatment at 10 DAA (SPAD10DAA), just after heat treatment at 13DAA (SPAD13DAA) and at two weeks after heat treatment at 27DAA (SPAD27DAA) (Fig. 2.1). Each value was the average of 10 measurements taken from the same leaf section (middle half), along the left-hand side of the flag leaf between the mid-rib and leaf margin. Change in chlorophyll content during the treatment period was calculated as SPAD13DAA - SPAD10DAA, for control and heat treated plants separately. Similarly, chlorophyll change during the period of treatment plus the two weeks after treatment was calculated as SPAD27DAA - SPAD10DAA.

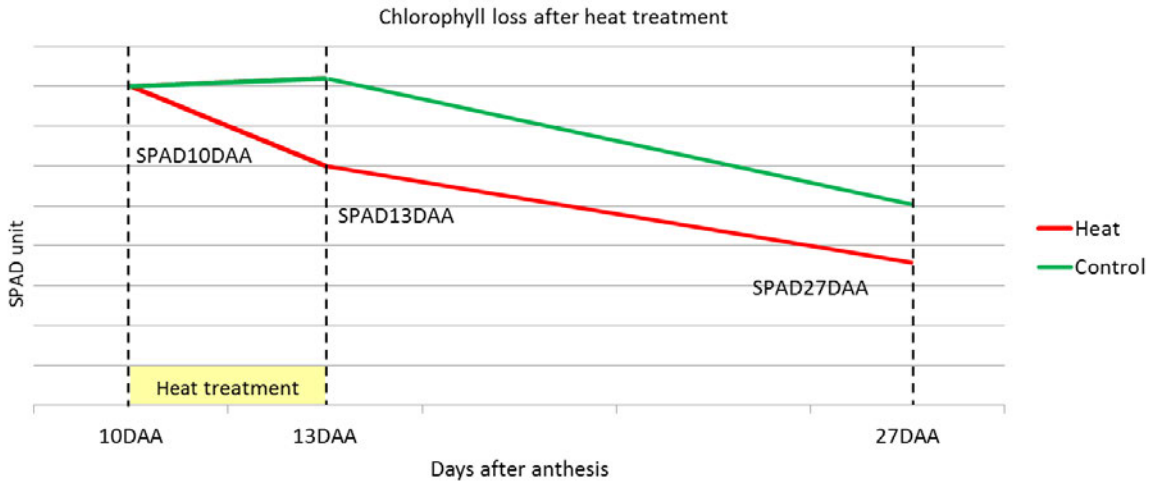


Fig. 2.1 Schematic presentation of trend of chlorophyll loss at 10, 13 and 27DAA under heat and control (Shirdelmoghanloo, Taylor, et al. 2016). Dotted lines and yellow box indicate sampling times and heat treatment period respectively.

Grain filling

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

Flag leaf senescence (FLSen): Days from anthesis to flag leaf senescence, representing the stay-green duration. The flag leaf was defined as fully senesced when they appeared ~ 95% yellow by visual scoring.

Grain filling duration (GFD): Days from anthesis to maturity. Plants were defined as mature when spikes became ~ 95% senesced and seeds became firm.

Number of developed or under developed spikelets per spike (DevSpklt and UnderdevSpklt): Developed and under developed spikelets were distinguished on the basis of awn length, to separate spikelets that may not normally form any grains from the others. Spikelets from the bottom of a spike having awn length less than half of the average awn length of the spike were defined as under developed spikelets. Total number of developed spikelets on a spike was defined as developed spikelet number spike^{-1} (DevSpklt).

Grain weight spike^{-1} (GnWSpk) and single grain weight (SingGW): The grains were left in the laboratory for ~4 weeks to reach a stable moisture content before being weighed. SingGW was calculated as GnWSpk / GnNoSpk.

Floret fertility

Grain number spike^{-1} (GnNoSpk) and grain number spikelet^{-1} (GnNoSpklt): Underdeveloped spikelets were removed, then the remainder of the spike divided into three equal parts (top, middle and bottom) for scoring floret fertility traits. Seeds at floret position 1 + 2 in each spikelet (the two most basal positions) and positions >2 were counted separately as the former develop before the latter (McMaster 1997). This gave GnNoSpklt for each third of the spike, and for each floret type. GnNoSpk was obtained by adding the component grain number measurements (for T2, T4 and T6), or was obtained simply by counting the seeds threshed from the entire spike after the basal underdeveloped spikelets had been removed (other treatments). GnNoSpklt for the whole spike was the average for all floret types across the spike.

Table 2.5 Traits scored in plants subjected to the various treatments.

Traits scored		Treatments						
		T1	T2	T3	T4	T5	T6	T7
	Heat	-	-	-	+	+	+	+
	Standing water	-	+	+	+	+	-	-
Grain size								
Anth		missed		yes		yes		yes
GnWSpk		yes		yes		yes		yes
SingGW		yes		yes		yes		yes
Floret fertility								
UnderdevSpklt		missed	yes		yes		yes	
DevSpklt		missed	yes		yes		yes	
GnNoSpk		yes	yes	yes	yes	yes	yes	yes
GnNoSpklt		missed	yes		yes		yes	
% fertility at top third part in floret 1+2 spike^{-1}		missed	yes		yes		yes	
% fertility at middle third part in floret 1+2 spike^{-1}		missed	yes		yes		yes	
% fertility at bottom third part in floret 1+2 spike^{-1}		missed	yes		yes		yes	
GnNoSpklt1&2		missed	yes		yes		yes	
GnNoSpklt>2		missed	yes		yes		yes	
Grain number at top third part in floret 1+2 spike^{-1}		missed	yes		yes		yes	
Grain number at middle third part in floret 1+2 spike^{-1}		missed	yes		yes		yes	
Grain number at bottom third part in floret 1+2 spike^{-1}		missed	yes		yes		yes	
Grain number at top third part in floret >2 spike^{-1}		missed	yes		yes		yes	
Grain number at middle third part in floret >2 spike^{-1}		missed	yes		yes		yes	
Grain number at bottom third part in floret >2 spike^{-1}		missed	yes		yes		yes	
Chlorophyll content								
SPAD10DAA		missed		yes		yes		yes
SPAD13DAA		missed		yes		yes		yes
SPAD27DAA		missed		yes		yes		yes

2.2.6 Data analysis

Types of data generated here included counts or continuous variables, percentages and repeated measurements. Measurements of all traits for different treatments were also compared. To analyze the data, several statistical modeling procedures were followed to minimize error and increase statistical power. Statistical models that were adopted for the various types of data were as follows:

Counts or continuous variables: Counts e.g. number of spikelets, grain number and continuous variable e.g. grain weight etc. were analyzed using linear mixed model:

$$y = X\tau + Zu + e$$

Where y is the vector of observations, τ is the vector of treatment, genotype and treatment by genotype fixed effects with design matrix X, u is the vector of block and whole plot random effects with design matrix Z, and e is the subplot random error.

Percentage variables: At floret positions 1+2, each developed spikelet can form a maximum 2 grains, so fertility could be expressed as a percentage (2 grains at this position being 100%). The empirical logistic transformation was used to transform the data onto the logit scale (McCullagh & Nelder 1989). The percentage of fertility was analyzed using the linear mixed model:

$$z = X\tau + Zu + e$$

Where z is the vector of transformed percentages, τ is the vector of treatment, genotype and treatment by genotype fixed effects with design matrix X, u is the vector of block and whole plot random effects with design matrix Z, and e is the subplot random error.

Repeated measurements over time: Repeated measurements were taken for traits e.g. chlorophyll content. Analysis of repeated measurement was done by the mode below:

$$y = X\tau + Z_b u_b + Z_w u_w + e$$

where y is the combined vector of chlorophyll content values at time 10, 13 and 27DAA, τ is the vector of treatment, genotype, time and all possible interaction effects with design matrix X, u_b is the vector of block with design matrix Z_b, u_w is the vector of whole plot random effects with design matrix Z_w and e is the random error.

Adjustment for multiple comparisons: Comparison of multiple means or hypotheses requires adjustment of *p* value as the probability of obtaining a false positive result increases with the number of comparisons. The Benjamini and Hochberg (1995) derived Bonferroni type multiple testing procedure was used to adjust the *p* value.

2.3 Results

Floret fertility

Plants destined for heat and standing water treatment for floret fertility reached the targeted stage (5cm AI) between the 25th of April and 13th of May 2015 (Fig. 2.2B). Daily maximum temperature in the greenhouses did not exceed 30 °C (Fig. 2.2A and B) for a single day during that time.

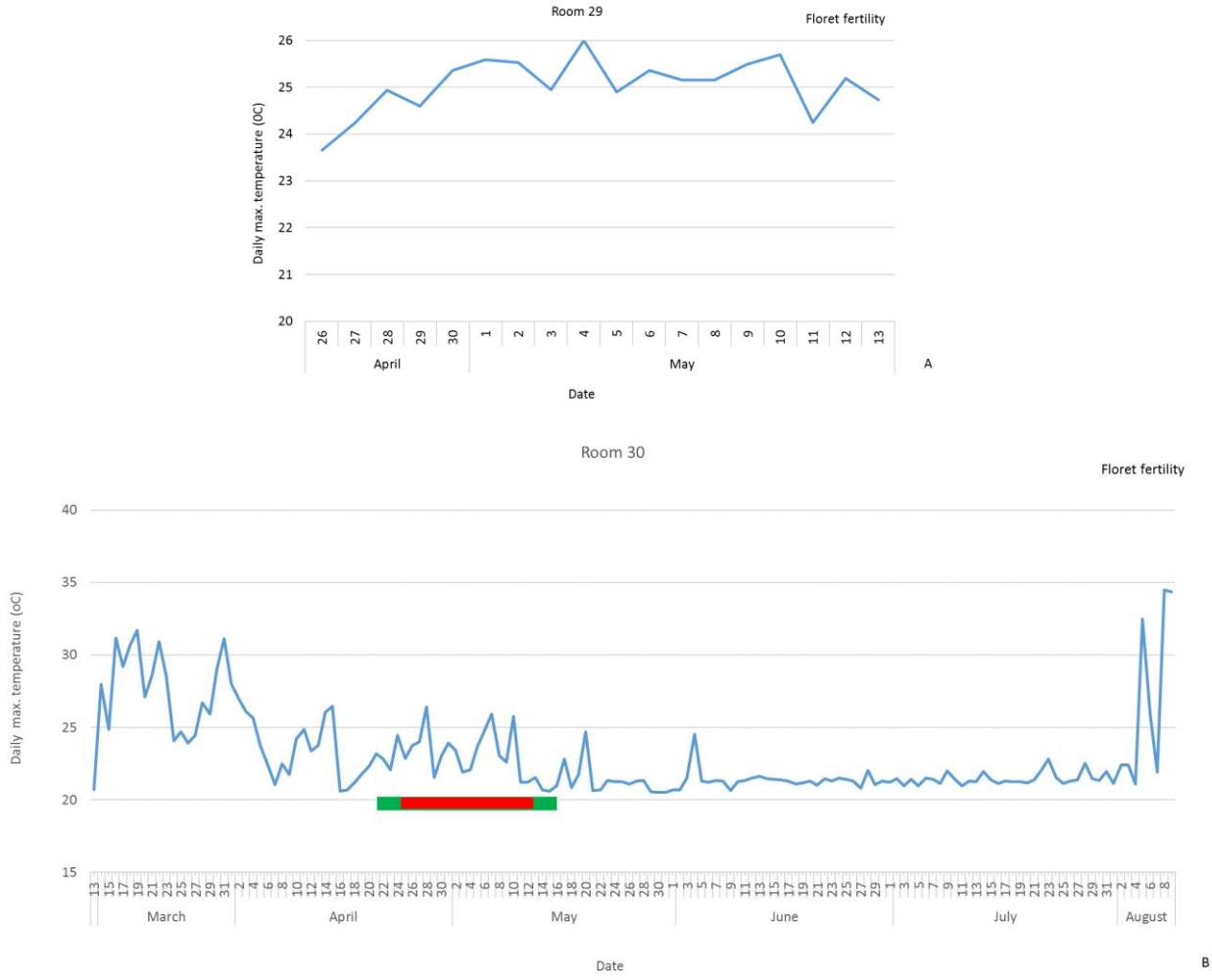


Fig. 2.2 A) Temperature of greenhouse room 29, with dates indicating the range at which plants were moved to room 29 at the sensitive tiller stage. B) Temperature of greenhouse room 30 from sowing to maturity. Red bar shows the dates at which plants reached the targeted growth stage and green extended bar indicate estimated time when plants were at sensitive stages to the effects of heat on fertility.

Heat treatment reduced GnNoSpk for all the varieties, and the effect was largely insensitive to the watering method (Fig. 2.3). GnNoSpk in HTWYT12 showed a strong response to heat but this genotype was previously classified as tolerant, while the magnitudes of the reactions of the other genotypes were consistent with their previous heat tolerance classifications, broadly speaking (Table 2.1). The only genotype in which the watering method made a significant difference was RAC875, for which a significant reduction in GnNoSpk due to heat was observed only under freely drained conditions.

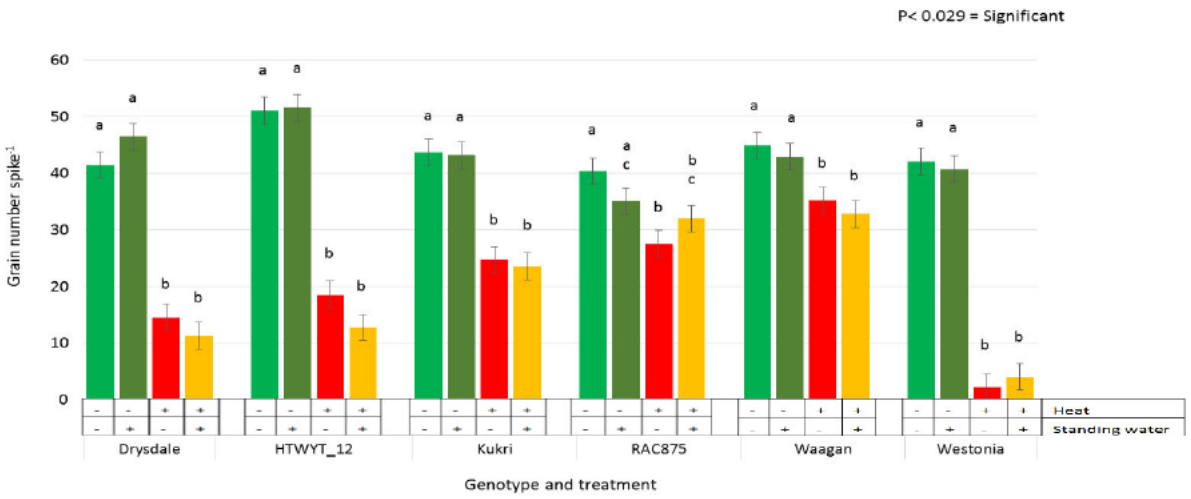


Fig. 2.3 GnNoSpk for six varieties under four treatments (bar below). Means with same letter code were not significantly different for comparisons between treatments within a genotype.

Similar trends were observed when GnNoSpk was separated into grains from floret positions 1+2 and grains from >2 positions (Fig. 2.4A and B). Fertility was reduced most in the floret 1+2 positions in some varieties (Drysdale, HTWYT_12, Kukri and Westonia), while in others it was reduced more in floret >2 positions (HTWYT_12), perhaps owing to varietal differences in the timing of spike developmental stage relative to AI elongation. Overall, there were fewer significant treatment effects observed at the floret >2 positions than at the floret 1+2 positions, which may at least partly be due to the former having the potential to set fewer grains (roughly half, compared to 1+2 positions).

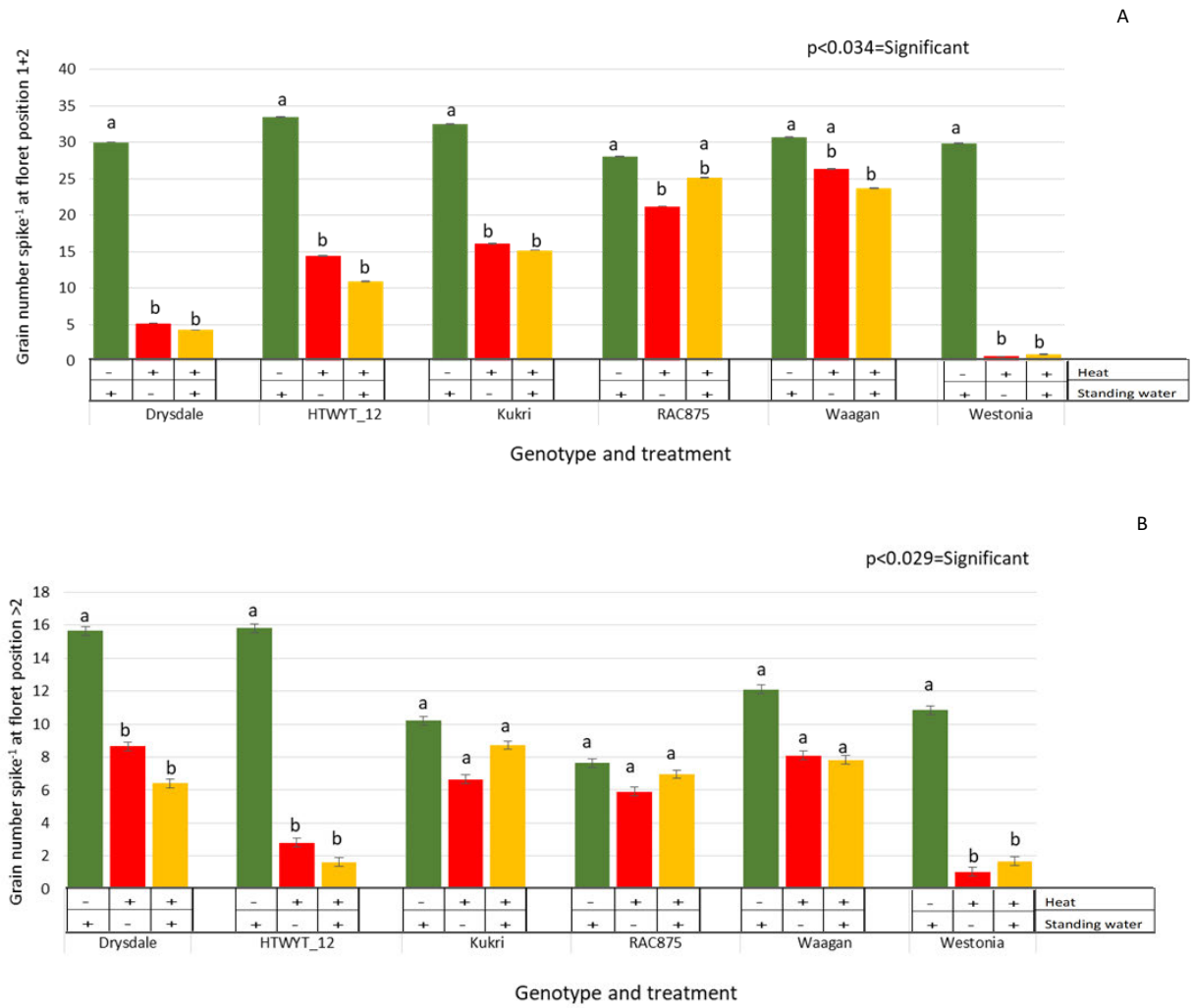


Fig. 2.4 Grain number at floret position 1+2 (A) and >2 (B) under three treatments. Means with same letter code were not significantly different for comparisons within a genotype and bars show SE.

In control plants of all genotypes, GnNoSpklt for florets 1+2 was close to 2.0 (Fig. 2.5A), confirming that temperatures in the greenhouse rooms used for control treatments (compartments 29 and 30) were not high enough to affect floret fertility.

GnNoSpklt at floret position 1+2 at top, middle and bottom spike positions were reduced in Drysdale, HTWYT_12, Kukri and Westonia but not in the highly and moderately tolerant varieties Waagan and RAC675, respectively (Fig. 2.5A). Standing water in the presence and absence of heat did not cause additional effects in any of the three parts of the spike.

In all three parts of the spike, GnNoSpklt in >2 floret positions (Fig. 2.5B) followed a similar trend as GnNoSpklt at floret positions 1+2.

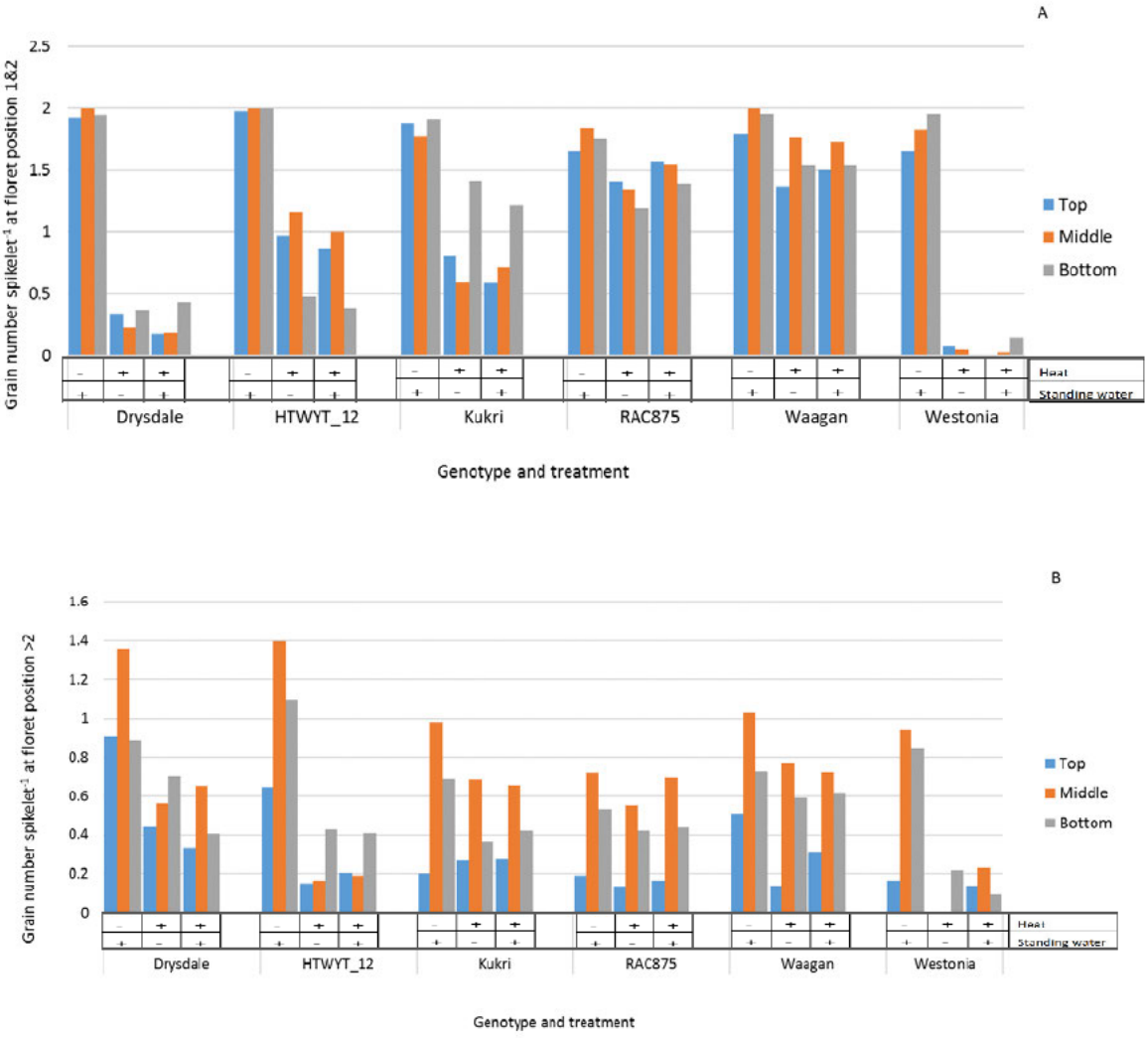


Fig. 2.5 GnNoSpklt in 1+2 (A) and >2 (B) position (top, middle and bottom) for standing water, heat and heat + standing water.

Fertility percentage at floret position 1+2 (Fig. 2.6) in the middle part of the spike was higher than in the other two parts under all treatment conditions. Heat reduced the fertility percentage of the middle part of the genotypes Drysdale, HTWYT_12 and Westonia. Standing water did not influence the heat effect significantly.

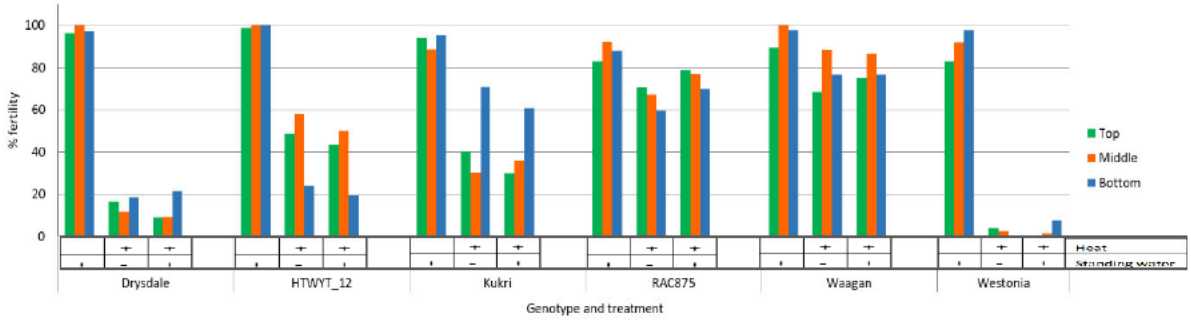


Fig. 2.6 Percentage fertility at floret position 1+2 at top, middle and bottom position under standing water, heat and heat + standing water.

Grain filling

The sensitive stage for grain filling is from five days before anthesis to 20 days after anthesis. Plants were at this stage from 29th of April to 5th of June and plants reached the target treatment stage (10DAA) between the 4th and 26th of May 2015 (Fig. 2.7A and B). Plants did not experience high temperature (> 30°C) at the sensitive stage for grain filling, even for a single day for this sub-experiment. Temperatures of room 30 were high at the beginning of August but this was well before anthesis occurred.

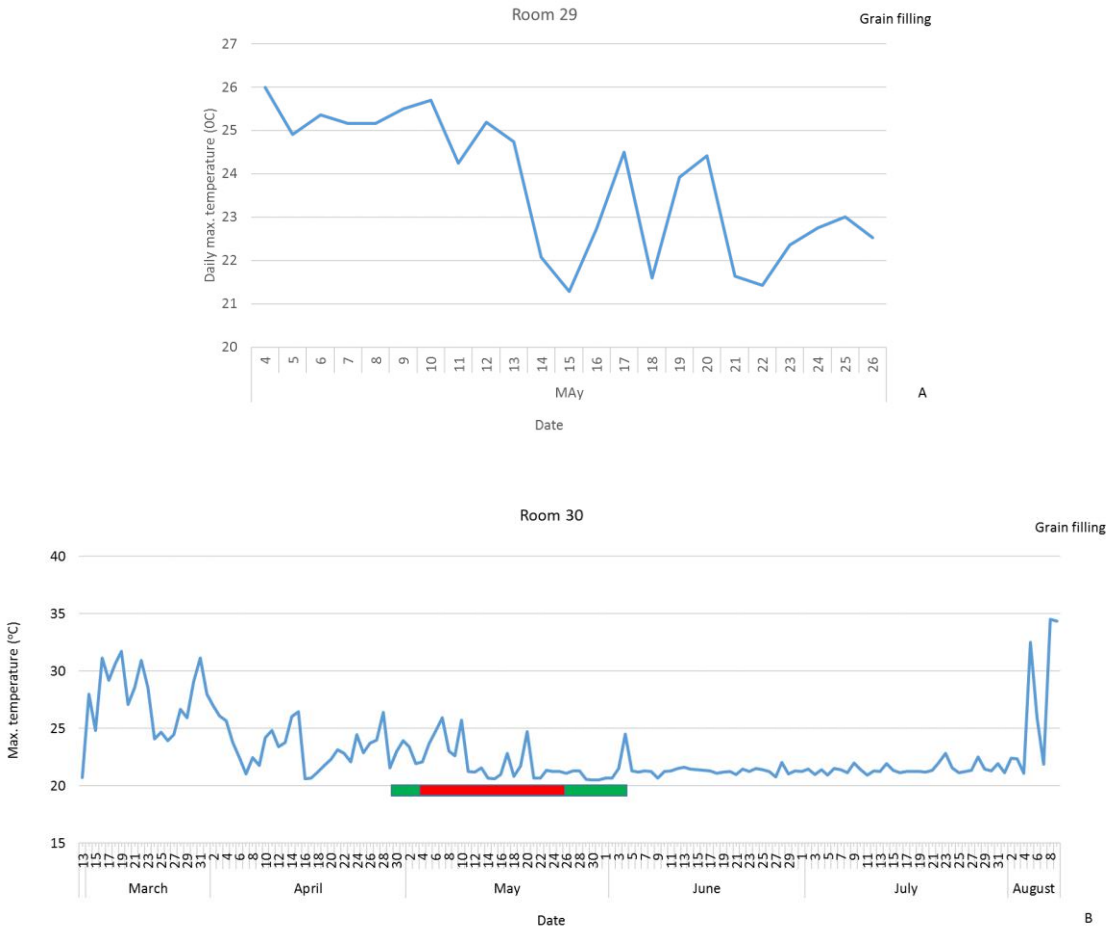


Fig. 2.7 Temperatures of two greenhouse rooms at the sensitive stage for grain filling (A, B). Red bar (B) shows the dates at which plants reached the targeted stage. Red bar shows the dates at which plants reached the targeted growth stage and the green extended bar indicates the range of time when the plants were at the sensitive stage (from five days before to 20 days after anthesis).

The magnitude of the responses seen for SingGW were not as great as those observed for floret fertility, which somewhat limited the power of the grain filling experiment to fulfil the purpose of the study. In Drysdale, SingGW was reduced significantly by heat, but was not further reduced by standing water. In HTWYT_12 and Westonia SingGW was reduced significantly only by the combined heat with standing water treatment (Fig. 2.8). The tolerant genotypes Waagan and

RAC875 showed an increase in SingGW with standing water, but only in the absence of heat. Direct comparisons between the heat treated plants (+/- standing water) showed no significant differences in SingGW or grain weight per spike due to the watering method. Therefore, evidence for any interaction effect between heat and watering treatments was weak for SingGW.

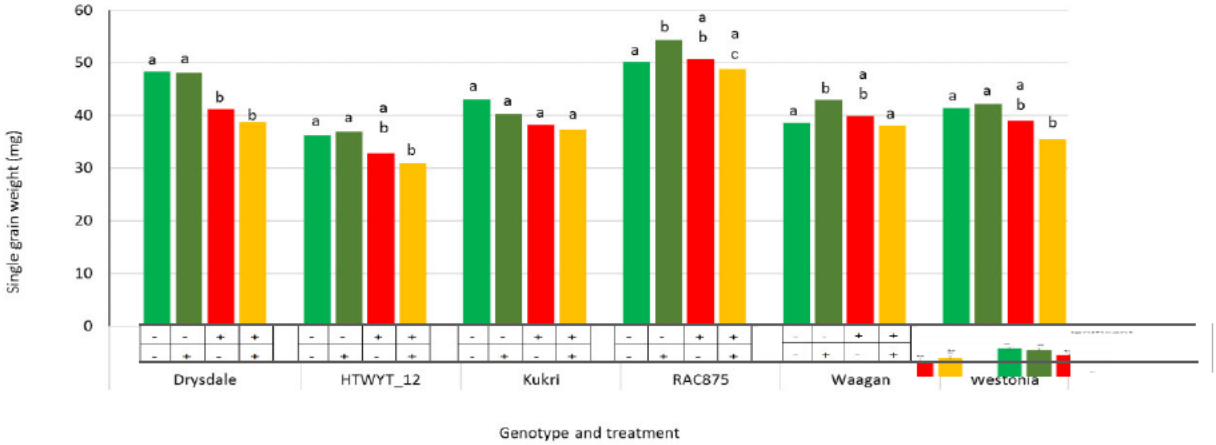


Fig. 2.8 SingGW in plants after receiving different combinations of watering and heat treatments. Means with same letter code were not significantly different for comparisons within a genotype.

General trends for GnWSpk were similar (Fig. 2.9), but this trait is more difficult to interpret, as it is subject to plant to plant variability in spikelet number per spike and grain number per spikelet.

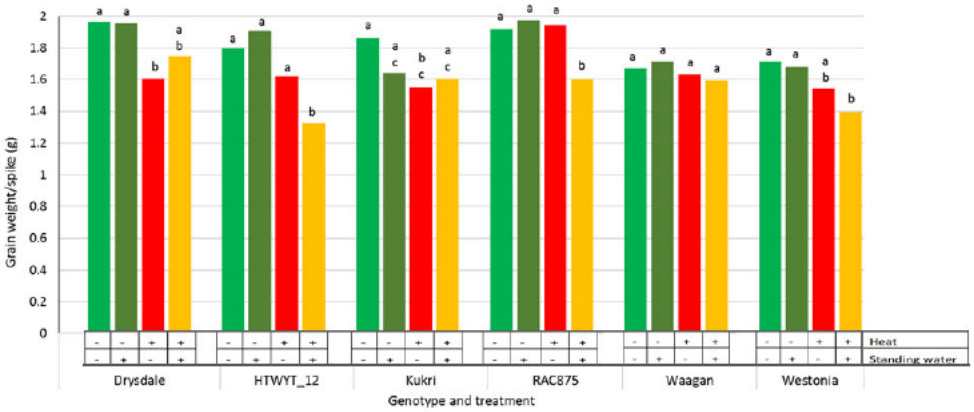


Fig. 2.9 Grain weight spike^{-1} in plants after receiving different combinations of watering and heat treatments. Means with same letter code were not significantly different for comparisons within a genotype.

As expected, flag leaf chlorophyll measured at 10DAA after anthesis (just before heat treatment) showed no significant differences between plants destined for the different treatments (Fig. 2.10A). RAC875 contained more chlorophyll than the other genotypes.

Just after the heat treatment (13DAA), chlorophyll content was significantly decreased by heat (relative to control plants) in HTWYT_12 and Kukri but only under freely drained conditions (Fig. 2.10B). By 27DAA, HTWYT_12 and Drysdale showed significant reductions in chlorophyll due to heat irrespective of watering method, while chlorophyll content in Kukri was only significantly decreased (relative to control) by the combined standing water plus heat treatment (Fig. 2.10C). Direct comparisons between the heat treated plants (+/- standing water) showed no significant differences in chlorophyll content due to the watering method, at either 13DAA or 27DAA. Hence, as for SingGW, flag leaf chlorophyll content showed no strong evidence for any interaction effect for heat and watering treatments.

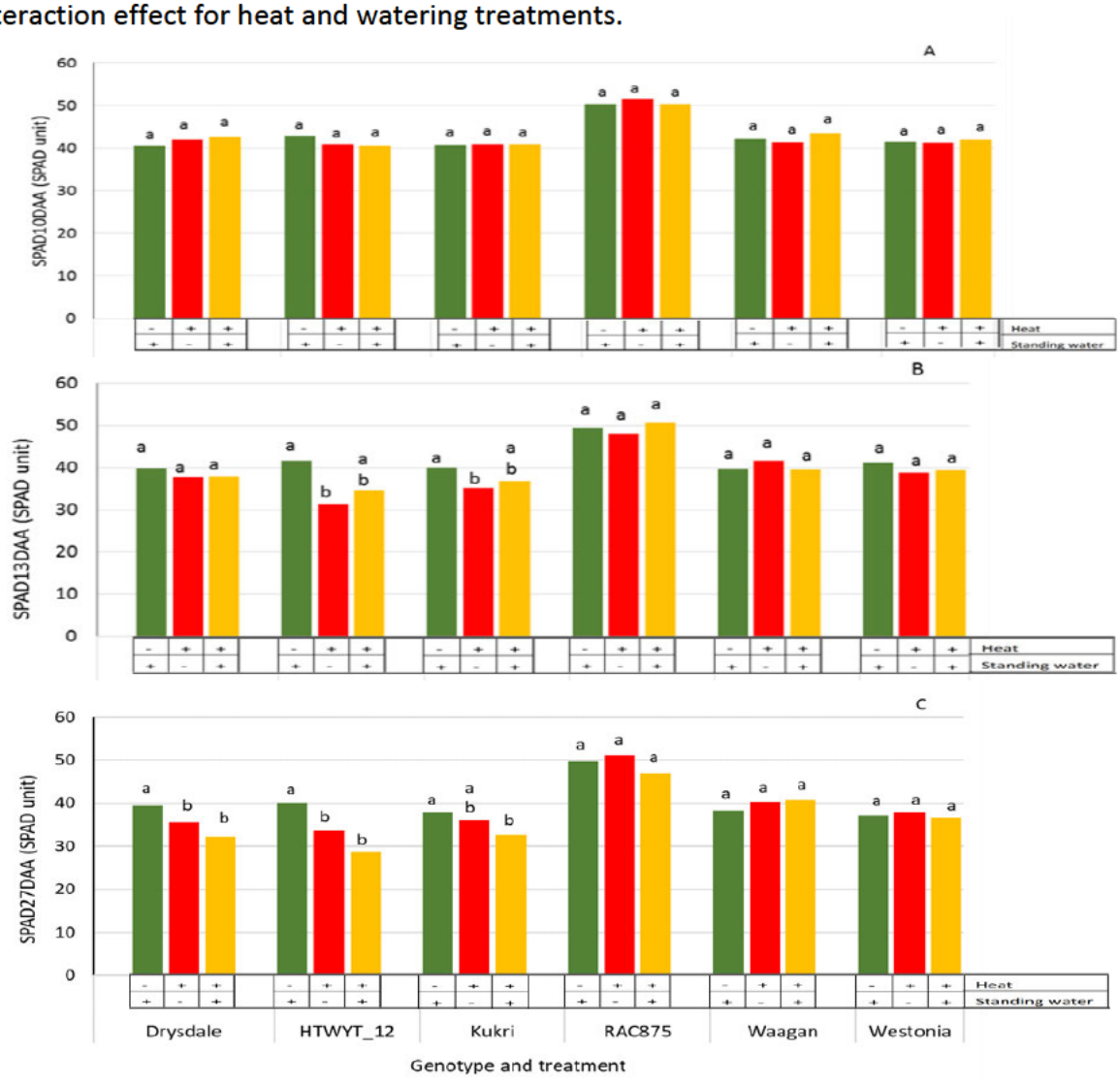


Fig. 2.10 SPAD measurement at 10DAA (A), 13DA (B) and 27 DAA (C). Means with same letter code were not significantly different for comparisons within a genotype.

The similarity between trends for SingGW and flag leaf chlorophyll heat responses, with respect to which genotypes were intolerant, combined with the lack of any strong effect of watering method on heat responses, is consistent with other studies that found these two traits and their heat responses to be strongly correlated genetically (Lopes & Reynolds 2012; Reynolds et al. 1994; Shirdelmoghanloo, Taylor, et al. 2016).

2.4 Conclusions

Heat treatment reduced GnNoSpk for all the varieties, and the effect was largely insensitive to the watering method. GnNoSpk at floret 1+2 and >2 positions were significantly reduced in heat susceptible genotypes Drysdale and Westonia along with tolerant genotype HTWYT_12. GnNoSpklt at floret position 1+2 and >2 in the three different sections of the spike (top, middle and bottom) showed that middle part of the spike gave more GnNoSpklt at both floret positions compared to other parts of spike. Heat reduced GnNoSpklt in heat susceptible genotype at both floret positions on all parts of the spike. Fertility percentage at floret positions 1+2 showed a similar trend and for all the traits standing water did not exacerbate the heat effect.

SingGW was reduced significantly in the intolerant genotype Drysdale under heat only condition but under the heat + standing water condition HTWYT_12 and Westonia (tolerant genotypes) showed a reduction in this trait. GnWSpk followed similar trends to SingGW. Chlorophyll content just after heat treatment, and at two weeks after heat treatment, were reduced in intolerant genotypes and showed similar trends to GnWSpk. Watering method had no significant impact on this trait.

Therefore, in this test of wheat genotypes of varying tolerance levels, standing water did not affect grain number or grain size responses to a three day heat treatments (or the traits under non-heated control conditions). Thus, these heat tolerance screening protocols can be used without concern that the method of watering will have a major influence on the plant responses. It can also be concluded that previously described heat experiments were unlikely to have been confounded by this type of watering method. As no consistent responses to the watering method itself was observed, the experiment shed no light on whether tolerance mechanisms for water logging and heat might be related or interact. A more severe waterlogging stress treatment would be needed to be used to address these questions.

Chapter 3: Selection and greenhouse evaluation of Near Isogenic Lines for QTL on chromosome 3B and 6B for grain filling and chlorophyll heat tolerance from the Drysdale × Waagan cross

3.1 Introduction

Two grain filling and chlorophyll heat tolerance QTLs, QTL11 (*QHsgw.aww-3B*) and QTL27 (*QChlr13.aww-6B*) were identified by Shirdelmoghanloo, Taylor, et al. (2016), on the short arm of chromosome 3B and on chromosome 6B, in Drysdale × Waagan mapping population under greenhouse/chamber conditions. *QHSGW.AWW-3B* on chromosome 3B was identified in two different phenotyping experiments and accounted for 11 to 22% variance for heat susceptibility index (HSI) of single grain weight (SingGW) and grain weight spike^{-1} (GnWSpk) in the whole population. The Waagan allele accounted for SingGW stability and reduced GnWSpk loss due to heat stress by 2.5 and 1.7 mg in the two experiments over the Drysdale allele. This locus also gave significant QTL effects under heat conditions for grain filling duration and shoot weight. *QHSGW.AWW-3B* was also the strongest for HSI of chlorophyll related traits e.g. response of absolute chlorophyll content after heat treatment and duration of flag leaf senescence after anthesis and accounted for ~13 to 40% of the variance for these traits. The Waagan allele at *QHSGW.AWW-3B* conferred greater chlorophyll stability and higher values *per se* in control plants for flag leaf chlorophyll content, both before (ChlC10DAA trait) and after (ChlC13DAA and ChlC27DAA traits) the heat treatment period. As *QHSGW.AWW-3B* showed heat tolerance effects for grain weight and chlorophyll stabilization in coupling, it indicated that heat tolerance for grain weight was associated with stay-green.

QTL 27 on chromosome 6B was identified in one experiment only, with weaker and less consistent effect for SingGW stability, chlorophyll loss and higher area under the SPAD curve (AUSC) *per se* under heat compared to *QHsgw.aww-3B*. The QTL contributed 8.9 to 12 % of the variation for these traits, with the Drysdale contributing the positive allele for heat tolerance on average, the Drysdale allele reduced heat-induced SingGW loss by 2.1 mg over the Waagan allele.

The two experiments used to define those QTLs were conducted in different seasons using a greenhouse for growth before and after heat treatments, and a growth chamber for heat

treatments. Using potted plants and a greenhouse and growth chamber allowed heat stress to be applied at a specific developmental stage. However, growth conditions of such experiments differ from those in the field in various ways which can lead to 'artifacts' (Passioura 2006). Quantitative traits observed in a greenhouse may be expressed differently in the field due to the different conditions. Polygenic quantitative traits may differ in expression depending on the environment due to interactions with various environmentally sensitive genes. Consistent expression of QTL under field conditions is important for their utility in marker assisted breeding. Field trials are time consuming and expensive and require replication to observe quantitative effects of a QTL in a whole population. Field trials for heat tolerance studies are difficult due to the unpredictability of heat waves. Nevertheless, field trials are necessary to validate QTL effects in the target production environment.

Near isogenic lines (NILs) offer a means to evaluate QTLs effects in detail under both controlled and field conditions, and are also useful for fine mapping, as they allow observation of QTL effects with fewer individuals, thereby increasing the statistical power of experiments. NILs can be produced by crossing F_1 individuals with a recurrent parent repeatedly for many generations, while maintaining selection for the QTL allele of interest (backcrossing), to provide a line (Singh, RP, Huerta-Espino & William 2005) that is genetically similar to the recurrent parent, except carrying a different version of the QTL chromosome region. Recombinant inbred lines (RILs) can also be used to generate NILs (herein referred to as RIL-NILs). Starting with an F_2 family, an inbred line is produced from each F_2 through successive generations of single-seed descent. RILs that are still heterogeneous for a target QTL can be identified using molecular markers, and markers applied to individuals of those lines to select out plants that are homozygous for each of the two contrasting alleles to produce a NIL pair. These can be used for further detailed study and validation of the QTL.

Phenotyping of multiple pairs of NILs differing slightly for the introgressed segment can allow the QTL interval to be narrowed down further. This process can involve generating new molecular markers in the region using various high throughput genotyping arrays, to delimit the boundaries of the introgressed chromosome segments, and hence further refine the QTL interval. This process

may ultimately lead to the identification of gene/s controlling the QTL of interest, and/or diagnostic markers suitable for use in breeding.

In this exercise, RIL-NILs were generated for the Drysdale × Waagan grain filling heat tolerance QTLs on chromosome 3B and 6B, and evaluated for heat tolerance phenotypes.

3.2 Materials and Methods

3.2.1 Plant materials

Plant materials used in this experiment were obtained from several previous experiments conducted since 2009. RIL and NIL generation from the cross between the varieties Drysdale and Waagan included a number of steps and trials.

(Background information regarding generation and selection of RIL-NILs was written by Nick Collins; Marker work prior to selection of homozygous NIL-RILs was done by Iman Lohraseb; selection of homozygous NIL-RILs and all subsequent steps were done by me)

Construction and initial field trialling of the Drysdale × Waagan RIL population was done by the group of Livinus Emebiri at the EH Graham Centre for Agricultural Innovation, NSW Department of Primary Industries, Wagga Wagga, as part of a project funded by the NSW BioFirst initiative. The number of generations of single-seed descent used to make the population was not recorded, although it was likely to have been around 5 ($F_{2:5}$ population). The population of 2,627 lines (derived from 43 F_1 plants) was subjected to several field trials at Wagga Wagga during 2009-2011, and plant height, flowering time and yield data used to identify ~604 most agronomic lines (semidwarfs, since the population segregates for both the *Rht-B1* and *Rht-D1* dwarfing genes, and excluding those with the most extreme early or late flowering time). Seed of those lines were sourced for further work, mostly from the first field trial (9E1; single row trial). It was assumed 9E1 was sown using seed of individual plants from the last SSD generation (not seed produced from a multi-plant bulk), but records were not available to verify this (Fig. 3.1).

DNA was extracted from each of the 604 lines (plus the parents, obtained from NSW-DPI), using a mixture of leaf tissue from 5 seedlings per line, to allow detection of any genetic heterogeneity. Six

Kompetitive Allele Specific PCR (KASP; LGC, Queens Road, Teddington, TW11 0LY, London, UK; www.lgcgroup.com) markers located around the genome, and known to have the same allele in Waagan and Drysdale, but polymorphic relative to many other varieties, were used to screen the DNA samples, identifying 8 lines (rate of 1.3%) showing non-Waagan/Drysdale alleles. These represent contamination (e.g. through stray seed, or outcrossing) and were discarded.

Nine SNP markers from the Illumina 9,000 SNP array (Cavanagh et al. 2013) that had been mapped around the 3B and 6B grain filling heat tolerance QTL (Shirdelmoghanloo, Lohraseb, et al. 2016) were used to design KASP marker assays (Table 2.1; Fig. 3.2) which were then used to screen the DNA samples. Sufficient numbers of lines heterogeneous for the QTL regions were identified from a single 96-well plate of DNA samples; hence all selections were made from the RIL lines represented in this plate. A total of 22 lines were identified for further work – 12 with potential heterogeneity for markers on chromosome 3B, and 10 with potential heterogeneity for markers on 6B, and one line with potential heterogeneity for both regions.

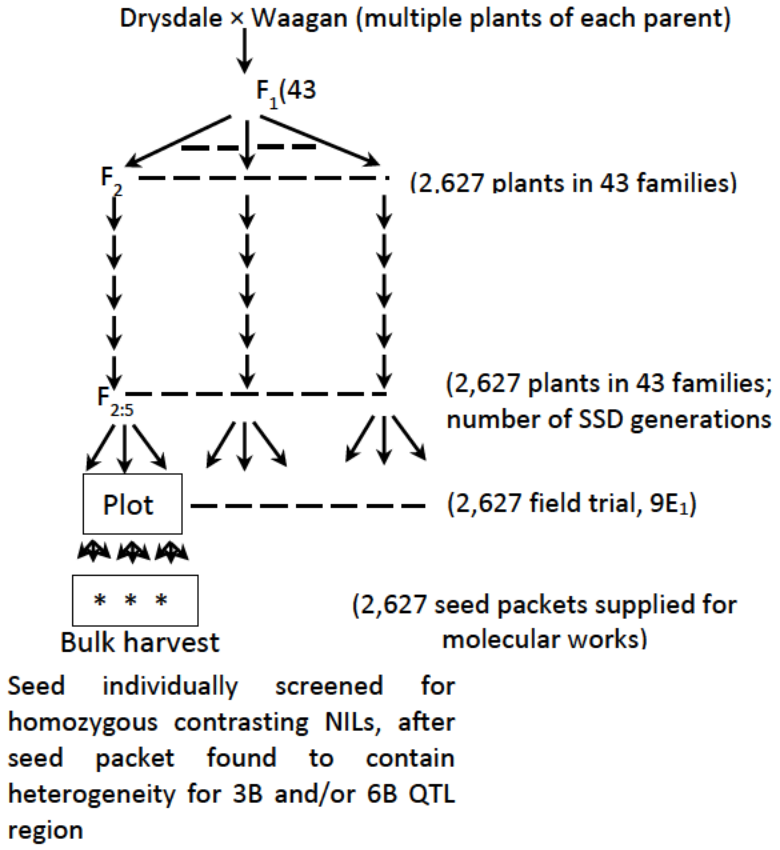


Fig. 3.1 Schematic diagram showing generation of RIL-NILs

Marker / Genotype	wsnp_Ra_c41135_48426638	wsnp_Ex_rep_c66331_64503065	wsnp_Ku_c12698_20441325	wsnp_Ex_c3005_5548573	wsnp_BE497169B_Ta_2_2	wsnp_Ku_c3817_7009093	wsnp_Ex_c11379_18370310	wsnp_Ex_c19525_28494827	wsnp_Ex_c45713_51429315
Linkage group	3B1	3B1	3B1	3B1	3B1	3B1	6B3	6B3	6B3
cM position	0.0	1.4	1.4	2.3	3.2	34.6	0.0	18.1	18.1
Drysdale	A:A	C:C	C:C	T:T	C:C	G:G	C:C	C:C	G:G
Waagan	G:G	T:T	T:T	C:C	A:A	G:G	T:T	T:T	A:A
WW30666	G:G	C:C	C:C	C:T ?	C:C	A:A	C:C	C:C	G:G
WW30674	G:G	C:T	C:T ?	C:C	C:C	G:G	C:C	T:T	A:A
WW30676	G:A	C:T	C:T	C:C	C:A	G:G	T:T	T:T	A:A
WW30692	A:A	C:T	C:T ?	C:T	C:C	G:G	T:T	C:C	G:G
WW30702	A:A	C:C	C:C	T:T	C:C	G:A	T:T	T:T	A:A
WW30709	A:A	C:T	C:T	C:T	C:A	G:G	T:T	T:T	A:A
WW30860	G:G	C:C	C:C	T:T	C:C	G:A	C:C	C:C	G:G
WW30883	G:G	C:C	C:C	T:T	C:C	G:A	C:C	C:C	G:G
WW30895	A:A	C:C	C:C	C:T ?	C:C	G:G	T:T	T:T	A:A
WW30913	G:A	T:T	C:T	C:C	C:A	G:G	T:T	T:T	A:A
WW30914	A:A	C:C	C:C	T:T	C:C	G:A	C:C	C:C	G:G
WW30764	G:A	C:T	C:T	C:T ?	C:A?	G:G	T:T	C:T	G:A
WW30655	A:A	C:C	C:C	T:T	C:C	G:G	C:T	C:T	G:A
WW30711	G:G	T:T	T:T	C:C	A:A	G:G	T:T	C:T	G:A
WW30845	A:A	C:C	C:C	T:T	C:C	G:G	C:T?	T:T	A:A
WW30852	A:A	C:C	C:C	T:T	C:C	A:A	T:T	C:T?	G:G
WW30874	G:G	C:C	C:C	T:T	C:C	G:G	C:T?	T:T	A:A
WW30875	A:A	C:C	C:C	T:T	C:C	G:G	C:T	C:C	G:G
WW30893	G:G	T:T	T:T	C:C	A:A	G:G	C:T	T:T	A:A
WW30900	G:G	T:T	T:T	C:C	A:A	G:G	T:T	C:T	A:A
WW30908	G:G	T:T	T:T	C:C	A:A	G:G	C:T	T:T	A:A
WW30915	A:A	C:C	C:C	T:T	C:C	A:A	T:T	C:T?	G:G

Fig. 3.2 Twenty-two Drysdale × Waagan RIL families were selected for homozygous NIL pair generation on the basis that they showed heterogeneity for QTL regions. Cell color designates Drysdale (pink), Waagan (Warrington, Dunstone & Green) or heterozygous (yellow) allele calls. White cells represent KASP calls different from parental alleles. Red and blue boxes highlight families that were selected for homozygous NIL pair generation for the 3B and 6B QTL regions, respectively. '?' indicates unsure KASP calls. Marker order was determined by (Shirdelmoghanloo, Taylor, et al. 2016) on DH lines.

3.2.2 Homozygous NIL pair selection

It was expected that RILs with heterozygous allele calls in the QTL regions would be heterogeneous and provide homozygous NILs. Seeds were taken from the same seed packets that were sown for the original DNA samples but this time DNA was extracted from individual plants. Seeds from these families were sown on 25th April, 2015 in a greenhouse with growth conditions similar to those discussed in paragraph (2.2.2).

Leaf tissue of ~50 mm long and 4mm wide segment from 2-week old plants were used for DNA extraction using the protocol of (Pallotta et al. 2003). DNA concentrations were measured by absorbance at 260 nm using NanoDrop (ND-1000 spectrophotometer; 3411 Silverside Rd, Tatnall Building Wilmington, DE 19810, USA) and integrity was tested by electrophoresis on 0.8% agarose gels.

KASP assay primers are shown in Table 3.1. Primers were supplied by Sigma-Aldrich (PO Box 970, Castle Hill NSW 1765, Australia), 100 µM primer stock was prepared by re-suspending in 10x µL MQ water to the amount (nmol) of DNA mentioned by the supplier. Genomic DNAs were diluted to concentration of ~5 ng/µL by adding Milli-Q water for DNA stamping. Five microliters of diluted DNA per sample was dispensed to each 384 well flat top black working plate (LGC, Labor GmbH Augsburg, Bgm. Schlosser-Str. 6 A, 86199 Augsburg, Germany) using an oKtopure robot (LGC, Queens Road, Teddington, Middlesex, TW11 0LY, UK; www.lgcgroup.com), spun briefly and oven dried for 1 hour or more at 65°C.

The KASP assay mix was prepared separately for each marker and contained one forward primer specific for each SNP allele and one common primer. KASP assay mix and master mix were added together to the DNA-containing 384 well flat top black plate using a Meridian liquid dispenser (LGC, London, UK; www.lgcgroup.com). Volumes of KASP master mix (TAQ polymerase, dNTPs, $MgCl_2$, FRET cassettes) and assay mix (primers) were calculated according to the standard protocol (https://www.lgcgroup.com/LGCGroup/media/PDFs/Products/Geno typing /KASP-genotyping-chemistry-User-guide.pdf?ext=.pdf). Plates were sealed using Kube heat sealing and Fusion3 laser sealing instruments (LGC, London, UK; www.lgcgroup.com), and spun briefly before hydrocycling.

Table 3.1 Primers for KASP assays used in RIL-NIL selection.

Marker name (from wheat Illumina 9,000 SNP array)	KASP Primer	Primer sequence (5'-3')	SNP allele specificity
For QTL on short arm of chromosome 3B			
wsnp_Ra_c41135_48426638	A1	GTCACTACAACACTACTCTTCT	A
	A2	AGGTCACTACAACACTACTCTTCC	G
	C	TCAGCGACAAGGGCGCCATCAA	
wsnp_Ex_rep_c66331_64503065	A1	CAAAGATCACTGCCACTGCTA	T
	A2	GCAAAGATCACTGCCACTGCTG	C
	C	GGGCATAGAAATTACTGATTTTGATGCCTT	
wsnp_Ku_c12698_20441325	A1	CCAACACCCTGGAGAACATCGTT	T
	A2	CAACACCCTGGAGAACATCGTC	C
	C	CCCCATCGATCTGAGAAGCAATGTT	
wsnp_Ex_c3005_5548573	A1	AAGTGCGAAACTCACACACAGAGT	T
	A2	GTGCGAAACTCACACACAGAGC	C
	C	CAGTAGTTATCCCAATTATTGCTGGCATA	
wsnp_BE497169B_Ta_2_2	A1	GCACATGCAGATCCATCATATGCAA	A
	A2	CACATGCAGATCCATCATATGCAC	C
	C	GAACAAGATGAAAGGGGCATTGAAATGAT	
wsnp_Ku_c3817_7009093	A1	CATGCCGGGTAGTATGCTTGGT	A
	A2	ATGCCGGGTAGTATGCTTGGC	G
	C	CGCCGATCCGAATACAGGAAATTCTA	
For QTL on chromosome 6B			
wsnp_Ex_c11379_18370310	A1	CCAGAAGTTCAGGATATCTTATGATGAA	T
	A2	CAGAAGTTCAGGATATCTTATGATGAG	C
	C	CTCAAAATCGGACATAGGTCATGTACTAT	
wsnp_Ex_c19525_28494827	A1	CGATGCAGATTGTCAGGCAGA	T
	A2	CGATGCAGATTGTCAGGCAGG	C
	C	GAACTATGCTACTACATACAGTAGTACAAT	
wsnp_Ex_c45713_51429315	A1	ATCATGTTGATGGACTTGATCTGACA	A
	A2	CATGTTGATGGACTTGATCTGACG	G
	C	CGGGAAGCATCTCATCATGYACAGAA	

A1 and A2 primers have GAAGGTGACCAAGTTCATGCTATAG and GAAGGTCGGAGTCAACGGATTC 5' extensions not shown here, that are complementary to FAM and HEX labelled oligonucleotides, respectively, that are in the KASP master mix.

A Hydrocycler16 (LGC, London, UK; www.lgcgroup.com) was used for hot start PCR amplification following the standard programs (https://www.lgcgroup.com/LGCGroup /media/PDFs/Products/Genotyping/KASP-genotyping-chemistry-User-guide.pdf?ext=.pdf) for thermal cycling and ~5 additional rounds of thermal cycling. After each cycle of amplification fluorescence was measured using a PHERAstar FSX plate reader (BMG LABTECH Pty. Ltd., 2/24 Carbine Way, Mornington, Victoria 3931, Australia; www.bmglabtech.com) and outputs were

processed by Kraken software (https://www.lgcgroup.com/products/genotyping-software/kraken/#.WL0AoW996Uk).

Once homozygous NIL plants were selected, these were grown up and seed collected. Five of these seeds per line were sown in 20 cm pots (all 5 plants in one pot) in a greenhouse. Two or three plants per line were randomly selected for marker analysis to verify the lines' genotype. Plants were grown to maturity and seed sent to NSW-DPI Wagga Wagga for field multiplication in the 2016 season, in preparation for validation trials in 2017.

3.2.3 NIL phenotyping

Seeds from selected NIL were sown for phenotyping on 31st March, 2016. Plants were grown and heat treated following similar growth and treatment conditions to those mentioned by Shirdelmoghanloo, Taylor, et al. (2016). In brief, two seeds were sown per pot in Room 30 at The Plant Accelerator (TPA, The University of Adelaide, Waite Campus, Adelaide). After germination plants were thinned to one per pot. Plants destined for heat treatment were moved to a growth chamber WI.5 (BDW120, Conviron Asia Pacific Pty Ltd. Melbourne, Australia). Greenhouse room conditions were as summarized in Table 3.2.

Heat treatments were applied at 10 days after anthesis (10DAA) as described by Shirdelmoghanloo, Taylor, et al. (2016). Pots were moved to the growth chamber set at 37^{o}C/27^{o}C day/night temperature for three days and then moved back to the greenhouse to grow up to maturity. The experiment used a split-plot design provided by Sabela Munoz-Santa (The University of Adelaide). A randomized complete block arrangement of 4 blocks was used to assign the two treatments (control and heat). For heat treatment eight (2 for each block) and four (1 for each block) replications for NILs and parental lines were used respectively but as control one plant for each of NILs and parental lines for each of the four blocks were used and were randomly distributed among four rows and 27 columns of each block. Pots from the same block in the greenhouse were placed in the same block in the growth chamber, so as to accumulate the variability due to blocks for the two different places.

Table 3.2 Details of greenhouse (room 30) temperature (°C) and relative humidity during the NIL phenotyping experiment. Set maximum-day/minimum-night temperatures were 20/16 °C. The stage for heat treatment (10DAA) was reached during May-June.

Month	Daily avg.	Avg. daily min.	Avg. daily max.	Days >30°C	Avg. % relative humidity
Mar	22.6	15.9	32.3	9	68.4
Apr	20.3	15.8	28.6	0	68.3
May	19.8	15.8	30.8	1	74.8
Jun	19.5	15.4	29.1	0	79.1
Jul	18.5	14.0	25.2	0	77.7
Aug	18.8	14.3	24.8	0	68.4
Sep	19.2	15.3	28.5	0	66.5

Data for six traits were collected. Chlorophyll traits were measured during growth, while the rest were measured at maturity. The primary tiller of each plant was tagged with colored tapes after reaching anthesis and after heat treatment, to identify the tiller to score afterwards and to confirm treatment status of the plants. Trait descriptions are described in Table 3.3.

Table 3.3 Traits evaluated in the NILs.

Trait	Abbreviation	Measurement method
Culm length (cm)	CulmLMat	Length of the culm from soil surface to the collar of the spike, at maturity.
Shoot weight (g)	ShootW	Measured at maturity only on the primary tiller. The tiller was cut off at the soil surface, the spike cut off at the collar, and the shoot (stem + leaves) was oven dried at 85^{o}C for 3 days before weighing.
Chlorophyll content (SPAD unit)	SPAD10DAA, SPAD13DAA and SPAD27DAA	Measured on flag leaf using a portable SPAD chlorophyll meter (SPAD-502; Minolta Co. Ltd., Japan) at 10 DAA (SPAD10DAA; before heat treatment), just after heat treatment (SPAD13DAA) and at two weeks after heat treatment (SPAD27DAA). Each value recorded was the average of 10 measurements taken from the same leaf area, along the left-hand side of the flag leaf between the mid-rib and leaf margin.
Days to anthesis	Anth	From time of sowing.
Grain weight spike^{-1} (g)	GnWSpk	Grains were left in the laboratory at room temperature for ~4 weeks to reach a stable moisture content before being weighed.
Single grain weight (g)	SingGW	GnWSpk/ GnNoSpk.

3.3 Results

3.3.1 Homozygous NIL pair selection and propagation

Homozygous NILs for the 3B and 6B QTL regions were identified by KASP assays in plants grown from the heterogeneous seed packets. These assays were performed on ~17 plants from each of the 22 families plus the parental varieties (total 380 plants). Results from seven families were not presented, as these families were either found to be not segregating, or segregating for too many recombinant chromosomes, making interpretation too difficult (not shown). Of the remaining 15 families, six were segregating for the 3B QTL and nine for the 6B QTL.

NILs were selected from five and four families for the 3B (Fig 3.3 A-B) and 6B (Fig 3.3 C-E) QTLs, respectively. Another six families showing no recombination in the intervals, or similar recombination to other families, were not phenotyped due to space limitation. Four plants were selected from each of these nine families: 2 homozygous for each of the two contrasting chromosome types (total 36 plants). Five seeds from each of the 36 selected RIL-NILs were grown for further seed propagation and verification of marker genotypes. DNA was extracted from three to two plants from each line and were used in KASP analysis except WW30676 family for which only two lines (WW30676_11 and WW30676_13) were used for phenotyping. KASP marker wsnp_Ku_c3817_7009093 was not used further due to its poor performance (Fig. 3.3). KASP assays were performed, giving clear genetic profiles of the RIL-NILs (Fig. 3.4).
Seeds from each of the five plants from each of eight families were collected. One to six grams of seed per RIL-NIL was sent to NSW-DPI Wagga Wagga research station for field propagation in the 2016 season.

3.3.1 RIL-NIL phenotyping

Thirty-four lines (excluding WW30676_9 and WW30676_17) from nine families (paragraph 3.3.1) were subjected to phenotypic evaluation of the 3B and 6B QTL effect in the greenhouse. Two NIL pairs (four lines) from each of five families for 3B and four families for 6B were selected but from family WW30676 only two contrasting lines were used.

Marker / Genotype	wsnp_Ra_c41135_48426638	wsnp_Ex_rep_c66331_64503065	wsnp_Ku_c12698_20441325	wsnp_Ex_c3005_5548573	wsnp_BE497169B_Ta_2_2	wsnp_Ku_c3817_7009093
chrom region	3B1	3B1	3B1	3B1	3B1	3B1
cM position	0.0	1.4	1.4	2.3	3.2	34.6
Drysdale	A:A	C:C	C:C	T:T	C:C	G:G
Waagan	G:G	T:T	T:T	C:C	A:A	G:G
WW30674	G:G	C:T	C:T?	C:C	C:C	G:G
WW30674_1	G:G	T:T	C:C	T:T	C:C	G:G
WW30674_2	G:G	T:T	T:T	C:C	C:C	G:G
WW30674_3	G:G	T:T	T:T	C:C	C:C	G:G
WW30674_4	G:G	T:T	T:T	C:C	C:C	G:G
WW30674_5	G:G	T:T	C:T	C:C	C:C	G:G
WW30674_6	G:G	T:T	C:C	T:T	C:C	G:G
WW30674_7	G:G	T:T	C:C	T:T	C:C	G:G
WW30674_8	G:G	T:T	C:T	C:C	C:C	G:G
WW30674_9	G:G	T:T	T:T	C:C	C:C	G:G
WW30674_10	G:G	T:T	T:T	C:C	C:C	G:G
WW30674_11	G:G	T:T	T:T	C:C	C:C	G:G
WW30674_12	G:G	T:T	C:T	C:C	C:C	G:G
WW30674_13	G:G	T:T	C:C	T:T	C:C	G:G
WW30674_14	G:G	T:T	T:T	C:C	C:C	G:G
WW30674_15	G:G	T:T	T:T	C:C	C:C	G:G
WW30674_16	G:G	T:T	T:T	C:C	C:C	G:G
WW30676	G:A	C:T	C:T	C:C	C:A?	G:G
WW30676_9	A:A	C:C	C:C	C:C	C:C	A:A
WW30676_10	A:A	C:C	C:C	C:C	C:C	A:A
WW30676_11	A:A	C:C	C:C	C:C	C:C	A:A
WW30676_3	A:A	C:C	C:C	C:C	C:C?	A:A
WW30676_5	A:A	C:C	C:C	C:C	C:C?	A:A
WW30676_4	A:A	C:C	C:C	C:C	C:C?	G:A
WW30676_6	A:A	C:C	C:C	C:C ?	C:C?	A:A
WW30676_14	A:A	C:C	C:C	C:T	C:C	A:A
WW30676_7	A:A	C:C	C:C	C:T	C:C	G:A
WW30676_16	Bad	NC	NC	NC	NC	NC
WW30676_12	G:G	T:T	C:T	NC	A:A?	G:G
WW30676_1	G:G	T:T	T:T	NC	A:A?	G:G
WW30676_8	G:G	T:T	T:T	C:C	A:A?	G:G
WW30676_13	G:G	T:T	T:T	C:C	A:A?	G:G
WW30676_2	G:G	T:T	T:T?	NC	A:A?	G:G
WW30676_17	G:G	T:T	T:T?	C:C	A:A	G:G
WW30676_15	G:G	T:T	T:T?	C:C	A:A?	G:G
WW30692	A:A	C:T	C:T?	C:T	C:C	G:G
WW30692_2	A:A	C:C	C:C	T:T	C:C	A:A
WW30692_4	A:A	C:C	C:C	T:T	C:C	A:A
WW30692_7	A:A	C:C	C:C	T:T	C:C	A:A
WW30692_8	A:A	C:C	C:C	T:T	C:C	A:A
WW30692_12	A:A	C:C	C:C	T:T	C:C	A:A
WW30692_14	A:A	C:C	C:C	T:T	C:C	A:A
WW30692_16	A:A	C:C	C:C	T:T	C:C	A:A
WW30692_6	A:A	C:T	C:C	NC	C:A	G:G
WW30692_9	A:A	C:T	C:T	C:T	C:C?	G:G
WW30692_10	G:A	C:T	C:C ?	C:C	A:A?	G:G
WW30692_1	G:A	C:T	C:T	C:C	C:A?	G:G
WW30692_5	G:A?	C:T	C:T	C:C	C:A	G:G
WW30692_15	G:A?	C:T	C:T	C:C	C:A	G:G
WW30692_11	G:G	T:T	T:T	C:C	A:A	G:G
WW30692_17	G:G	T:T	T:T	C:C	A:A	G:G
WW30692_13	G:G	T:T	T:T?	C:C	A:A	G:G
WW30692_3	G:G	T:T	T:T?	C:C	A:A?	G:G

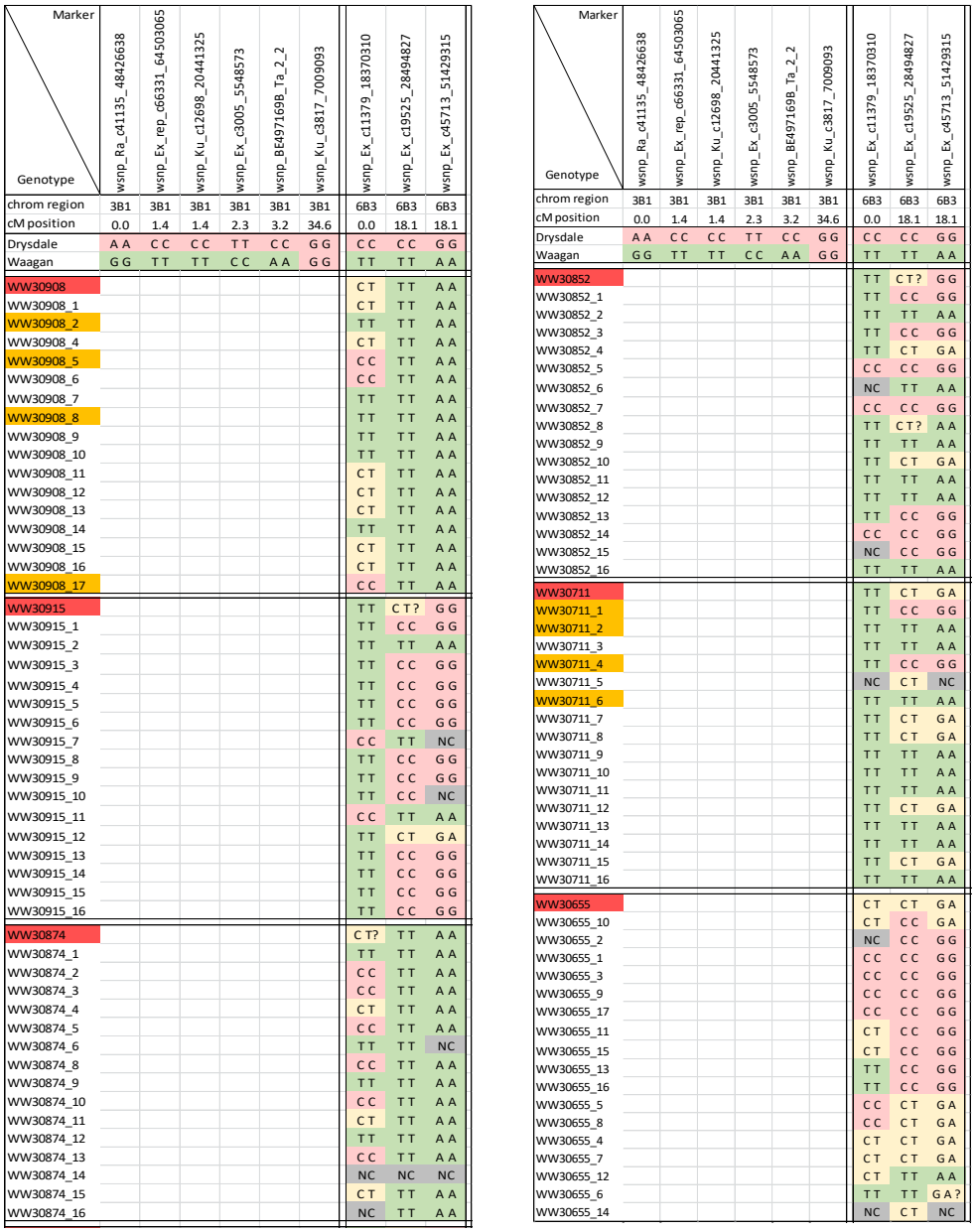
A

Marker / Genotype	wsnp_Ra_c41135_48426638	wsnp_Ex_rep_c66331_64503065	wsnp_Ku_c12698_20441325	wsnp_Ex_c3005_5548573	wsnp_BE497169B_Ta_2_2	wsnp_Ku_c3817_7009093	wsnp_Ex_c11379_18370310	wsnp_Ex_c19525_28494827	wsnp_Ex_c45713_51429315
chrom region	3B1	3B1	3B1	3B1	3B1	3B1	6B3	6B3	6B3
cM position	0.0	1.4	1.4	2.3	3.2	34.6	0.0	18.1	18.1
Drysdale	A:A	C:C	C:C	T:T	C:C	G:G	C:C	C:C	G:G
Waagan	G:G	T:T	T:T	C:C	A:A	G:G	T:T	T:T	A:A
WW30709	A:A	C:T	C:T	C:T	C:A	G:G			
WW30709_9	A:A	C:C	C:C	T:T	C:C	A:A			
WW30709_12	A:A	C:C	C:C	T:T	C:C	A:A			
WW30709_16	A:A	C:C	C:C	T:T	C:C	A:A			
WW30709_1	A:A	C:C	C:C	T:T	C:C	G:A			
WW30709_3	A:A	C:C	C:C	T:T	C:C	G:G			
WW30709_8	A:A	C:C	C:C	T:T	C:C	G:G			
WW30709_10	A:A	C:C	C:C	T:T	C:C	G:G			
WW30709_2	A:A	C:C	T:T?	T:T	C:C	G:G			
WW30709_15	A:A	C:T	C:T	C:T	C:A	G:G			
WW30709_4	G:A	C:T	C:T	C:T	C:A	G:G			
WW30709_11	G:A	C:T	C:C?	C:C	A:A?	G:G			
WW30709_6	G:A	C:T	C:T	C:C	C:A	G:G			
WW30709_14	G:A	C:T	C:T	C:C	C:A?	G:G			
WW30709_7	G:A?	C:T	C:T	C:C	C:A	G:G			
WW30709_17	G:G	T:T	T:T	C:C	A:A	G:G			
WW30709_5	G:G	T:T	T:T	C:C	A:A?	G:G			
WW30709_13	G:G	T:T	T:T	C:C	A:A?	G:G			
WW30913	G:A	T:T	C:T	C:C	C:A	G:G			
WW30913_5	A:A	C:C	C:C	T:T	C:C	A:A			
WW30913_12	A:A	C:C	C:C	T:T	C:C	A:A			
WW30913_10	A:A	C:C	C:C	T:T	C:C	G:G			
WW30913_14	A:A	C:C	C:C	T:T	C:C	G:G			
WW30913_15	A:A	C:C	C:C	T:T	C:C	G:G			
WW30913_13	A:A	C:T ?	C:T	C:C?	C:A	G:G			
WW30913_17	G:A	C:T	C:C?	C:C	C:A	G:G			
WW30913_2	G:A	C:T	C:T	C:C	A:A?	G:G			
WW30913_3	G:A	C:T	C:T	C:C	C:C?	G:G			
WW30913_8	G:A	C:T	T:T?	C:C	C:A	G:G			
WW30913_11	G:A?	C:T	C:T	C:T ?	C:A	G:G			
WW30913_7	G:G	T:T	C:C?	C:C	A:A	G:G			
WW30913_6	G:G	T:T	C:T	C:C	A:A?	G:G			
WW30913_9	G:G	T:T	T:T	C:C	A:A	G:G			
WW30913_16	G:G	T:T	T:T	C:C	A:A	G:G			
WW30913_4	G:G	T:T	T:T	C:T	A:A	G:G			
WW30913_1	G:G ?	C:T ?	C:C	C:T ?	C:A	G:G			
WW30764	G:A	C:T	C:T	C:T ?	C:A?	G:G	T:T	C:T	G:A
WW30764_1	G:G	T:T	T:T	C:C	A:A	G:G	T:T	C:C	G:G
WW30764_4	G:G	T:T	T:T	C:C	A:A	G:G	T:T	C:C	G:G
WW30764_10	G:G	T:T	T:T	C:C	A:A	G:G	T:T	C:C	G:G
WW30764_12	G:G	T:T	T:T	C:C	A:A	G:G	T:T	C:C	G:G
WW30764_16	A:A	C:C	C:C	T:T	C:C	G:G	T:T	C:C	G:G
WW30764_5	G:G	T:T	T:T	C:C	A:A?	G:G	T:T	NC	A:A
WW30764_2	G:G	T:T	T:T	C:C	A:A	G:G	T:T	T:T	A:A
WW30764_13	G:G	T:T	T:T	NC	A:A	G:G	T:T	T:T	A:A
WW30764_17	G:G	T:T	T:T	C:C	A:A	G:G	T:T	T:T	A:A
WW30764_18	G:G	T:T	T:T	C:C	A:A	G:G	T:T	T:T	A:A
WW30764_7	G:A	C:T	C:T	C:C	C:A	G:A	T:T	T:T	A:A
WW30764_9	G:A	C:T	T:T?	C:C?	C:A	G:G	T:T	T:T	A:A
WW30764_15	G:A	C:T	T:T?	C:C	C:A	G:G	T:T	T:T	A:A
WW30764_8	A:A	C:C	C:C	T:T	C:C	G:G	T:T	C:T	G:A
WW30764_3	G:G	T:T	T:T	C:C	A:A	G:G	T:T	C:T	G:A
WW30764_6	G:G	T:T	T:T	C:C	A:A	G:G	T:T	C:T	G:A
WW30764_11	G:G	T:T	T:T	C:C	A:A	G:G	T:T	C:T	G:A
WW30764_14	G:G	T:T	T:T	C:C	A:A	G:G	T:T	C:T	G:A

B

Marker / Genotype	wsnp_Ra_c41135_48426638	wsnp_Ex_rep_c66331_64503065	wsnp_Ku_c12698_20441325	wsnp_Ex_c3005_5548573	wsnp_BE497169B_Ta_2_2	wsnp_Ku_c3817_7009093	wsnp_Ex_c11379_18370310	wsnp_Ex_c19525_28494827	wsnp_Ex_c45713_51429315
chrom region	3B1	3B1	3B1	3B1	3B1	3B1	6B3	6B3	6B3
cM position	0.0	1.4	1.4	2.3	3.2	34.6	0.0	18.1	18.1
Drysdale	A:A	C:C	C:C	T:T	C:C	G:G	C:C	C:C	G:G
Waagan	G:G	T:T	T:T	C:C	A:A	G:G	T:T	T:T	A:A
WW30875							C:T	C:C	G:G
WW30875_1							T:T	NC	G:G
WW30875_2							C:T	C:C	G:A
WW30875_3							T:T	C:C	G:G
WW30875_4							C:T	C:C	G:G
WW30875_5							T:T	C:C	G:G
WW30875_6							T:T	C:C	G:G
WW30875_7							T:T	C:C	G:G
WW30875_8							C:C	C:C	G:G
WW30875_9							C:T	C:C	G:G
WW30875_10							C:C	C:C	G:G
WW30875_11							C:C	C:C	G:G
WW30875_12							T:T	C:C	G:G
WW30875_13							C:C	C:C	G:G
WW30875_14							T:T	C:C	G:G
WW30875_15							T:T	C:C	G:G
WW30875_16							NC	C:C	G:G
WW30875_17							NC	C:C	G:G
WW30893							C:T	T:T	A:A
WW30893_1							C:T	T:T	A:A
WW30893_2							C:C	NC	A:A
WW30893_3							C:T	T:T	A:A
WW30893_4							C:C	T:T	A:A
WW30893_5							T:T	T:T	A:A
WW30893_6							T:T	T:T	A:A
WW30893_7							T:T	T:T	A:A
WW30893_8							T:T	T:T	A:A
WW30893_9							T:T	T:T	A:A
WW30893_10							C:T	T:T	A:A
WW30893_11							C:T	T:T	A:A
WW30893_12							T:T	T:T	A:A
WW30893_13							C:T	T:T	A:A
WW30893_14							C:C	T:T	A:A
WW30893_15							T:T	T:T	A:A
WW30893_16							C:C	T:T	A:A
WW30893_17							C:T	T:T	A:A
WW30900							T:T	C:T	A:A
WW30900_1							T:T	C:T	G:A
WW30900_2							T:T	C:T	G:A
WW30900_3							T:T	C:T	G:A
WW30900_4							T:T	C:C	G:G
WW30900_5							T:T	C:T	G:A
WW30900_6							T:T	C:C	G:G
WW30900_7							T:T	C:C	G:G
WW30900_8							T:T	C:C	G:G
WW30900_9							NC	NC	NC
WW30900_10							T:T	T:T	A:A
WW30900_11							T:T	C:C	G:G
WW30900_12							T:T	C:T	G:A
WW30900_13							T:T	C:T	G:A
WW30900_14							T:T	C:T	G:A
WW30900_15							T:T	T:T	A:A
WW30900_16							NC	NC	NC
WW30900_17							T:T	C:T	G:A

C



Marker / Genotype	wsnp_Ra_c41135_48426638	wsnp_Ex_rep_c66331_64503065	wsnp_Ku_c12698_20441325	wsnp_Ex_c3005_5548573	wsnp_BE497169B_Ta_2_2	wsnp_Ku_c3817_7009093	wsnp_Ex_c11379_18370310	wsnp_Ex_c19525_28494827	wsnp_Ex_c45713_51429315
chrom region	3B1	3B1	3B1	3B1	3B1	3B1	6B3	6B3	6B3
cM position	0.0	1.4	1.4	2.3	3.2	34.6	0.0	18.1	18.1
Drysdale	A A	C C	C C	T T	C C	G G	C C	C C	G G
Waagan	G G	T T	T T	C C	A A	G G	T T	T T	A A
WW30908							C T	T T	A A
WW30908_1							C T	T T	A A
WW30908_2							T T	T T	A A
WW30908_4							C T	T T	A A
WW30908_5							C C	T T	A A
WW30908_6							C C	T T	A A
WW30908_7							T T	T T	A A
WW30908_8							T T	T T	A A
WW30908_9							T T	T T	A A
WW30908_10							T T	T T	A A
WW30908_11							C T	T T	A A
WW30908_12							C T	T T	A A
WW30908_13							C T	T T	A A
WW30908_14							T T	T T	A A
WW30908_15							C T	T T	A A
WW30908_16							C T	T T	A A
WW30908_17							C C	T T	A A
WW30915							T T	C T ?	G G
WW30915_1							T T	C C	G G
WW30915_2							T T	T T	A A
WW30915_3							T T	C C	G G
WW30915_4							T T	C C	G G
WW30915_5							T T	C C	G G
WW30915_6							T T	C C	G G
WW30915_7							C C	T T	NC
WW30915_8							T T	C C	G G
WW30915_9							T T	C C	G G
WW30915_10							T T	C C	NC
WW30915_11							C C	T T	A A
WW30915_12							T T	C T	G A
WW30915_13							T T	C C	G G
WW30915_14							T T	C C	G G
WW30915_15							T T	C C	G G
WW30915_16							T T	C C	G G
WW30874							C T ?	T T	A A
WW30874_1							T T	T T	A A
WW30874_2							C C	T T	A A
WW30874_3							C C	T T	A A
WW30874_4							C T	T T	A A
WW30874_5							C C	T T	A A
WW30874_6							T T	T T	NC
WW30874_8							C C	T T	A A
WW30874_9							T T	T T	A A
WW30874_10							C C	T T	A A
WW30874_11							C T	T T	A A
WW30874_12							T T	T T	A A
WW30874_13							C C	T T	A A
WW30874_14							NC	NC	NC
WW30874_15							C T	T T	A A
WW30874_16							NC	T T	A A

D

Marker / Genotype	wsnp_Ra_c41135_48426638	wsnp_Ex_rep_c66331_64503065	wsnp_Ku_c12698_20441325	wsnp_Ex_c3005_5548573	wsnp_BE497169B_Ta_2_2	wsnp_Ku_c3817_7009093	wsnp_Ex_c11379_18370310	wsnp_Ex_c19525_28494827	wsnp_Ex_c45713_51429315
chrom region	3B1	3B1	3B1	3B1	3B1	3B1	6B3	6B3	6B3
cM position	0.0	1.4	1.4	2.3	3.2	34.6	0.0	18.1	18.1
Drysdale	A A	C C	C C	T T	C C	G G	C C	C C	G G
Waagan	G G	T T	T T	C C	A A	G G	T T	T T	A A
WW30852							T T	C T ?	G G
WW30852_1							T T	C C	G G
WW30852_2							T T	T T	A A
WW30852_3							T T	C C	G G
WW30852_4							T T	C T	G A
WW30852_5							C C	C C	G G
WW30852_6							NC	T T	A A
WW30852_7							C C	C C	G G
WW30852_8							T T	C T ?	A A
WW30852_9							T T	T T	A A
WW30852_10							T T	C T	G A
WW30852_11							T T	T T	A A
WW30852_12							T T	T T	A A
WW30852_13							T T	C C	G G
WW30852_14							C C	C C	G G
WW30852_15							NC	C C	G G
WW30852_16							T T	T T	A A
WW30711							T T	C T	G A
WW30711_1							T T	C C	G G
WW30711_2							T T	T T	A A
WW30711_3							T T	T T	A A
WW30711_4							T T	C C	G G
WW30711_5							NC	C T	NC
WW30711_6							T T	T T	A A
WW30711_7							T T	C T	G A
WW30711_8							T T	C T	G A
WW30711_9							T T	T T	A A
WW30711_10							T T	T T	A A
WW30711_11							T T	T T	A A
WW30711_12							T T	C T	G A
WW30711_13							T T	T T	A A
WW30711_14							T T	T T	A A
WW30711_15							T T	C T	G A
WW30711_16							T T	T T	A A
WW30655							C T	C T	G A
WW30655_10							C T	C C	G A
WW30655_2							NC	C C	G G
WW30655_1							C C	C C	G G
WW30655_3							C C	C C	G G
WW30655_9							C C	C C	G G
WW30655_17							C C	C C	G G
WW30655_11							C T	C C	G G
WW30655_15							C T	C C	G G
WW30655_13							T T	C C	G G
WW30655_16							T T	C C	G G
WW30655_5							C C	C T	G A
WW30655_8							C C	C T	G A
WW30655_4							C T	C T	G A
WW30655_7							C T	C T	G A
WW30655_12							C T	T T	A A
WW30655_6							T T	T T	G A ?
WW30655_14							NC	C T	NC

E

Fig. 3.3 Selection of homozygous RIL-NILs using KASP markers. Cell color designates homozygous Drysdale (pink), homozygous Waagan (green) or heterozygous (yellow) marker allele calls. The original marker profile of the 5-plant DNA bulk (highlighted in blue for 3B and red for 6B) is shown for reference above the scores for the single plants, grown from the same seed packet. Selected homozygous RIL-NILs lines are marked in orange. NC: No call (unscorable) Tentative KASP calls are marked with '?'. Markers outlined in blue performed poorly; White cells represent KASP scores different from parental alleles. Blank cells mean that the marker was not run. In some families, plants were re-ordered to aid interpretation. Marker order was determined by (Shirdelmoghanloo, Taylor, et al. 2016) on DH lines.

Marker / Genotype	wsnp_Ra_c41135_48426638	wsnp_Ku_c12698_20441325	wsnp_Ex_rep_c66331_64503065	wsnp_Ex_c3005_5548573	wsnp_BE497169B_Ta_2_2
Linkage group	3B1	3B1	3B1	3B1	3B1
cM position	0.0	1.4	1.4	2.3	3.2
Drydale	A:A	C:C	C:C	T:T	C:C
Drydale	A:A	C:C	C:C	T:T	C:C
Drydale	A:A	C:C	C:C	T:T	C:C
Drydale	A:A	C:C	C:C	T:T	C:C
Waagan	G:G	T:T	T:T	C:C	A:A
Waagan	G:G	T:T	T:T	C:C	A:A
Waagan	G:G	T:T	T:T	C:C	A:A
Waagan	G:G	T:T	T:T	C:C	A:A
WW30674_13_1	G:G	T:T	C:C	T:T	C:C
WW30674_13_2	G:G	T:T	C:C	T:T	C:C
WW30674_13_3	G:G	T:T	C:C	T:T	C:C
WW30674_1_1	G:G	T:T	C:C	T:T	C:C
WW30674_1_2	G:G	T:T	C:C	T:T	C:C
WW30674_1_3	G:G	T:T	C:C	T:T	C:C
WW30674_3_1	G:G	T:T	T:T	C:C	C:C
WW30674_3_2	G:G	T:T	T:T	C:C	C:C
WW30674_3_3	G:G	T:T	T:T	C:C	C:C
WW30674_4_1	G:G	T:T	T:T	C:C	C:C
WW30674_4_2	G:G	T:T	T:T	C:C	C:C
WW30674_4_3	G:G	T:T	T:T	C:C	C:C
WW30692_12_1	A:A	C:C	C:C	T:T	C:C
WW30692_12_2	A:A	C:C	C:C	T:T	C:C
WW30692_12_3	A:A	C:C	C:C	T:T	C:C
WW30692_4_1	A:A	C:C	C:C	T:T	C:C
WW30692_4_2	A:A	C:C	C:C	T:T	C:C
WW30692_4_3	A:A	C:C	C:C	T:T	C:C
WW30692_17_1	G:G	T:T	T:T	C:C	A:A
WW30692_17_2	G:G	T:T	T:T	C:C	A:A
WW30692_17_3	G:G	T:T	T:T	C:C	A:A
WW30692_3_1	G:G	T:T	T:T	C:C	A:A
WW30692_3_2	G:G	T:T	T:T	C:C	A:A
WW30692_3_3	G:G	T:T	T:T	C:C	A:A
WW30709_8_1	A:A	C:C	C:C	T:T	C:C
WW30709_8_2	A:A	C:C	C:C	T:T	C:C
WW30709_8_3	A:A	C:C	C:C	T:T	C:C
WW30709_9_1	A:A	C:C	C:C	T:T	C:C
WW30709_9_2	A:A	C:C	C:C	T:T	C:C
WW30709_9_3	A:A	C:C	C:C	T:T	C:C
WW30709_5_1	G:G	T:T	T:T	C:C	A:A
WW30709_5_2	G:G	T:T	T:T	C:C	A:A
WW30709_5_3	G:G	T:T	T:T	C:C	A:A
WW30709_17_1	G:G	T:T	T:T	C:C	A:A
WW30709_17_2	G:G	T:T	T:T	C:C	A:A
WW30709_17_3	G:G	T:T	T:T	C:C	A:A
WW30913_12_1	A:A	C:C	C:C	T:T	C:C
WW30913_12_2	A:A	C:C	C:C	T:T	C:C
WW30913_10_1	A:A	C:C	C:C	T:T	C:C
WW30913_10_2	A:A	C:C	C:C	T:T	C:C
WW30913_10_3	A:A	C:C	C:C	T:T	C:C
WW30913_16_1	G:G	T:T	T:T	C:C	A:A
WW30913_16_2	G:G	T:T	T:T	C:C	A:A
WW30913_9_1	G:G	T:T	T:T	C:C	A:A
WW30913_9_2	G:G	T:T	T:T	C:C	A:A
WW30913_9_3	G:G	T:T	T:T	C:C	A:A

Marker / Genotype	wsnp_Ex_c11379_18370310	wsnp_Ex_c19525_28494827	wsnp_Ex_c45713_51429315
Linkage group	6B3	6B3	6B3
cM position	0.0	18.1	18.1
Drydale	C:C	C:C	G:G
Drydale	C:C	C:C	G:G
Drydale	C:C	C:C	G:G
Drydale	C:C	C:C	G:G
Waagan	T:T	T:T	A:A
Waagan	T:T	NC	A:A
Waagan	T:T	NC	A:A
Waagan	T:T	T:T	A:A
WW30711_1_1	T:T	C:C	G:G
WW30711_1_2	T:T	C:C	G:G
WW30711_4_1	T:T	C:C	G:G
WW30711_4_2	T:T	C:C	G:G
WW30711_4_3	T:T	C:C	G:G
WW30711_2_1	T:T	T:T	A:A
WW30711_2_2	T:T	T:T	A:A
WW30711_16_1	T:T	T:T	A:A
WW30711_16_2	T:T	T:T	A:A
WW30711_16_3	T:T	T:T	A:A
WW30875_13_1	C:C	C:C	G:G
WW30875_13_2	C:C	C:C	G:G
WW30875_10_1	C:C	C:C	G:G
WW30875_10_2	C:C	C:C	G:G
WW30875_10_3	C:C	C:C	G:G
WW30875_5_1	T:T	C:C	G:G
WW30875_5_2	T:T	C:C	G:G
WW30875_7_1	T:T	C:C	G:G
WW30875_7_2	T:T	C:C	G:G
WW30875_7_3	T:T	C:C	G:G
WW30908_5_1	C:C	T:T	A:A
WW30908_5_2	C:C	T:T	A:A
WW30908_17_1	C:C	T:T	A:A
WW30908_17_2	C:C	T:T	A:A
WW30908_17_3	C:C	T:T	A:A
WW30908_2_1	T:T	T:T	A:A
WW30908_2_2	T:T	T:T	A:A
WW30908_8_1	T:T	T:T	A:A
WW30908_8_2	T:T	T:T	A:A
WW30908_8_3	T:T	T:T	A:A
WW30900_11_1	T:T	C:C	G:G
WW30900_11_2	T:T	C:C	G:G
WW30900_6_1	T:T	C:C	G:G
WW30900_6_2	T:T	C:C	G:G
WW30900_6_3	T:T	C:C	G:G
WW30900_10_1	T:T	T:T	A:A
WW30900_10_2	T:T	T:T	A:A
WW30900_15_1	T:T	T:T	A:A
WW30900_15_2	T:T	T:T	A:A
WW30900_15_3	T:T	T:T	A:A

Fig. 3.4 Results from KASP assays to verify the genotypes of selected homozygous RIL-NILs for 3B and 6B (A and B respectively) QTL set. Two to three progenies of each selected homozygous plant were analyzed. Pink, and green cells represent Drysdale and Waagan alleles respectively. NC: No call (uncallable).

3.3.1.1 Single grain weight (SingGW)

The NILs were largely unaffected by the heat treatment for SingGW; only four lines from the 3B set showed significant effects (reductions): one carrying Waagan QTL alleles, and three carrying Drysdale alleles (Fig. 3.5).

In the 3B NIL set, heat treated plants carrying Drysdale alleles showed reduced SingGW relative to those carrying Waagan alleles, in four of the five families (Fig. 3.6). In the 6B NIL set, two of the lines in the WW30711 family had lower SingGW than the other two lines under heat treatment, but this did not relate to the genotype at the 6B locus (Fig. 3.6). The parent varieties Drysdale and Waagan did not show any significant effects of heat treatment on SingGW, nor did they significantly differ under control conditions (Fig 3.5 and 3.6) perhaps reflecting the lower number of replications used in the experiment or chance variation.

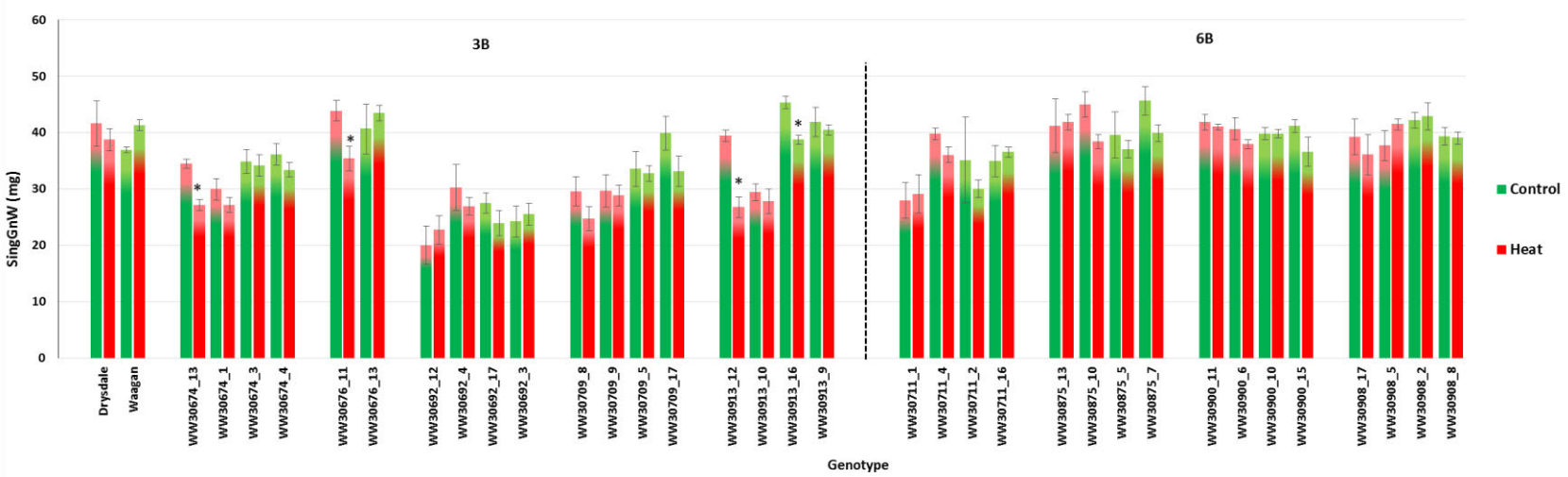


Fig. 3.5 Final single grain weight (SingGW) of NILs. Green and red bars represent control and heat treated plants, respectively. Bars with pink and light green tips represents lines harbouring Drysdale and Waagan marker alleles, respectively, at the target loci. NIL pairs for 3B and 6B QTL are separated by the dotted line. Bars are SE and "*" shows where there was a significant difference between the control and heat treated plants of the same line, at α=0.05.

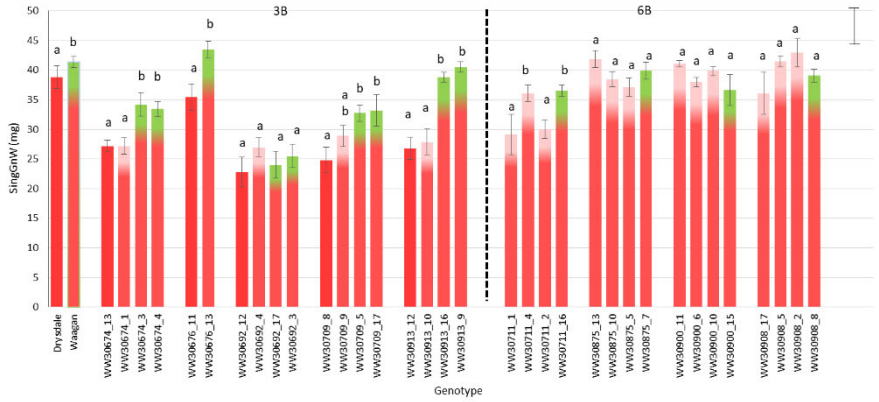


Fig. 3.6 Final single grain weight (SingGW) of NIL lines subjected to heat treatment (same means as shown in Fig 3.5, except only the heat treated plants). The vertical bar indicates the LSD=5.246 at α = 0.05 for within–family comparisons. In each family, pairs of bars with different letters were significantly different. Columns with pink and light green tip represent lines harbouring Drysdale and Waagan alleles, respectively, at the target QTLs.

3.3.1.2 Final shoot dry weight (ShootW)

ShootW was essentially unaffected by the heat treatment; only one line (WW30692) showed an effect with heat treatment (increase) (Fig. 3.7).

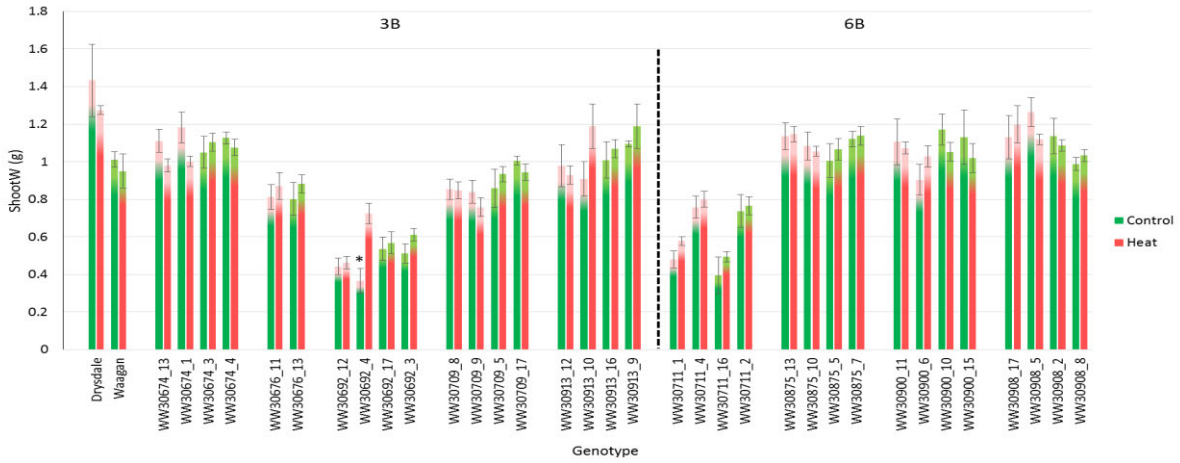


Fig. 3.7 Final shoot weight ShootW of NILs. Green and red bars represent control and heat treated plants, respectively. Bars with pink and light green tips represent lines harbouring Drysdale and Waagan marker alleles at the target loci, respectively. NIL sets for 3B and 6B QTL are separated by the dotted line. Lines on the bars show SE and "*" shows where there were significant differences between control and heat treated plants of the same line, at α=0.05.

3.3.1.3 Relative chlorophyll content

Relative chlorophyll content (SPAD) was measured at three time points, just before heat treatment (10DAA), just after heat treatment (13DAA) and two weeks after heat treatment (27DAA). Heat responses were then assessed based on the change in SPAD during the heat treatment period (Fig. 3.8 A; 10DAA vs. 13DAA) or between just before treatment to 2 weeks after the heat treatment (Fig. 3.8 B; 10DAA vs. 27DAA).

In the 3B QTL set, heat had little effect on SPAD change during the treatment period (10-13 DAA), but it had large effects by 27DAA which were generally related to the alleles at the 3B QTL; Marker alleles from Drysdale (which contributes the intolerance allele at this QTL) were significantly associated with greater reductions in SPAD in four of the five families, and in the fifth, significant reductions were observed in both lines carrying the Drysdale and Waagan alleles, but the former showed a greater absolute reduction.

In the 6B set, some significant reductions in SPAD were observed due to heat and these responses tended to be weakly associated with genotype at the 6B QTL markers. In family WW30900, significant heat responses were consistently associated with Waagan alleles (in line with Waagan contributing the susceptibility allele at this QTL), while in family WW30908, this was only the case for responses during the heat treatment period. In family WW30875, heat reduced SPAD in all lines, about equally in those carrying the two allele types.

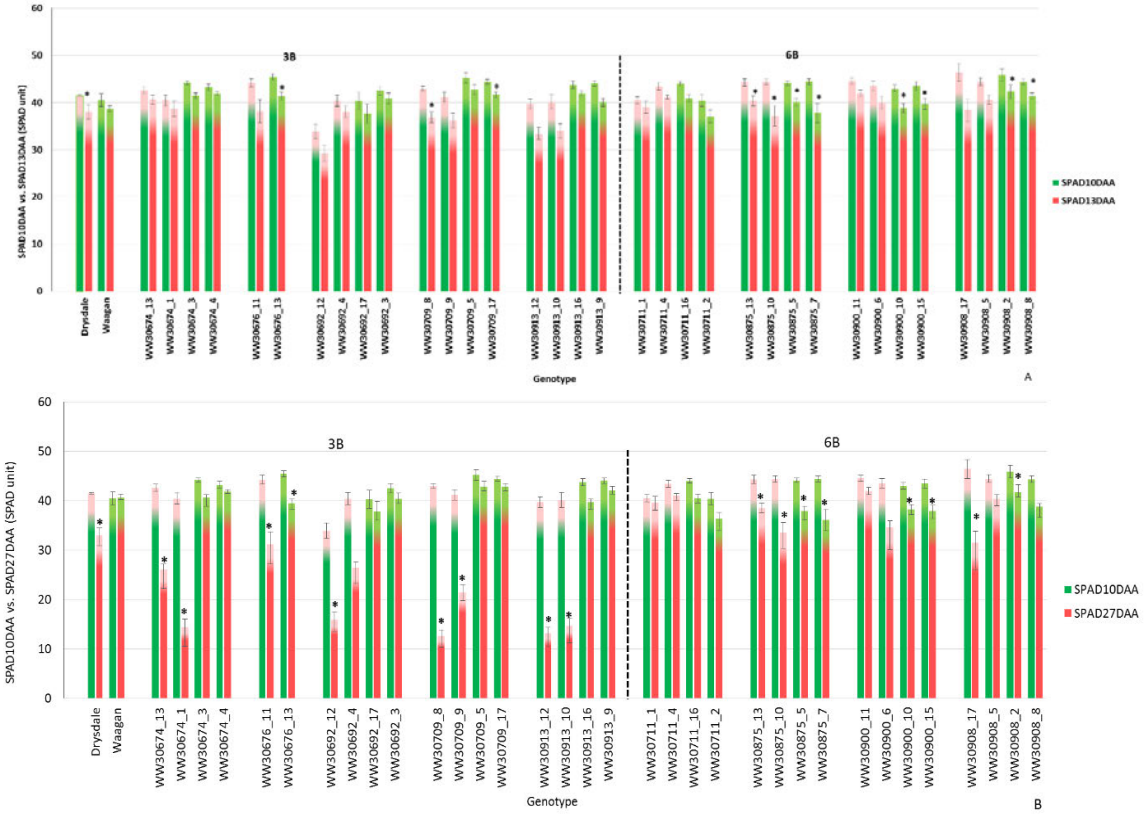


Fig. 3.8 Relative chlorophyll content of NILs. A: Chlorophyll content of the same plants at 10DAA (before heat treatment; green bars) and 13DAA (just after heat treatment; red bars). B: Chlorophyll content of same plants at 10DAA (before heat treatment; green bars) and at 27DAA (two weeks after heat treatment; red bars). Bars with pink and light green tips represent lines harbouring Drysdale and Waagan alleles at target QTL, respectively. NIL sets for 3B and 6B QTL are separated by the dotted line. Bars show SE.

Relative chlorophyll contents of the control plants at 10DAA, 13DAA and 27DAA are represented in Fig. 3.9 A-C. In the 3B set, Drysdale marker alleles at the QTL were associated with lower chlorophyll content in three of the five families. In the 6B set, there were some significant

differences in chlorophyll content between lines, but these differences were not consistently related with marker alleles at the 6B QTL in any of the families.

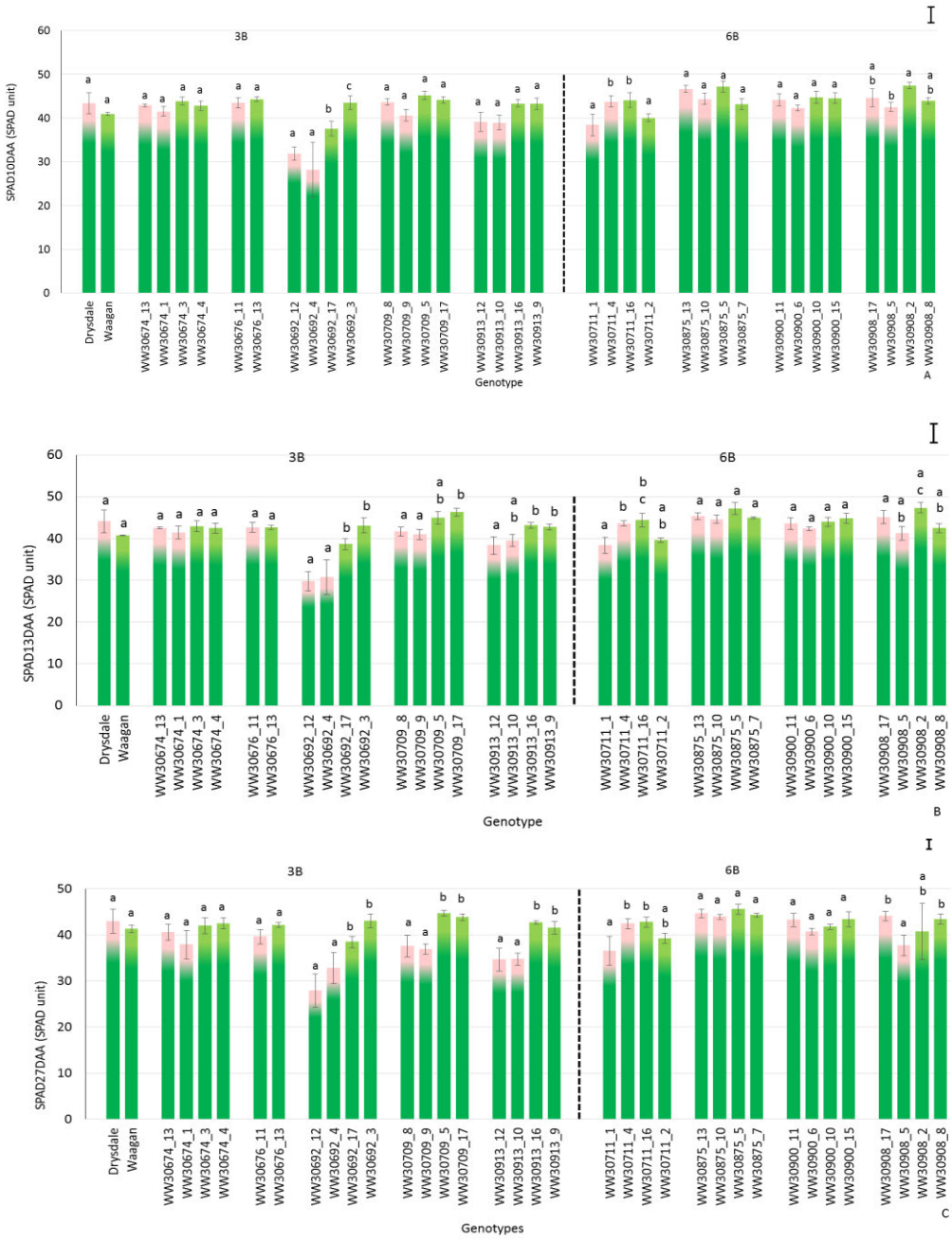


Fig. 3.9 Relative chlorophyll content of NILs under control conditions, at 10DAA (A), 13DAA (B) and 27DAA (C). Plants harbouring Drysdale (pink tip) and Waagan (light green tip) alleles at the target QTLs are represented by the bars with the pink and light green tips, respectively. Bars with different letters show where means were significantly different at p=0.05 for within family comparisons of the same trait (LSDs were 4.851, 4.259 and 5.658 for Figure A, B and C respectively). NIL sets for 3B and 6B QTL are separated by the dotted line. Lines on the bars show SE.

3.3.1.4 Culm length at maturity (CulmLMat)

One line (WW30692_4) showed significant increase in culm length after heat treatment while others remained unaffected (Fig. 3.10). This may have been due to segregation for an *Rht* gene in this line, caused by outcrossing. Family WW307711 also showed similar pattern and seems they differ for *Rht* genes, likely due to segregation for *Rht* genes in the original seed packet

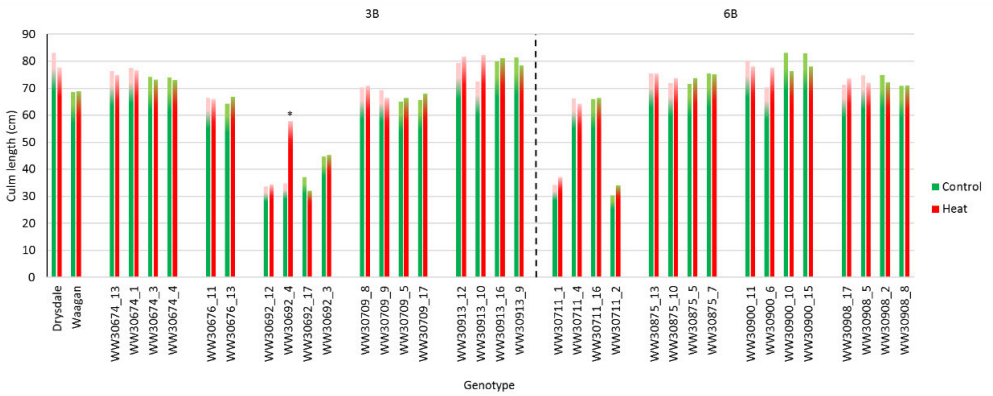


Fig. 3.10 Culm length of NILs at maturity. Green and red bars represent control and heat-treated plants, respectively. Bars with pink and light green tips represent lines with Drysdale and Waagan alleles at target loci, respectively. NIL sets for 3B and 6B QTL are separated by the dotted line. "*" shows where there were significant differences between control and heat-treated plants of the same line, at α=0.05.

3.3.1.5 Days to anthesis (Rane & Nagarajan)

No significant difference was observed in any line for days to anthesis between control and heat treated plants (Fig. 3.11), which was expected, as heat treatments were applied after anthesis. The allelic variation in different lines also did not affect the date of anthesis.

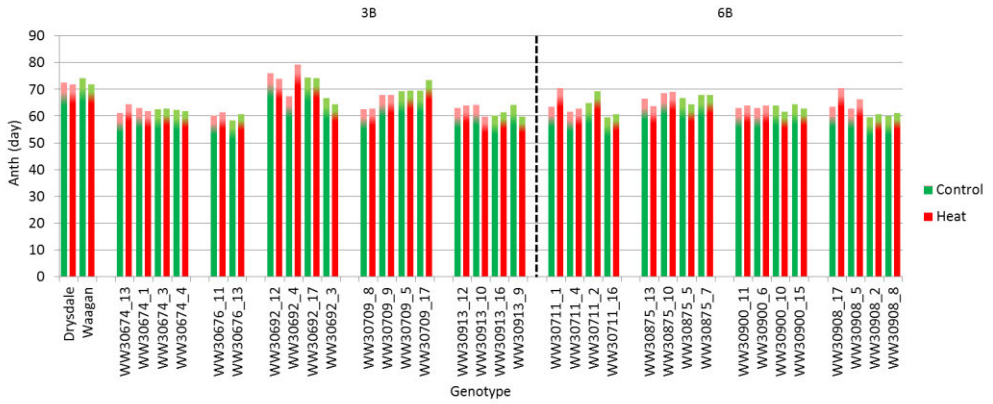


Fig. 3.11 Days to anthesis of NILs. Green and red bars represent control and heat-treated plants, respectively. Bars with pink and light green tips represent lines with Drysdale and Waagan alleles at target loci, respectively. NIL sets for 3B and 6B QTL are separated by the dotted line.

3.3.1.6 Association of phenotypic and genotypic scores of the RIL-NILs

As previously highlighted, in all five RIL-NILs sets for the 3B locus, the lines carrying Drysdale marker alleles at the 3B QTL showed greater loss of chlorophyll due to the heat treatment (by just after or two weeks after heat treatment) than the lines carrying the Waagan alleles, confirming that the effect of the 3B tolerance locus was closely linked to these markers, or spanned by them. This can also be visualized in Fig. 3.12, which shows the phenotype data in colored conditional formatting, alongside the marker genotypes of the lines. As these lines were confirmed as differing for the 3B tolerance locus genotype, they were shown to be suitable for future molecular/physiological/field evaluation of the 3B tolerance locus effects.

Contrasting chromosomes in the WW30674 RIL-NIL set were the result of a (different) recombination event in the region spanned by the 3B markers. The contrasting tolerance phenotypes between these lines (significant difference for chlorophyll loss by two weeks after heat treatment and SingGW traits and decreasing trend of the Drysdale types being less tolerant for ShootW and loss of chlorophyll just after heat treatment) suggested that the 3B tolerance locus is positioned in the 1.8 cM interval between the markers wsnp_Ku_c12698_20441325 and wsnp_BE497169B_Ta_2_2 (Fig. 3.12, between red arrows), however, this position needs to be confirmed by further mapping work. There was a large gap of 31.4 cM between wsnp_BE497169B_Ta_2_2 wsnp_Ku_c3817_7009093 markers mapped by Shirdelmoghanloo et al. (2016). Later in this thesis (Section 4.4) possible reason for this large gap is discussed. The Drysdale types showed greater loss of chlorophyll between 10 and 27DAA and greater loss of SingGW and ShootW. Hence, the WW30674 set of RIL-NILs helped to further delimit the 3B tolerance locus, in addition to providing another set of 3B locus NILs that could be used in functional evaluation of the locus effects.

As already mentioned, the 6B QTL sets showed no consistent tolerance phenotype differences associated with the genotypes of the markers in the 6B QTL region (Fig 3.12). Therefore, these RIL-NILs could not be confirmed as differing for a 6B tolerance locus.

Marker / Genotype	wsnp_Ra_c41135_48426638	wsnp_Ku_c12698_20441325	wsnp_Ex_rep_c66331_6450306	wsnp_Ex_c3005_5548573	wsnp_BE497169B_Ta_2_2	wsnp_Ku_c3817_7009093	wsnp_Ex_c11379_18370310	wsnp_Ex_c19525_28494827	wsnp_Ex_c45713_51429315	Loss of chlorophyll (%) between 10 and 13DAA	Loss of chlorophyll (%) between 10 and 27DAA	Loss of SingGnW (%)	Loss of shootw(%)
Linkage group	3B1	3B1	3B1	3B1	3B1	3B1	6B3	6B3	6B3				
cM position	0.0	1.4	1.4	2.3	3.2	34.6	0.0	18.1	18.1				
Drysdale	A:A	C:C	C:C	T:T	C:C	G:G	T:T	T:T	A:A	-8.31	-20.42	-2.23	-11.94
Waagan	G:G	T:T	T:T	C:C	A:A	G:G	C:C	C:C	G:G	-4.51	0.25	4.49	-6.68
WW30674_13	G:G	T:T	C:C	T:T	C:C	G:G	C:C			-4.72	-38.64	-21.21	-11.91
WW30674_1	G:G	T:T	C:C	T:T	C:C	G:G	C:C			-4.30	-64.27	-9.32	-15.26
WW30674_3	G:G	T:T	T:T	C:C	C:C	G:G	C:C			-6.19	-8.00	-2.05	5.33
WW30674_4	G:G	T:T	T:T	C:C	C:C	G:G	C:C			-3.12	-3.32	-7.60	-4.51
WW30676_11	A:A	C:C	C:C	C:C	C:C	A:A	T:T			-13.68	-29.40	-19.28	7.21
WW30676_13	G:G	T:T	T:T	C:C	A:A?	G:G	T:T			-8.94	-13.15	6.83	10.18
WW30692_12	A:A	C:C	C:C	T:T	C:C	A:A	T:T			-13.79	-53.01	13.41	4.51
WW30692_4	A:A	C:C	C:C	T:T	C:C	A:A	T:T			-5.75	-34.55	-10.87	98.34
WW30692_17	G:G	T:T	T:T	C:C	A:A	G:G	T:T			-6.52	-6.05	-12.80	6.02
WW30692_3	G:G	T:T	T:T?	C:C	A:A?	G:G	T:T			-3.65	-4.94	5.16	19.28
WW30709_8	A:A	C:C	C:C	T:T	C:C	G:G	T:T			-14.28	-70.60	-16.21	-0.75
WW30709_9	A:A	C:C	C:C	T:T	C:C	A:A	T:T			-12.08	-47.97	-2.61	-9.69
WW30709_5	G:G	T:T	T:T	C:C	A:A?	G:G	T:T			-5.42	-5.14	-2.51	8.92
WW30709_17	G:G	T:T	T:T	C:C	A:A	G:G	T:T			-6.14	-3.41	-16.90	-6.10
WW30913_12	A:A	C:C	C:C	T:T	C:C	A:A	T:T			-15.80	-66.80	-32.11	-5.00
WW30913_10	A:A	C:C	C:C	T:T	C:C	G:G	T:T			-15.31	-63.40	-5.39	13.81
WW30913_16	G:G	T:T	T:T	C:C	A:A	G:G	T:T			-4.17	-9.31	-14.52	6.16
WW30913_9	G:G	T:T	T:T	C:C	A:A	G:G	T:T			-9.02	-4.57	-3.37	8.54
WW30711_1							T:T	C:C	G:G	-3.67	-2.13	4.12	20.21
WW30711_4							T:T	C:C	G:G	-7.05	-7.84	-9.35	3.67
WW30711_2							T:T	T:T	A:A	-8.38	-10.14	-14.53	25.20
WW30711_16							T:T	T:T	A:A	-5.04	-5.47	4.58	5.51
WW30875_13	A:A	C:C	C:C	T:T	C:C	G:G	C:C	C:C	G:G	-8.78	-13.06	1.58	0.86
WW30875_10	A:A	C:C	C:C	T:T	C:C	G:G	C:C	C:C	G:G	-16.30	-24.47	-14.60	-2.55
WW30875_5	A:A	C:C	C:C	NC	C:C ?	G:G	T:T	C:C	G:G	-9.12	-13.96	-6.41	6.08
WW30875_7	A:A	C:C	C:C	NC	C:C	G:G	T:T	C:C	G:G	-15.12	-18.72	-12.62	1.51
WW30900_11	G:G	T:T	T:T	C:C	A:A	G:G	T:T	C:C	G:G	-5.83	-5.80	-1.98	-2.96
WW30900_6	G:G	T:T	T:T	C:C	A:A	G:G	T:T	C:C	G:G	-8.12	-20.49	-6.62	13.78
WW30900_10	G:G	T:T	T:T	C:C	A:A	G:G	T:T	T:T	A:A	-9.62	-10.99	0.06	-10.08
WW30900_15	G:G	T:T	T:T	C:C	A:A	G:G	T:T	T:T	A:A	-8.65	-12.93	-11.06	-9.85
WW30908_17	G:G	T:T	T:T	C:C	A:A	G:G	C:C	T:T	A:A	-17.30	-32.09	-8.05	6.03
WW30908_5	G:G	T:T	T:T	C:C	A:A	G:G	C:C	T:T	A:A	-8.75	-9.22	10.02	-11.59
WW30908_2	G:G	T:T	T:T	C:C	A:A	G:G	T:T	T:T	A:A	-7.71	-8.94	1.73	-4.32
WW30908_8	G:G	T:T	T:T	C:C	A:A	G:G	T:T	T:T	A:A	-6.74	-12.54	-0.81	4.44

Fig. 3.12 Summary of genotypes and phenotypes of NIL sets. Pink and green cells represent Drysdale and Waagan marker alleles respectively. NC: No call (uncallable). White cells designate KASP scores different from parental alleles and '?' for unsure calls. Green to red cell colors indicate increasing loss (%) of chlorophyll between 10 and 13DAA, 10 and 27DAA, SingGW and ShootW and represent the same data as in Fig 3.8 (A and B), Fig 3.5 and Fig 3.7 respectively. Red arrows show potential position of 3B QTL.

3.4 Discussion

3.4.1 Homozygous RIL-NIL pair selection and propagation

Homozygous RIL-NIL pairs differed from each other for small chromosome segments at the QTL regions and provide the opportunity to study QTL effects using a small population of lines. In this study, homozygous RIL-NIL pairs were selected for the 3B and 6B QTL regions using KASP markers, from 22 families heterogeneous for those loci. Phenotyping of different RIL-NIL pairs was expected to reveal phenotypic contributions of different chromosome segments in the QTL regions to aid fine mapping. RIL-NILs are required to be tested in the field or close to the production environment for assessing the breeding value of the QTL. Seeds from the RIL-NILs were field multiplied for trialling using late vs. timely sown trials with irrigation to assess heat tolerance, as part of GRDC project UA-00147, in 2017 and 2018 seasons, at sites at Wagga Wagga, Leeton and Condobolin, NSW. The NILs will also be trialed under rain fed conditions as part of the ARC Industrial Transformation Research Hub for Wheat in a hot and dry climate.

3.4.2 RIL-NIL phenotyping

3.4.2.1 Single grain weight (SingGW), final shoot dry weight (ShootW), culm length at maturity (CulmLMat) and days to anthesis (Rane & Nagarajan)

SingGW was mostly unaffected by heat in the RIL-NILs. Three lines carrying Drysdale alleles at the 3B locus showed reduction in SingGW as expected (Shirdelmoghanloo, Taylor, et al. 2016). Lines with the Drysdale allele at the 6B locus were expected to be heat tolerant. However, the NIL sets for the 6B locus showed weak associations between 6B marker genotypes and SingGW. Most plants reached the sensitive stage for heat stress during May and June. Plants in the greenhouse were exposed to high temperature (>30^{o}C, Table 2.2) for one day at that period (due to high outside temperatures) which might have affected SingGW in control plants and explained the weak/lack of correlation with genotype at the tolerance loci.

ShootW was also largely unaffected by the heat treatment, so the correlation between SingGW and ShootW heat responses identified by Shirdelmoghanloo, Lohraseb, et al. (2016) could not be tested in this instance.

CulmLMat was also not affected by heat and no effect of the 3B or 6B tolerance loci on anthesis date was observed, consistent with the findings of (Shirdelmoghanloo, Taylor, et al. 2016).

3.4.2.2 Relative chlorophyll content

RIL-NIL pairs carrying Drysdale marker alleles at the 3B locus showed small reductions in chlorophyll during the treatment, but they showed much greater heat-attributed chlorophyll loss than the Waagan types by two weeks after heat treatment. Shirdelmoghanloo, Taylor, et al. (2016) observed that the 3B QTL accounted for ~13 to 40 % variance for chlorophyll retention and that the Waagan allele conferred greater chlorophyll stability than the Drysdale allele. It was also reported that chlorophyll retention, or stay green, was associated with higher ShootW and longer grain filling duration. In this study, this correlation was not strongly observed. The RIL-NILs selected for 6B QTL showed no consistent association with the traits.

The RIL-NIL phenotype analysis showed that lines with Drysdale and Waagan alleles in the WW30674 family expressed contrasting phenotypes under short term heat stress. This family resulted in recombination (Fig. 3.12) in the region and allowed further delimitation of the 3B locus. These recombinant chromosomes could be used to further delimit the locus by scoring the WW30674 NILs with additional markers in the region.

Chapter 4: Further mapping of the 3B QTL for grain filling

4.1 Introduction

Narrowing down a QTL interval can be done using molecular markers and repeated phenotyping. The markers used to define a major grain filling heat tolerance QTL, *QHsgw.aww-3B* on chromosome 3B in a Drysdale x Waagan DH population (Shirdelmoghanloo, Taylor, et al. 2016) covered 34.6 cM. The most closely associated markers were the distal most markers on the map of the short arm of chromosome 3B, so the boundary of the QTL interval was only defined on the lower (proximal side). This region could correspond to one gene with major effect or, less likely, a cluster of genes. The chromosome 3B sequence pseudomolecule is predicted to contain 7264 coding genes (Choulet, Alberti, Theil, Glover, Barbe, Daron, Pingault, Sourdille, Couloux, Paux, et al. 2014) with a gene density of one gene per 104 kb (Choulet, Frédéric et al. 2010). Further mapping of the 3B locus for grain filling heat tolerance could lead to the cloning of the gene/s controlling the trait and development of a diagnostic marker to enable breeders to select for heat tolerance. In this region, Shirdelmoghanloo, Taylor, et al. (2016) defined six genetically non redundant loci using SNP markers from the 9K SNP array, at positions 0, 1.4, 2.3, 3.2 and 34.6 cM on linkage group 3B1 of the Drysdale x Waagan DH map. It may be possible to delimit the 3B locus further by mapping additional markers closer to the telomere, or in the large gap in the map between positions 3.2 and 34.6 cM. Available genetic maps with marker information for parental lines Drysdale and Waagan e.g. 9K SNP array, 35K Axiom array, 90K SNP array, and markers used by other researches in the same region, could provide a source of additional markers. This chapter describes work to further delimit the 3B heat tolerance locus using the aforementioned approaches.

4.2 Materials and methods

Markers mapped in the vicinity of the 3B QTL were identified in other genetic maps: Maps examined included genetic consensus/biparental genetic maps supplied for the 9K SNP array (Cavanagh et al. 2013), 90K SNP array (Wang et al. 2014), and the 35K Axiom array (http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB /axiom_download.php). Sequenced RFLP probes or sequenced SSR markers reported to be mapped by others close to heat tolerance

related traits in this region (Xbarc133, Xcfa2226, Xbrac102, Xbarc75 and Xgwm493; (Shirdelmoghanloo, Taylor, et al. 2016) , and references therein) were also evaluated.

Potential markers were also identified along the IWGSC whole genome assembly of the cv. Chinese Spring through the wheat genomics platform DAWN (Diversity Among Wheat geNome). The IWGSC assembly used in DAWN was the version made using the DeNovoMAGICTM software from NRgene. DAWN (Ute Baumann, unpublished) is a tool created by The University of Adelaide Bioinformatics group to visualize diversity among wheat genotypes by aligning the IWGSC reference sequence of cv. Chinese Spring to the 10x genomic shotgun sequences from Drysdale and 15 other (mostly Australian) wheat varieties generated in a collaboration with Bioplatforms Australia (BPA) (Edwards et al. 2012). The tool also aligns the genomic sequences with gene annotation and gene expression data, and with marker information from different sources including the 35K Axiom array (Allen et al. 2017). Markers from the 9k SNP array (Cavanagh et al. 2013), mapped by Shirdelmoghanloo, Taylor, et al. (2016), were used to search the IWGSC genomic reference sequence using the BLAST portal of ACPFG (The University of Adelaide) to obtain the corresponding scaffold numbers (Table 4.2). The scaffolds in the large gap between the markers at 3.2 and 34.6 cM, and distal to the last markers on the short arm of the map, were identified with the help of ACPFG Bioinformatics group using the marker scaffolds as reference. The scaffolds were selected with an aim to generate one marker per cM. Preference was given to the markers reported to be polymorphic for Drysdale/Waagan in those scaffolds, according to the information provided for the 35K Axiom array on the aforementioned website. As there was only BPA sequence for Drysdale and not Waagan, Drysdale and Chinese Spring were compared in DAWN to identify SNP markers for mapping.

When possible, primer sets for KASP assays were designed to be 3B sub-genome specific. The SNP source sequences were BLASTed against the Chinese Spring genomic reference sequence to identify polymorphisms between the (usually 3) homoeologous sequence copies, which were subsequently used to position the 3’ ends of the common primers so as to make them more locus-specific. Primer performance was predicted using the NetPrimer online tool (http://www.premierbiosoft.com/netprimer/) so that primers predicted to give strong hairpin structure, self-dimers or cross dimers, could be avoided.

4.3 Results and Discussion

Targeted marker generation and mapping using Drysdale x Waagan DH lines.

Genetic locations of the markers on the 90k SNP array consensus map and the 9k SNP array map of the CSIRO 4-way MAGIC population were mostly on chromosome 3B, but otherwise the order and locations of the markers were quite inconsistent between these maps and the 9K SNP Drysdale x Waagan genetic map (Table 4.1). Possible reasons for this inconsistency may include use of different plant materials (parent with divergent haplotypes), difficulties in achieving correct marker orders with consensus maps, and differences in algorithms used for constructing the various maps. The order of the IWGSC genomic scaffolds agreed most with marker orders of the 9K SNP Drysdale x Waagan genetic map (Table 4.1), so the IWGSC genomic scaffolds were used as the main basis for identifying SNPs located in useful positions.

Three scaffolds in the IWGSC whole genome assembly (92936, 20580 and 98002) contained markers mapped to the respective positions 0, 2.29 and 3.15 cM in the Drysdale x Waagan genetic map (Table 4.1). Among those three scaffolds, the positions of last two markers were not in the same order as in the Drysdale x Waagan genetic map. Other IWGSC scaffolds matching markers in the region were not allocated to chromosomes or were in different orders on the assembly relative to the Drysdale x Waagan genetic map (Table 4.1), reflecting limitations in the accuracy and completeness of the genome assembly in this region.

With the help of the Bio-informatics group at ACPFG, scaffolds potentially located in and around the QTL region were identified (Table 4.2). These scaffolds were visualized in DAWN to identify SNPs polymorphic for Drysdale/CS. From these, a subset of SNPs was chosen for KASP assay development, giving preference to those shown to be Drysdale/Waagan polymorphic in the publicly available database of 35K Axiom array SNP scores of wheat varieties. Twenty-six primer sets were designed and tested on the parents (Table 4.4). Twelve primer sets were found to give clear polymorphism, nine were monomorphic and five failed to produce clear amplification (Table 4.3).

Table 4.1 Position of marker sequences in the 3B QTL region in various genetic maps and the genomic assembly of cv. Chinese Spring.

Marker	W×D 9K SNP map		90K SNP array consensus		CSIRO 4-way MAGIC RIL population 9K SNP map		IWGSC scaffolds and their positions		
	Linkage group	cM	Linkage group	cM	Linkage group	cM	Linkage group	cM	Scaffold
wsnp_Ra_c41135_48426638	3B	0	3B, 3D	204.26, 13.29	3B	177.4	3B	8.1	92936
Xbarc75	-	-	-	-	-	-	3B	8.1	92936
wsnp_Ex_c30368_39293103	3B	0	3B	35.9	3B	177.4	3B	8.1	92936
wsnp_Ex_c1375_2633027	3B	1.4	1B	102.4	-	-	1D	8.6	37555
wsnp_Ex_rep_c67107_65584404	3B	1.4	3B	47.3	3B	173	1D	8.6	37555
wsnp_Ex_rep_c66331_64502363	3B	1.4	3D	11.7	3B	173	UA	8	134957
wsnp_Ex_c12875_20407926	3B	1.4	3A	47.9	3A,3B	-	3A	9.3	38898
Xbarc133	-	-	-	-	-	-	3B	11.4	117960
wsnp_Ku_c12698_20441325	3B	1.4	3B	43.5	3B	11.6	UA	NA	204247
wsnp_Ex_rep_c66331_64503065	3B	1.4	3B	11.5	3B	173.3	UA	8	134957
wsnp_Ex_c3005_5548573	3B	2.3	3B	56.4	3B	19.2	3B	24.6	20580
Xgwm493	-	-	-	-	-	-	3B	18.7	126088
wsnp_BE497169B_Ta_2_1	3B	3.2	3A,3B	156.40, 54.4	3B	7.1	3B	21.5	98002
Xcfa2226	-	-	-	-	-	-	3B	21.5	98002
Xbrac102	-	-	-	-	-	-	3B	21.5	98002
wsnp_BE497169B_Ta_2_2	3B	3.2	3B	121.3	3B	7.1	3B	21.5	98002
wsnp_Ex_c25636_34897348	3B	3.2	-	-	2A	24.8	2A	91.8	20969
wsnp_Ku_c3817_7009093	3B	34.6	-	-	3A,3B	0.9	3A	10	28042
wsnp_Ex_c44375_50444756	3B	46.3	-	-	3A,3B	-	3A	9.2	99374
wsnp_Ra_c5532_9788185	3B	46.4	3D	11.8	3A,3B	-	3A	9.2	99374

Markers with "wsnp" prefixes are markers from the 9K SNP mapped on the Drysdale x Waagan DH population by Shirdelmoghanloo, Taylor, et al. (2016) and markers beginning with "X" were mapped to heat related QTL in other studies. Purple highlighted cells represent the scaffolds used as reference for identification of remaining scaffolds. "-" means no information was available and "UA" refers to scaffolds not allocated to a chromosome. Some markers were identified on chromosomes other than 3B but those genetic locations were not used. cM positions of IWGSC scaffolds were mostly defined using PopSeq data. Conditional formatting was used on the cM locations to help visualize the order of the markers on 3B.

Table 4.2 IWGSC reference genome scaffolds reported around the 3B locus position and their genetic locations.

Scaffold No.	cM	Scaffold No.	cM	Scaffold No.	cM	Scaffold No.	cM	Scaffold No.	cM
120622	0.7	55789-1	22.8	64394	36.2	1676	42.6	30202	46.6
65694	2.5	58481	22.7	54738	36.7	124144	43.8	6897	47.0
2536	1.0	20580	24.6	58580	37.7	9470	41.9	82468-1	46.8
16578	8.5	134957	8.0	153200	37.9	118191	42.7	28382	46.7
76726	7.5	204247	NA	145471	38.4	682	43.1	73826	46.7
92936	8.1	87992	20.0	50269	40.6	30207-1	43.0	21513	46.7
133866	9.1	98002	21.5	1649	40.4	140960	43.1	33973	46.6
27539	7.3	126152	22.8	45978	39.9	90058	43.6	70209	46.7
117960	11.4	55789-1	22.8	88454	40.5	16628-2	43.0	95739	46.7
15984	13.2	58481	22.7	51241	41.3	114067	44.1	127963	46.7
67011	18.0	20580	24.6	38168	41.1	61887	44.0	140033	46.7
6769	18.9	127841	30.7	127231	42.2	2599	44.2	19863	46.6
126088	18.7	39148	32.0	14004	42.2	48217	44.3	61520	46.7
87992	20.0	51931	36.4	20	43.9	48216	45.2	82468-1	46.8
98002	21.5	44202	33.1	11305	42.4	25340	45.6	103980	74.1
126152	22.8	30362	33.0	80536	42.9	72581	46.5	139052	121.0

Table 4.3 KASP marker assays tested. Markers with a 35k Axiom SNP ID had been reported to be polymorphic between Drysdale and Waagan in the database of 35K Axiom array SNP scores for wheat varieties. The last column indicates whether the developed KASP assay detected clear polymorphism between Drysdale and Waagan.

Marker name	Sequence	IWGSC reference genome sequence		35K Axiom SNP ID	Drysdale/Waagan Polymorphic?
		Scaffold	Position (cM)		
KASParmar2	GAATCATTGAAAGAGGACATTACCCAGAC CATTTTACTAAACGTACAGCAGGTTC[C/T] GCACAAGTTCATCTACTAGATTACTACTAA AGTTACATGAATCTA	scaffold2536	1.0	AX-94396258	Yes
KASParmar8	AAGTGTGGAACGCACCTCTTATTCGGCCT GTAATCCACCCTCATTTTCGGCCTTC[G/T] AAACGGGCCCTGTTTTAGAGTTGAGCAGA GCACCAAGCTGCCCTGTTTGTCGGCC	scaffold16578	8.5	AX-94433176	Yes
KASParmar10	TGACACCACAGCGCTCCGTCATCTGGCGA TCCATGTCGGTCAGGGGGGACTTCTT[C/G]TTGATCGCCTTGAACGCGTCCTCACACAT ATCACCAGCGTCCTTCGCGAGCAAGA	scaffold133866	9.1	AX-94744906	Yes
KASParmar11	TAAACCTGGGCCTTTTGAAGGGGTAAATG ACTCCGCGTTGGGGAAATTAGCTGTA[C/G]TTCCGCTCCCAGGAGTACCGTTTCCTACA GATGAACAAACGCTAATCACAACACC	scaffold117960	11.4	AX-94705969	Yes
KASParmar12	CATTCTGGTACGGTTCGTACCCTACGGCCT TAGCCTTTTCCAGCAAATTCTCCAA[C/T]A ATAGATACAACTCTGTAGCTTGTGGGTGA GTCATGTCATCAGCTCTGAAGAAAT	scaffold117960	11.4	AX-94388729	Yes
KASParmar13	TCAGTATAGTATAAGTTTCAGGACTAGTTA GGGAAGGGAAGAAGGCCTGAGCACT[A/ G]CCGGGGTGACAAAAGGAGACGAAACC CACCCCCATTTTGCAGCGAAGAGTTTGGT	scaffold15984	13.2	AX-94691217	Yes
KASParmar15	TTGAACGTACTCCTACCTTTTTAACTACTAG TGTTTCAAGCGTGCGATGCGACCA[A/G]G AAGAAGAAGACCCGTACTAGTTGACGAT GTGAACTGCCAAACACACTCAGACAT	scaffold6769	18.9	AX-94809409	Yes
KASParmar16	ACAAGCCAGCGGCCAGCCGCACCACCATC AAGGGAGCGCACCTCGGCGGGGTCCA[C/ T]GGGATCTCCTTCGCCGACAACGACACCC TGGTGACCGCCGGCGAGGACGCATGCA	scaffold98002	21.5	AX-95000416	Yes
KASParmar17	TCGAAACGATTTGATCCAAGTTGTCAAATA CGGAGAAGCAAGGATAGAGTAGGTG[G/ T]CCCATTTCATCACTGTCACGCTACTACG ACATCTCTTTTCTTGTCCGAAGGCAGG	scaffold126152	22.8	AX-94430973	Yes
KASParmar21	ATATGGTGACTTCTTAGTCAGCTTTGACTT AACATACTTGGATATGGCCTCTTGGAGG[C/T]ATTCAACCGTGTATGTTTTTCTATGTT TTTGTCTGCCTTAGGGCTTCAGACT	scaffold153200	37.9	_	Yes

Table 4.3 continued

Marker name	Sequence	IWGSC reference genome sequence		35K Axiom SNP ID	Drysdale/Waagan Polymorphic?
		Scaffold	Position (cM)		
KASParmar4	TTGGAGGAGCACAAGGATAAGATTCCTGAGATCAC[G/T]GCGGCCATCTTGTCAGCAGGCACTGATTTAATGGA	scaffold118191	42.7	AX-95169625	Yes?
KASParmar4_1	TGCAGAAGAGCAGACACATCAAGAAGGCTTGCTCATGTATTTGGAGGAGCACAAGGATAAGATTCCTGAGATCAC[T/G]GCGGCCATCTTGTCAGCAGGCACTGATTTAATGGAGTCGCCGAAGAAGGATGAAGACGGCAGCAGAAGCAGCACC	scaffold118191	42.7	AX-95169625	Monomorphic
KASParmar3	CGTAGCGACCCAAACCGAGCTCCTCACGGGGAGCAGAGTTACGAGACTAGAGATGTTCC[T/G]AATAGGCAGCTAGTGTGCAAAGCTGGACGAATTGGTTATTGGCCCGTCGAGCATC	scaffold88454	40.5	_	Monomorphic?
KASParmar3_1	ATGTTGACCACTTTGGGCCAGCAACGGAGGAAGAGATGGAGGCTGACCTGATGAGGGTAGATGCCATGGAAGATCAAGAAGTCACCTCTCGCCTTCGAGC[C/T]GGGTTTACGATGGGGGAACTATAGAGCTCAGCTATTCCAAATTCGGTTATCCCTTCCAATGTTAGATTTCTTTCCTATGAAAATATGAGGAGGAGTGTCT	scaffold88454	40.5	_	Monomorphic
KASParmar1	GAGGCTTGATATCGAAATGGACAATACGAGTATTACATCTATG[T/G]TGTAAGTACTCAAGGCCACGAGCAATCCCG	scaffold30362	33.0	_	Monomorphic
KASParmar7	TCACATCATCTTGCACCAACTCTTCCGACGAATCCGCAACTAACTCAGCTTCCTC[A/G]GGCACCTCCACATTTATGACCTGAACTTTGAACTCGGGAATCCGGGACTTGAAAA	scaffold120622	0.7	AX-95218138	Monomorphic
KASParmar9	GGTACATGGCTCCAGAGTATGCATCCAGTGGCAAGCTGACTGAGAAATCTGATGT[C/G]TTCTCTTTTGGCGTTGTCCTCTTAGAGCTCATTACTGGTAGAAAGCCTGTCGATG	scaffold76726	7.5	AX-95081630	Monomorphic
KASParmar14	CGAGTGCAACATGTGGAAAGAGATTGGCATCCATTGGGTTGGACAACACCGAAGT[G/T]AACCGCCAGGCTTACAGGCAGCTGTTGCTGACCACTGCTGGTCTTGGTGAATATA	scaffold67011	18.0	AX-95072897	Monomorphic
KASParmar19	ATCAAGATAAGAGAAGAGCTTTGACGAATTATCATTGTACGTAACATTATAGTTATG[A/G]TAGTTGAAATGATGGAATGTGTAGCACTAACCTGTTTTCTTGTCGTGTAGTATA	scaffold20580	24.6	_	Monomorphic

Table 4.3 continued..

Marker name	Sequence	IWGSC reference genome sequence		35K Axiom SNP ID	Drysdale/Waagan Polymorphic?
		Scaffold	Position (cM)		
KASParmar20	CACCATTGACCTCGACATCCTCGACTGCCCCGTCTGCTACCTGC[C/T]CCTGCGCCCTCCCATCTTCCAGGTACCCCCTGCACCTGTCTTGAT	scaffold127841	30.7	_	Monomorphic
KASParmar22	TCGCGAAAGTCCTACAGCCACATCCTCCAGTTTGC[C/T]GTGTGCCTCGGTCAGCTCTACGGATGCATCGTCTA	scaffold48217	44.3	AX-94895411	Did not work
KASParmar22_1	ACAAGAAATCCAATGCACAAAAGTTGGATGAAAAG[A/C]AGCTCATAGTAGAACTCCGAAAATTACTTGAGAAT	scaffold48217	44.3	AX-95223462	Did not work
KASParmar5	TCGGGTGATGGTTGGGATAGGGAGAAATCCCTGTCGGCCTGTCCGAGACT[G/A]ACGTGGTGGCGTGTGAGGGTGCCGCCGGGCCTTCCTGAAGGGCG	scaffold20	43.9	_	Did not work
KASParmar5_1	GTGGCGTAGCCGGCCAGCGAAGTAGGGTGCGCCGCCTGATCTGAGCTTGGAATTTCACCTCCTGCTCCGCCACCGCTGTTTTCTCGCCCAAATCCACTGCCTGCC[G/T]CCGTCCGTCTCATTCAGAGCTTGGTCAACACACTGCTGACCTCTTTTTTCCACAAAAATATAACCCTCATAAAAACTGACCCACTAGGTCCTTCCAGCAG	scaffold20	43.9		Did not work
KASParmar6	CAGACCGCGCACCCCCTAGCCCGCCGAATCTGGTTGGGCT[T/C]TCGGTAATGGAGTTCACCGCTGCAGATATCTTCCAACACTCGCCCT	scaffold72581	46.5	_	Did not work
KASParmar6_1	CTCGTGGCGGTGCAGACGCCGTGGAGCCGCCATGG[C/T]GGGGATTTTACGCTGGTCAAGGTGTCCAACATGTC	scaffold72581	46.5	AX-94807379	Yes

Markers without 35k Axiom SNP ID were selected as polymorphic for Drysdale and Chinese Spring.

Table 4.4 Names and sequences of primers.

KASP Primer Name	Sequence	KASP Primer Name	Sequence
KASParmar1A1	AAATGGACAATACGAGTATTACATCTATGT	KASParmar10C	TCATCTGGCGATCCATGTCGGTC
KASParmar1A2	GGACAATACGAGTATTACATCTATGG	KASParmar11A1	GCGTTGGGGAAATTAGCTGTAC
KASParmar1C	GATTGCTCGTGGCCTTGAGTACTTA	KASParmar11A2	GCGTTGGGGAAATTAGCTGTAG
KASParmar2A1	AGTAATCTAGTAGATGAACTTGTGCG	KASParmar11C	GGAAACGGTACTCCTGGG
KASParmar2A2	GTAGTAATCTAGTAGATGAACTTGTGCA	KASParmar12A1	CCTTTTCCAGCAAATTCTCCAAC
KASParmar2C	AGACCATTTTACTAAACGTACAGCAGGTT	KASParmar12A2	CCTTTTCCAGCAAATTCTCCAAT
KASParmar3A1	GAGTTACGAGACTAGAGATGTTCCT	KASParmar12C	AGAGCTGATGACATGACTCACCCA
KASParmar3A2	AGTTACGAGACTAGAGATGTTCCG	KASParmar13A1	AAGGGAAGAAGGCCTGAGCACTA
KASParmar3C	CAGCTTTGCACACTAGCTGCCTATT	KASParmar13A2	AAGGGAAGAAGGCCTGAGCACTG
KASParmar3_1A1	TTCCCCCATCGTAAACCCG	KASParmar13C	AATGGGGGTGGGTTTCGTCTCCTTT
KASParmar3_1A2	TTCCCCCATCGTAAACCCA	KASParmar14A1	CCTGTAAGCCTGGCGGTTC
KASParmar3_1C	GAAGATCAAGAAGTCACCTCTC	KASParmar14A2	CCTGTAAGCCTGGCGGTTA
KASParmar4A1	CAAGGATAAGATTCCTGAGATCACG	KASParmar14C	GGAAAGAGATTGGCATCCATTGGG
KASParmar4A2	ACAAGGATAAGATTCCTGAGATCACT	KASParmar15A1	GCGTGCGATGCGACCAA
KASParmar4C	CATTAAATCAGTGCCTGCTGACAAGAT	KASParmar15A2	GCGTGCGATGCGACCAG
KASParmar4_1A1	ACAAGGATAAGATTCCTGAGATCACT	KASParmar15C	CAGTTCACATCGTCAACTAGTACGG
KASParmar4_1A2	ACAAGGATAAGATTCCTGAGATCACG	KASParmar16A1	ACCTCGGCGGGGTCCAC
KASParmar4_1C	CTGCTGACAAGATGGCCGCC	KASParmar16A2	ACCTCGGCGGGGTCCAT
KASParmar5A1	CCTCACACGCCACCACGTC	KASParmar16C	CGGCGGTCACCAGG
KASParmar5A2	CCCTCACACGCCACCACGTT	KASParmar17A1	CGTGACAGTGATGAAATGGGC
KASParmar5C	GGTGATGGTTGGGATAGGGAGAAAT	KASParmar17A2	CGTGACAGTGATGAAATGGGA

Table 4.4 continued…

KASP Primer Name	Sequence	KASP Primer Name	Sequence
KASParmar5_1A1	CCAAATCCACTGCCTGCCG	KASParmar17C	GTCAAATACGGAGAAGCAAGGAT
KASParmar5_1A2	CCAAATCCACTGCCTGCCT	KASParmar18A1	CGTGACAGTGATGAAATGGGC
KASParmar5_1C	TGGGTCAGTTTTTATGAGGGTTATA	KASParmar18A2	CGTGACAGTGATGAAATGGGA
KASParmar6A1	CAGCGGTGAACTCCATTACCGAA	KASParmar18C	TGTCAAATACGGAGAAGCAAGGAT
KASParmar6A2	AGCGGTGAACTCCATTACCGAG	KASParmar19A1	TGCTACACATTCCATCATTTCAACTAC
KASParmar6C	CCTAGCCCGCCGAATCTGGTT	KASParmar19A2	TGCTACACATTCCATCATTTCAACTAT
KASParmar6_1A1	CGTGGAGCCGCCATGGC	KASParmar19C	GACGAATTATCATTGTACGTAACATT
KASParmar6_1A2	CGTGGAGCCGCCATGGT	KASParmar20A1	ATGGGAGGGCGCAGGG
KASParmar6_1C	GACATGTTGGACACCTTGACC	KASParmar20A2	ATGGGAGGGCGCAGGA
KASParmar7A1	CGCAACTAACTCAGCTTCCTCA	KASParmar20C	GCCGCCGATGTCACCATT
KASParmar7A2	CGCAACTAACTCAGCTTCCTCG	KASParmar21A1	ACATAGAAAAACATACACGGTTGAATG
KASParmar7C	GGATTCCCGAGTTCAAAGTTCAG	KASParmar21A2	ACATAGAAAAACATACACGGTTGAATA
KASParmar8A1	TGTAATCCACCCTCATTTTCGGCCTTCG	KASParmar21C	TAACATACTTGGATATGGCCTCTTG
KASParmar8A2	TGTAATCCACCCTCATTTTCGGCCTTCT	KASParmar22A1	TAGAGCTGACCGAGGCACACG
KASParmar8C	TCTGCTCAACTCTAAAACAGG	KASParmar22A2	TAGAGCTGACCGAGGCACACA
KASParmar9A1	AAGAGGACAACGCCAAAAGAGAAC	KASParmar22C	TACAGCCACATCCTCCAGTTT
KASParmar9A2	AAGAGGACAACGCCAAAAGAGAAG	KASParmar22_1A1	AATGCACAAAAGTTGGATGAAAAGA
KASParmar9C	CATCCAGTGGCAAGC	KASParmar22_1A2	AATGCACAAAAGTTGGATGAAAAGC
KASParmar10A1	GCGTTCAAGGCGATCAAC	KASParmar22_1C	ATTCTCAAGTAATTTTCGGAGTTCTACT
KASParmar10A2	GCGTTCAAGGCGATCAAG		

5’ complementary sequence to fluor-labelled oligos: FAM: GAAGGTGACCAAGTTCATGCT, HEX: GAAGGTCGGAGTCAACGGATT were added to each A1 and A2 primers respectively and are not shown here.

New KASP assays were scored on the 144 Drysdale x Waagan DH lines (Shirdelmoghanloo, Taylor, et al. 2016) originally used to map the *QHSGW.AWW-3B* heat tolerance QTL. Markers were placed in order by graphical genotyping, in such a way as to minimize the requirement for double recombination events (Fig 3.1). One sub population of DH lines (Derived from F_1 individual R) was found to behave differently to the other DH lines with respect to segregation of some of the markers; a Drysdale or Waagan plant that contained an atypical haplotype in this region was probably used for crossing to make this F_1 plant. For this reason, DH lines from this sub-population were not used in construction of the revised map. After obtaining the optimal order for the new and old markers, new centimorgan (cM) distances were calculated on the basis of numbers of recombination events between markers and the Kosambi mapping function (Kosambi 1943) was used to convert recombination frequencies to cM distances. Two new markers were positioned four recombinants (3.1 cM) distal of the most distal markers from the previous genetic map (previous 0 cM location). Four new markers were positioned at the previously defined 1.4 cM location. Two new markers were located in the gap between the previous 3.2 and 34.6 cM positions, but close (9.1 and 9.9 cM) to the distal boundary (Fig 3.2). The last three new markers were located below the previous 34 cM position.

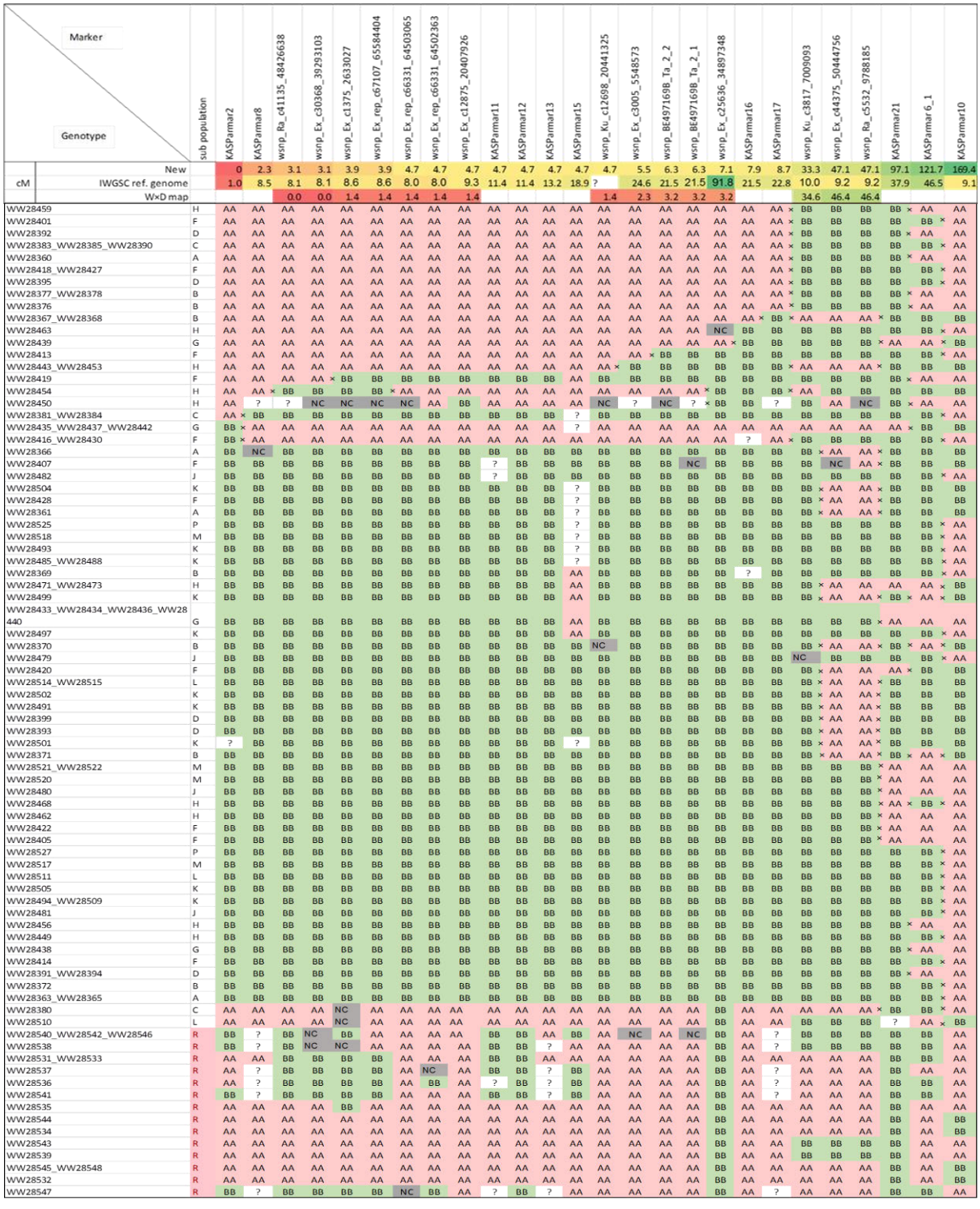
Unfortunately, most of the markers intended to fill the gap between 3.2 to 34.6 cM were either monomorphic or performed inadequately (Table 4.3). The paucity of markers in this region may be due to one or a combination of factors: (a) the presence of the same/similar haplotype for this region in cvs. Drysdale and Waagan, (b) the presence of the same/similar haplotype for this region in cvs. Drysdale and Chinese Spring (since polymorphism between them was a pre-requisite for identifying SNPs for marker generation), (c) sequence duplication, insertion/deletion of chromosomal segment in this interval, or (d) poor coverage of the Drysdale BPA genomic sequence in parts of the region, as revealed by variation in read depth along the chromosome visualized in DAWN.

All the developed KASP markers were scored on 16 of the RIL-NILs for *QHsgw.aww-3B* (that had been selected in Chapter 2) by scoring two or three progeny of each plant originally selected as being homozygous for contrasting alleles of *QHsgw.aww-3B* (Fig. 3.3). Markers were arranged in

the same order as those determined using the DH lines in Fig 3.3 to understand the recombination patterns in the RIL-NILs. The wsnp_Ku_c12698_20441325 marker score for the WW30674_13 plants showed an unexpected Drysdale allele call as in the previous generation (for selection of heterozygous lines, Chapter 2, Fig. 2.3) This line was the only one derived from the F_1 plant 21, therefore one of the parent plants used to produce this F_1 plant may have contained a slightly different haplotype in the region to other parent plants of the same variety. KASParmar2 showed unexpected heterozygous scores in all tested plants from the four families WW30692_12, WW30692_4, WW30709_8, WW30709_9 (Fig. 3.3). The three tested WW30913_9 plants showed three different classes of KASParmar2 calls. A likely explanation seems to be that this marker detected two polymorphic loci, only one of which was in the 3B QTL region. The two single plant selections of Drysdale also differed for their KASParmar21 scores (Fig. 3.3), suggesting that these two selections of Drysdale may contain slightly different haplotypes in the region.

Marker / Genotype		sub population	KASParmar2	KASParmar8	wsnp_Ra_c41135_48426638	wsnp_Ex_c30368_39293103	wsnp_Ex_c1375_2633027	wsnp_Ex_rep_c67107_65584404	wsnp_Ex_rep_c66331_64503065	wsnp_Ex_rep_c66331_64502363	wsnp_Ex_c12875_20407926	KASParmar11	KASParmar12	KASParmar13	KASParmar15	wsnp_Ku_c12698_20441325	wsnp_Ex_c3005_5548573	wsnp_BE497169B_Ta_2_2	wsnp_BE497169B_Ta_2_1	wsnp_Ex_c25636_34897348	KASParmar16	KASParmar17	wsnp_Ku_c3817_7009093	wsnp_Ex_c44375_50444756	wsnp_Ra_c5532_9788185	KASParmar21	KASParmar 6_1	KASParmar10
	New		0	2.3	3.1	3.1	3.9	3.9	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	5.5	6.3	6.3	7.1	7.9	8.7	33.3	47.1	47.1	97.1	121.7	169.4
cM	IWGSC ref. genome		1.0	8.5	8.1	8.1	8.6	8.6	8.0	8.0	9.3	11.4	11.4	13.2	18.9	?	24.6	21.5	21.5	91.8	21.5	22.8	10.0	9.2	9.2	37.9	46.5	9.1
	W×D map				0.0	0.0	1.4	1.4	1.4	1.4	1.4					1.4	2.3	3.2	3.2	3.2			34.6	46.4	46.4			
Drysdale185			AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
Drysdale 186			AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
Waagan192			BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
Waagan194			BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
WW28475		J	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28470_WW28472		H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28469		H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28461_WW28465		H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28460_WW28467		H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28447_WW28458		H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB
WW28446		H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28441		G	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28432		G	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28415_WW28424_WW28429		F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28408		F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28387		C	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28523		M	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28519		M	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28483_WW28484		J	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28452_WW28464		H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28388		C	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB
WW28512		L	AA	AA	AA	AA	AA	AA	AA	AA	AA	?	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB
WW28466		H	?	?	?	AA	AA	AA	AA	AA	AA	?	?	?	?	AA	?	?	AA	AA	?	?	AA	AA	AA	?	AA	?
WW28362		A	?	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28382		C	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	?	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28374		B	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	?	AA	NC	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28403		F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	?	AA	AA	AA	AA ×	BB	BB	BB
WW28498		K	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28496		K	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB ×	AA ×	BB
WW28492		K	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28487_WW28495		K	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB ×	AA ×	BB
WW28474		J	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28451		H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28423		F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28404		F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28400		F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28397		D	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB ×	AA ×	BB
WW28396		D	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28386		C	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28379		B	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28375		B	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB ×	AA ×	BB
WW28359		A	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28529		P	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB ×	AA ×	BB
WW28524		M	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28503_WW28508		K	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28398		D	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB ×	AA ×	BB
WW28389		C	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28373		B	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB ×	AA ×	BB
WW28364		A	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28478		J	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB ×	AA	AA	AA
WW28477		J	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB ×	AA ×	BB ×	AA
WW28476		J	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB ×	AA	AA	AA
WW28455		H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB ×	AA	AA	AA
WW28411		F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB ×	AA ×	BB ×	AA
WW28410		F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB ×	AA ×	BB ×	AA
WW28402		F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB ×	AA	AA	AA
WW28448_WW28457_WW28506		H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB ×	AA	AA	AA
WW28444_WW28445		H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB ×	AA	AA	AA
WW28421		F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB ×	AA	AA	AA
WW28417		F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB ×	AA	AA	AA
WW28412_WW28426		F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB ×	AA	AA	AA
WW28409		F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB ×	AA	AA	AA
WW28486		K	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	?	AA ×	BB	BB	BB	BB	BB ×	AA
WW28526_WW28528		P	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB	BB ×	AA	AA
WW28513		L	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB	BB	BB ×	AA
WW28500_WW28507		K	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB	BB ×	AA	AA
WW28490		K	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB	BB	BB ×	AA
WW28459		H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB	BB ×	AA	AA

Fig. 4.1 continued..



Marker / Genotype		sub population	KASParmar2	KASParmar8	wsnp_Ra_c41135_48426638	wsnp_Ex_c30368_39293103	wsnp_Ex_c1375_2633027	wsnp_Ex_rep_c67107_65584404	wsnp_Ex_rep_c66331_64503065	wsnp_Ex_rep_c66331_64502363	wsnp_Ex_c12875_20407926	KASParmar11	KASParmar12	KASParmar13	KASParmar15	wsnp_Ku_c12698_20441325	wsnp_Ex_c3005_5548573	wsnp_BE497169B_Ta_2_2	wsnp_BE497169B_Ta_2_1	wsnp_Ex_c25636_34897348	KASParmar16	KASParmar17	wsnp_Ku_c3817_7009093	wsnp_Ex_c44375_50444756	wsnp_Ra_c5532_9788185	KASParmar21	KASParmar 6_1	KASParmar10
	New		0	2.3	3.1	3.1	3.9	3.9	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	5.5	6.3	6.3	7.1	7.9	8.7	33.3	47.1	47.1	97.1	121.7	169.4
cM	IWGSC ref. genome		1.0	8.5	8.1	8.1	8.6	8.6	8.0	8.0	9.3	11.4	11.4	13.2	18.9	?	24.6	21.5	21.5	91.8	21.5	22.8	10.0	9.2	9.2	37.9	46.5	9.1
	W×D map				0.0	0.0	1.4	1.4	1.4	1.4	1.4					1.4	2.3	3.2	3.2	3.2			34.6	46.4	46.4			
WW28459		H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB	BB × AA	AA
WW28401		F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB	BB × AA	AA
WW28392		D	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB	BB × AA	AA
WW28383_WW28385_WW28390		C	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB	BB × AA	AA
WW28360		A	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB	BB × AA	AA
WW28418_WW28427		F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB	BB × AA	AA
WW28395		D	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB	BB × AA	AA
WW28377_WW28378		B	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB	BB × AA	AA
WW28376		B	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB	BB × AA	AA
WW28367_WW28368		B	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB × AA	AA	AA ×	BB	BB	BB
WW28463		H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	NC	BB	BB	BB	BB	BB	BB	BB ×	AA
WW28439		G	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB	BB	BB ×	AA	AA ×	BB
WW28413		F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA
WW28443_WW28453		H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB	BB	BB	BB	BB × AA	AA	AA ×	BB	BB	BB	BB
WW28419		F	AA	AA	AA	AA ×	BB	BB	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA
WW28454		H	AA	AA ×	BB	BB	BB	BB ×	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB ×	AA	BB	BB	BB	BB	BB
WW28450		H	AA	?	?	NC	NC	NC	NC	AA	BB	AA	AA	AA	AA	NC	?	NC	? ×	BB	BB	?	BB	AA	NC	BB ×	AA	AA
WW28381_WW28384		C	AA ×	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA
WW28435_WW28437_WW28442		G	BB ×	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	?	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB
WW28416_WW28430		F	BB ×	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	?	AA ×	BB	BB	BB	BB ×	AA	AA
WW28366		A	BB	NC	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA ×	BB	BB	BB	BB
WW28407		F	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	NC	BB	BB	BB	BB	NC	AA ×	BB	BB	BB
WW28482		J	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	
WW28504		K	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA ×	BB	BB	BB
WW28428		F	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	BB ×	AA	AA ×	BB	BB	BB	BB
WW28361		A	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	BB ×	AA	AA ×	BB	BB	BB	BB
WW28525		P	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	
WW28518		M	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	
WW28493		K	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	
WW28485_WW28488		K	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	
WW28369		B	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB ×	AA	
WW28471_WW28473		H	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA	AA	AA ×	BB
WW28499		K	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA ×	BB ×	AA ×	BB
WW28433_WW28434_WW28436_WW28440		G	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA	AA	
WW28497		K	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	
WW28370		B	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	NC	BB	BB	BB	BB	BB	BB	BB ×	AA	AA ×	BB ×	AA ×	BB
WW28479		J	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	NC	BB	BB	BB	BB ×	AA
WW28420		F	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA	AA ×	BB	BB
WW28514_WW28515		L	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA ×	BB	BB	BB
WW28502		K	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA ×	BB	BB	BB
WW28491		K	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA ×	BB	BB	BB
WW28399		D	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA ×	BB	BB	BB
WW28393		D	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA ×	BB	BB	BB
WW28501		K	?	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA ×	BB	BB	BB
WW28371		B	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA ×	BB ×	AA ×	BB
WW28521_WW28522		M	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA	AA	
WW28520		M	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA	AA	
WW28480		J	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA	AA	
WW28468		H	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA ×	BB ×	AA	
WW28462		H	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA	AA	
WW28422		F	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA	AA	
WW28405		F	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA	AA	
WW28527		P	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	
WW28517		M	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	
WW28511		L	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	
WW28505		K	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	
WW28494_WW28509		K	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	
WW28481		J	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	
WW28456		H	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	
WW28449		H	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	
WW28438		G	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	
WW28414		F	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	
WW28391_WW28394		D	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA	
WW28372		B	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	
WW28363_WW28365		A	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	
WW28380		C	AA	AA	AA	AA	NC	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA ×	BB	BB ×	AA
WW28510		L	AA	AA	AA	AA	NC	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	BB	BB	BB	?	AA ×	BB
WW28540_WW28542_WW28546		R	BB	?	BB	NC	BB	AA	AA	AA	AA	BB	BB	AA	BB	AA	NC	AA	NC	BB	AA	?	BB	BB	BB	BB	BB	AA
WW28538		R	BB	?	BB	NC	NC	AA	AA	AA	AA	BB	BB	?	AA	AA	AA	AA	AA	BB	AA	?	BB	BB	BB	BB	BB	AA
WW28531_WW28533		R	AA	AA	BB	BB	BB	BB	AA	AA	AA	BB	BB	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	AA
WW28537		R	AA	?	BB	BB	BB	BB	AA	NC	AA	BB	BB	?	BB	AA	AA	AA	AA	BB	AA	?	AA	AA	AA	BB	AA	AA
WW28536		R	AA	?	BB	BB	BB	BB	AA	BB	AA	?	BB	?	BB	AA	AA	AA	AA	BB	AA	?	AA	AA	AA	BB	BB	AA
WW28541		R	BB	?	BB	BB	BB	AA	AA	AA	AA	BB	BB	?	BB	AA	AA	AA	AA	BB	AA	?	AA	AA	AA	BB	BB	AA
WW28535		R	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	BB	AA	AA
WW28544		R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	BB	AA	BB
WW28534		R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	BB	AA	BB
WW28543		R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	BB	BB	BB	BB	AA	AA
WW28539		R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	BB	BB	BB	BB	AA	AA
WW28545_WW28548		R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	BB	AA	BB
WW28532		R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	BB	AA	BB
WW28547		R	BB	?	BB	BB	BB	BB	NC	BB	AA	?	BB	?	AA	AA	AA	AA	AA	BB	AA	?	AA	AA	AA	BB	BB	AA

Fig. 4.1 Scores for 9k SNP markers and new KASP markers on the Drysdale x Waagan DH lines. Cell color designates Drysdale (pink), Waagan (green) alleles, bad call (black), '?' for unsure, and 'X'es indicate recombination points. Singleton marker scores for markers otherwise closely linked to surrounding markers were suspected of being errors and were not counted in making the genetic map.

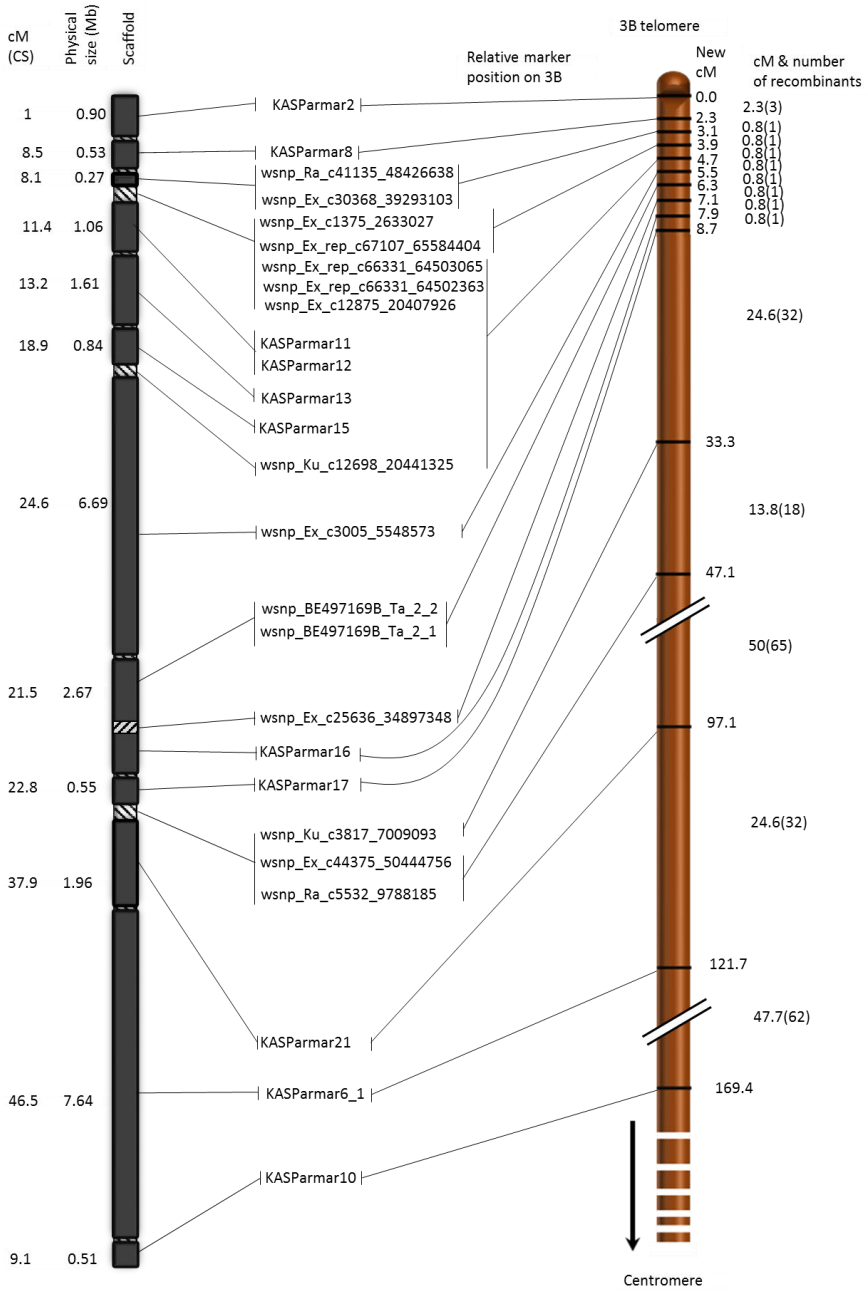


Fig. 4.2 Genetic and physical map of the 3B QTL region. Relative size and positions of the IWGSC whole genome assembly scaffolds presented by black boxes with possible gaps (diagonal stripes). Relative marker position in D x W DH lines and 9K SNP array also incorporated with number of recombination (in brackets).

Marker / Genotype		F1 parent used	KASParmar2	KASParmar8	wsnp_Ra_c41135_48426638	wsnp_Ex_rep_c66331_64503065	KASParmar11	KASParmar12	KASParmar13	KASParmar15	wsnp_Ku_c12698_20441325	wsnp_Ex_c3005_5548573	wsnp_BE497169B_Ta_2_2	KASParmar16	KASParmar17	KASParmar21	KASParmar6_1	KASParmar10
cM	New		0	2.3	3.1	4.7	4.7	4.7	4.7	4.7	4.7	5.5	6.3	7.9	8.7	97.1	121.7	169.4
	IWGSC ref. genome		1.0	8.5	8.1	8.0	11.4	11.4	13.2	18.9	?	24.6	21.5	21.5	22.8	37.9	46.5	9.1
	W×D map				0.0	1.4					1.4	2.3	3.2					
Drysdale 185			C:C	T:T	A:A	C:C	C:C	T:T	G:G	G:G	C:C	T:T	C:C	C:C	T:T	C:C	T:T	C:C
Drysdale 185			C:C	T:T	A:A	C:C	C:C	T:T	G:G	G:G	C:C	T:T	C:C	C:C	T:T	C:C	T:T	C:C
Drysdale 186			C:C	T:T	A:A	C:C	C:C	T:T	G:G	G:G	C:C	T:T	C:C	C:C	T:T	T:T	T:T	C:C
Drysdale 186			C:C	T:T	A:A	C:C	C:C	T:T	G:G	G:G	C:C	T:T	C:C	C:C	T:T	T:T	T:T	C:C
Waagan 192			T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	A:A	T:T	G:G	C:C	C:C	G:G
Waagan 192			T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	A:A	T:T	G:G	C:C	C:C	G:G
Waagan 194			T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	A:A	T:T	G:G	C:C	C:C	G:G
Waagan 194			T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	A:A	T:T	G:G	C:C	C:C	G:G
WW30674_13_1		21	T:T	G:G	G:G	C:C	C:C	T:T	G:G	G:G	T:T*	T:T	C:C	C:C	T:T	T:T	C:C	C:C
WW30674_13_2		21	T:T	G:G	G:G	C:C	C:C	T:T	G:G	G:G	T:T*	T:T	C:C	C:C	T:T	T:T	C:C	C:C
WW30674_13_3		21	T:T	G:G	G:G	C:C	C:C	T:T	G:G	G:G	T:T*	T:T	C:C	C:C	T:T	T:T	C:C	C:C
WW30674_1_1		21	T:T	G:G	G:G	C:C	C:C	T:T	G:G	G:G	T:T*	T:T	C:C	C:C	T:T	T:T	C:C	C:C
WW30674_1_2		21	T:T	G:G	G:G	C:C	C:C	T:T	G:G	G:G	T:T*	T:T	C:C	C:C	T:T	T:T	C:C	C:C
WW30674_1_3		21	T:T	G:G	G:G	C:C	C:C	T:T	G:G	G:G	T:T*	T:T	C:C	C:C	T:T	T:T	C:C	C:C
WW30674_3_1		21	T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	C:C	C:C	T:T	T:T	C:C	C:C
WW30674_3_2		21	T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	C:C	C:C	T:T	T:T	C:C	C:C
WW30674_3_3		21	T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	C:C	C:C	T:T	T:T	C:C	C:C
WW30674_4_1		21	T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	C:C	C:C	T:T	T:T	C:C	C:C
WW30674_4_2		21	T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	C:C	C:C	T:T	T:T	C:C	C:C
WW30674_4_3		21	T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	C:C	C:C	T:T	T:T	C:C	C:C
WW30692_12_1		22	T:C*	T:T	A:A	C:C	C:C	T:T	G:G	G:G	C:C	T:T	C:C	C:C	T:T	T:T	T:T	G:G
WW30692_12_2		22	T:C*	T:T	A:A	C:C	C:C	T:T	G:G	G:G	C:C	T:T	C:C	?	T:T	T:T	T:T	G:G
WW30692_12_3		22	T:C*	T:T	A:A	C:C	C:C	T:T	G:G	G:G	C:C	T:T	C:C	C:C	T:T	T:T	T:T	G:G
WW30692_17_1		22	T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	A:A	T:T	G:G	T:T	T:T	G:G
WW30692_17_2		22	T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	A:A	T:T	G:G	T:T	T:T	G:G
WW30692_17_3		22	T:T	G:G	G:G	T:T	G:G	C:C	A:A	G:G*	T:T	C:C	A:A	T:T	G:G	T:T	T:T	G:G
WW30692_3_1		22	T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	A:A	T:T	G:G	T:T	T:T	G:G
WW30692_3_2		22	T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	A:A	T:T	G:G	T:T	T:T	G:G
WW30692_3_3		22	T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	A:A	T:T	G:G	T:T	T:T	G:G
WW30692_4_1		22	T:C*	T:T	A:A	C:C	C:C	T:T	G:G	G:G	C:C	T:T	C:C	C:C	T:T	T:T	T:T	G:G
WW30692_4_2		22	T:C*	T:T	A:A	C:C	C:C	T:T	G:G	G:G	C:C	T:T	C:C	C:C	T:T	T:T	T:T	G:G
WW30692_4_3		22	T:C*	T:T	A:A	C:C	C:C	T:T	G:G	G:G	C:C	T:T	C:C	C:C	T:T	T:T	T:T	G:G
WW30709_17_1		22	T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	A:A	T:T	G:G	C:C	T:T	G:G
WW30709_17_2		22	T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	A:A	T:T	G:G	C:C	T:T	G:G
WW30709_17_3		22	T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	A:A	T:T	G:G	C:C	T:T	G:G
WW30709_5_1		22	T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	A:A	T:T	G:G	C:C	T:T	G:G
WW30709_5_2		22	T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	A:A	T:T	G:G	C:C	T:T	G:G
WW30709_5_3		22	T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	A:A	T:T	G:G	C:C	T:T	G:G
WW30709_8_1		22	T:C*	T:T	A:A	C:C	C:C	T:T	G:G	G:G	C:C	T:T	C:C	C:C	T:T	C:C	T:T	C:C
WW30709_8_2		22	T:C*	T:T	A:A	C:C	C:C	T:T	G:G	G:G	C:C	T:T	C:C	C:C	T:T	C:C	T:T	C:C
WW30709_8_3		22	T:C*	T:T	A:A	C:C	C:C	C:C*	G:G	G:G	C:C	T:T	C:C	C:C	T:T	C:C	T:T	C:C
WW30709_9_1		22	T:C*	T:T	A:A	C:C	C:C	T:T	G:G	G:G	C:C	T:T	C:C	C:C	T:T	C:C	T:T	G:G
WW30709_9_2		22	T:C*	T:T	A:A	C:C	C:C	T:T	G:G	G:G	C:C	T:T	C:C	C:C	T:T	C:C	T:T	G:G
WW30709_9_3		22	T:C*	T:T	A:A	C:C	C:C	T:T	G:G	G:G	C:C	T:T	C:C	C:C	T:T	C:C	T:T	G:G
WW30913_10_1		25	C:C	T:T	A:A	C:C	C:C	T:T	G:G	G:G	C:C	T:T	C:C	C:C	T:T	C:C	T:T	C:C
WW30913_10_2		25	C:C	T:T	A:A	C:C	C:C	T:T	G:G	G:G	C:C	T:T	C:C	C:C	T:T	C:C	T:T	C:C
WW30913_10_3		25	C:C	T:T	A:A	C:C	C:C	T:T	G:G	G:G	C:C	T:T	C:C	C:C	T:T	C:C	T:T	G:G
WW30913_12_1		25	C:C	T:T	A:A	C:C	C:C	T:T	G:G	G:G	C:C	T:T	C:C	C:C	T:T	C:C	T:T	G:G
WW30913_12_2		25	C:C	T:T	A:A	C:C	C:C	T:T	G:G	G:G	C:C	T:T	C:C	C:C	T:T	C:C	T:T	G:G
WW30913_16_1		25	T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	A:A	T:T	G:G	C:C	T:T	C:C
WW30913_16_2		25	T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	A:A	T:T	G:G	C:C	T:T	C:C
WW30913_9_1		25	C:C*	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	A:A	T:T	G:G	C:C	T:T	G:G
WW30913_9_2		25	T:C*	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	A:A	T:T	G:G	C:C	T:T	C:C
WW30913_9_3		25	T:T	G:G	G:G	T:T	G:G	C:C	A:A	A:A	T:T	C:C	A:A	T:T	G:G	C:C	T:T	C:C

Fig. 4.3 Scores of markers on RIL-NILs. Pink, green and yellow cells represent Drysdale, Waagan and heterozygous marker calls, respectively. '?' means unsure and '*' unexpected calls. Marker order was followed as observed in DH lines (Fig. 4.1).

4.4 Conclusions

- Two new markers were identified distal of the distal-most markers from the previous map, four new markers were mapped 1.5 cM proximal of the previous most distal marker, and two were generated in the upper part of the previous 31.4 cM gap between positions 3.2 and 34.6 cM.
- Markers were designed for the 31.4 cM gap in the previous map but these were mostly found to be monomorphic or did not perform well. This may indicate that this region had the same/similar haplotypes in Drysdale and Waagan, sequence duplications in Drysdale and Waagan, or errors in the IWGS genomic sequence assembly.
- Sequencing of cv Waagan and alignment of its sequence to the Drysdale sequence could help to identify more SNP markers in the target region, including the large gap.
- KASP markers are high throughput and cheap to run, and hence are good for further genetic studies of this locus (Chapter 5). From IWGS genomic sequence assembly it is now possible to predict distance between the markers but at the time of this study the wheat genome was assembled in scaffolds.

Chapter 5: Genes potentially associated with the chromosome 3B grain filling heat tolerance locus

5.1 Introduction

Wheat chromosome 3B contains 5326 genes with a density of 145 genes kb^{-1} (Choulet, Alberti, Theil, Glover, Barbe, Daron, Pingault, Sourdille, Couloux, Paux, et al. 2014) including a number of disease resistance genes e.g. *Sr2,* located on the short arm of this (Hare & McIntosh 1979). Stem rust (or black rust) of wheat is one of the devastating fungal diseases and is caused by the obligate parasite *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn. A total of 58 stem rust resistance genes were listed by McIntosh R. A. (2013), of which five confer adult plant resistance (APR), which is typically non-race-specific (broad-spectrum). *Sr2* is one of the APR genes and is located on the short arm of chromosome 3B (Hare & McIntosh 1979). This gene was transferred from tetraploid emmer wheat *Triticum turgidum* L. ssp. *dicoccum* Schrank ex Schübler (accession 'Yaroslav Emmer') into hexaploid wheat by crossing with the stem rust susceptible hexaploid wheat cv. Marquis (McFadden 1930). Since then it has been widely deployed. It is present in many currently grown cultivars and is *considered* as one of the most important disease resistance genes for wheat breeding (McIntosh, Wellings & Park 1995).

Sr2 stem rust resistance is non-hypersensitive and is only detectable in adult wheat plants (Roelfs 1988). Recessive inheritance (McIntosh, Wellings & Park 1995) and the partial nature of the resistance conferred by *Sr2* also hamper its selection. Its resistance effect can also be masked by the presence of other resistance genes, environmental factors or genetic background effects. Pleiotropic effects of the *Sr2* resistance locus, namely pseudo-black chaff (PBC) (Hare & McIntosh 1979; Kota et al. 2006) and enhancement of high-temperature-induced seedling chlorosis (at >25 ^{o}C; HTISC) (Brown 1997) have been used to assist selection of *Sr2* in breeding programs. Adult plant chlorosis associated with PBC has also been reported by (Sheen, Ebeltoft & Smith 1968). PBC is characterized by a dark pigmentation on the stem internodes and glumes. Expression of PBC is partially dominant and influenced by genotype and environment (Bhowal & Narkhede 1981) which hinders its selection. HTISC is recessively expressed (Brown 1997) which also makes it non-ideal for selection**.** It has been proposed that *Sr2* might have a yield

penalty in the absence of rust, due to the PBC phenotype (Hare & McIntosh 1979; Kota et al. 2006; Sheen, Ebeltoft & Smith 1968). Kota et al. (2006) failed to separate *Sr2* and PBC through recombination in a fine mapping study using 1340 F_2 plants. PBC and HTISC could therefore be pleiotropic effects of the *Sr2* resistance gene, or due to separate but closely linked genes.

Sr2 also shows tight linkage with rust resistance genes *Lr27* (Singh, R & McIntosh 1984) with the resistance alleles being inherited together in coupling phase (Singh, RP, Huerta-Espino & William 2005). *Lr27* is a major race specific leaf (brown) rust (caused by *Puccinia triticina*) seedling resistance gene (Nelson et al. 1997). Expression of *Lr27* resitance requires the presence of a complementary gene *Lr31* on chromosome 4BL (Singh, R & McIntosh 1984). In one study, (Singh, D, Park & McIntosh 1999) the *Sr2* and *Lr27* genes co-segregated in a high resolution family derived from over 3000 gametes from the cross between cv. CS and a CS derivative containing a 3B chromosome substitution from cv. Hope. *Lr27* resistance can therefore be used as a surrogate for following the inheritance of *Sr2*. *Sr2* and *Lr27* may therefore represent the same resistance gene effective against multiple rust types, or separate but closely linked resistance genes.

Sr2 was positioned between markers *CA640157* and *gwm533* using a cv. Chinese Spring (CS) x CS (Hope3B) mapping population by Kota et al. (2006) and localized to the wheat cv. CS genomic sequence contig 11 (1.2 Mb; 3B specific BAC library, contig ctg0011b, Genbank accession FN645450) by Choulet, F. et al. (2010). The SSR marker *gwm533* (Röder et al. 1998) is tightly linked to *Sr2* and through fine mapping *Sr2* was narrowed down further to a 0.07 cM interval (approximately 570 kb) (Mago, R., Tabe, et al. 2011) using the same mapping cross. This research group also developed the cleaved amplified polymorphic sequence (CAPS) marker *csSr2* by exploriing 3B specific sequence variation in cv. CS and 'Hope'. It produces one of three DNA banding patterns upon digestion of the PCR product with *Bsp*HI: null (no amplification), three fragments of 172, 112 and 53 bp (CS (Hope 3B) type) and two fragments of 225 and 112 bp (Marquis type; due to a A→G SNP that abolishes a *Bsp*HI restriction site). The CS (Hope 3B) marker allele is associated with the presence of *Sr2* resistance, whereas the null and Marquis marker alleles are associated with its absence. In a set of 205 genotypes, the null and Marquis *csSr2*

alleles predicted absence of *Sr2* with 100% and 95% accuracy, respectively, whereas the CS (Hope 3B) predicted its presence with 95% accuracy (Mago, R. et al. 2011).

At the grain filling heat tolerance locus *QHsgw.aww-3B* identified on chromosome 3B in the Drysdale x Waagan mapping population (Shirdelmoghanloo, Taylor, et al. 2016), grain weight stability was positively associated with stay-green. *QHsgw.aww-3B* accounted for ~13-40% variance in the heat susceptibility index (HSI) of traits relating to chlorophyll content after heat treatment, senescence rate and duration of flag leaf senescence after anthesis. The stay green and grain filling tolerance phenotypes were proposed to be encoded by the same gene, in line with a model involving common control of heat triggered senescence in both the leaves and grains (Shirdelmoghanloo, Cozzolino, et al. 2016).

In BLAST searches of the wheat cv. CS genomic sequence, markers mapped to *QHsgw.aww-3B* were found to be located in the same region of 3BS as those mapped to *Sr2*, indicating that *QHsgw.aww-3B*and *Sr2* were in a similar location (N. Collins, unpublished data). *Sr2* has been documented to be present in cv. Drysdale but not cv. Waagan (www.wheatpedigree.net). The Drysdale x Waagan DH mapping population has been subjected to several trials in NSW which were affected by rust, where rust infection levels and PBC was scored. QTLs were detected in the *QHsgw.aww-3B* region, with the Drysdale QTL alleles conferring PBC and rust resistance (Livinus Emebiri and Hamid Shirdelmoghanloo, unpublished data). The HTISC and flag leaf chlorophyll effects of *QHsgw.aww-3B* conceivably represent the same biological phenomenon. These factors suggest that the same gene controls the effects at *QHsgw.aww-3B* and *Sr2*, with the allele for heat intolerance (both grain weight and chlorophyll instability) being one and the same as the *Sr2* rust resistance gene.

In the current chapter, closer examination of the aforementioned data from the Drysdale x Waagan DH mapping population, and scoring for the *Sr2* diagnostic marker, are used to further test the hypothesis that *QHsgw.aww-3B* is *Sr2*. Furthermore, grain filling and chlorophyll heat tolerance data from five previous screens of wheat genotypes, performed in the group of Nick Collins (Shirdelmoghanloo, Lohraseb, et al. 2016); and unpublished data), and scoring of the *CsSr2*

diagnostic marker in these lines, are used to test the idea that *Sr2* controls variation for these heat tolerance traits in wheat germplasm more broadly.

Alternatively, the grain filling heat tolerance QTL on 3BS might have as its basis a gene involved in nitrogen use. Leaf senescence is a natural phenomenon which could either be triggered by internal hormonal signals or external stresses e.g. disease or nitrogen deficiency. Adequate nitrogen supply positively influences leaf growth and delays senescence (Diaz et al. 2005; Vos, J & Van der Putten 1998) but deficiency accelerates senescence (Schulte auf'm Erley et al. 2007). In barley, senescence was slowed or even reversed by supplying additional nitrogen at the onset of senescence (Schildhauer, Wiedemuth & Humbeck 2008).

Plants take up nitrogen from the soil in the form of nitrate (NO_3^-) and ammonium (NH_4^+). Candidate genes for nitrogen use efficiency include genes for nitrate and ammonium transporters. Nitrate transporter gene families include genes for high and low affinity transport, known as *NRT1* and *NRT2* respectively. By comparing publicly available wheat cDNA sequences with characterized *NRT* genes in *Arabidopsis***,** *Brachypodium* and maize, Guo et al. (2014) identified seven *NRT1* isoforms (*NRT1.1- NRT1.5, NRT1.7, NRT1.8*) and five *NRT2* isoforms (*NRT2.1- NRT2.5*) in wheat. Through BLAST searches of these genes in the wheat cv. CS genomic sequence we established that *NRT2.5* (only) (GenBank accession number-GH727959.1) was located on 3BS. In this chapter, genetic mapping is used to test the hypothesis that the *NRT2.5* gene could be responsible for the chlorophyll and grain size heat tolerance effects controlled by *QHsgw.aww-3B* on 3BS.

Higher plants directly take up ammonium using ammonium transporters in the roots, or they generate it from nitrite by reduction of nitrate by nitrate reductase, followed by assimilation as amide group into amino acids through GS/GoGat (Glutamine synthase/glutamine-2-oxoglutarate aminotransferase). In a meta analysis of NUE (nitrogen use efficiency) QTLs on wheat chromosome 3B, *GoGat* was identified as a candidate gene for NUE loci on 3B (Quraishi et al. 2011). This group also found *GoGat* to be conserved in its location across co-linear chromosomal segments of wheat chromosome 3B, rice chromosome 1, sorghum chromosome 3 and maize chromosomes 3 and 8. Two *GoGat* genes (*Fd-GoGAT, NADH-GoGAT*) were identified on rice

chromosome 1, syntenic to wheat chromosome 3B (Kurata et al. 1994). *Fd-GoGAT* is active in photorespiration (Ireland & Lea 1999) and *NADH-GoGAT* is active in non-green leaves and developing grains (Yamaya et al. 1992). Wheat NADH-GoGAT shows 95% similarity with the rice NADH-GoGAT protein and has been asserted to play a major role in NUE in wheat (Salse et al. 2011). This group also patented a technology for improving yield of wheat through over expression of *NADH-GoGAT* in transgenic wheat. In the current chapter, the hypothesis that *GoGat* gene is *QHsgw.aww-3B* is tested by genetic mapping.

5.2 Materials and Methods

5.2.1 Marker design

New markers were designed to determine the positions of the candidate genes using the Drysdale x Waagan DH mapping population, and thereby establish their relationship with *QHsgw.aww-3B*.

An EST for the wheat *NRT2.5* gene sequence was obtained from GenBank (www.ncbi.nlm.nih.gov/nucest/GH727959). It was then used to identify a scaffold in the IWGSC reference genome sequence using the ACPFG (Australian Centre for Plant Functional Genomics) BLAST portal. SNPs were identified in the scaffold using the wheat genomics platform DAWN (Diversity Among Wheat geNome). DAWN (Ute Baumann, unpublished) helps to visualize diversity among wheat genotypes by aligning the IWGSC reference sequence of cv. Chinese Spring to the 10x genomic shotgun sequences of Drysdale and 15 other (mostly Australian) wheat varieties that had been generated in a collaboration with Bioplatforms Australia (BPA) (Edwards et al. 2012). The tool does not include genomic shotgun sequence of Waagan (one of the parental lines). Therefore, SNPs that were within the gene and polymorphic between Drysdale and CS were selected for marker generation. KASP primers were designed based on the SNPs following the procedure described in section 3.2.1. The new KASP markers were then tested on the parental lines to see if they would detect polymorphism between Drysdale and Waagan.

The sequence of the rice *GoGat* gene positioned at the orthologous position in rice chromosome 1, represented by the gene model *LOC_Os01g48960* (rice genome annotation project,

http://rice.plantbiology.msu.edu/cgi-bin/ORF_infopage.cgi?orf=LOC_Os01g48960.1, GenBank sequence accession no. AB001916) was used to identify the IWGSC reference genome sequence scaffold containing the corresponding gene on 3BS. Wheat marker assays were then generated using the same methodologies as described for the *NRT2.5* gene.

Markers used by Mago, R., Tabe, et al. (2011) to fine map *Sr2*, including *CA640157* and *gwm533* (0.4 cM apart) that flank the *Sr2* gene (Kota et al. 2006), and markers *RGA_1*, *DOX_1*, *BEX_2*, *CD882879*, *CA746621*, *RKO_2*, *MSF_2* and *RKO_1* that co-segregated with *Sr2*, were used as the basis for designing KASP markers closely linked to *Sr2*. BLAST searches with the marker sequences identified various scaffolds in the CS sequence assembly. These included 206172, 186348, 271041, 182457, 125609, 56755 and 20622 but these were all un-allocated (UA) to chromosomes. Only the *CA746621* marker detected seqeunces on scaffold 16578 located to 3B but no SNPs between Drysdale and CS were indentified in this scaffold sequence. Hence, IWGSC reference genome scaffold 206172 corresponding to contig 11 sequence was selected for KASP marker design. A SNP marker polymorphic for Drysdale and CS which is also polymorphic for Drysdale and Waagan according to the 35K Axiom array (http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB /axiom_download.php) data was selected. The primers were designed following similar methods as above.

5.2.2 *csSr2* marker scoring

Scoring of the *csSr2* marker on 101 hexaploid wheat varieties (mostly Australian; Table 5.1) and 184 DH lines of the Drysdale x Waagan population was done using previously extracted DNA. The former was done with DNA from the study of Shirdelmoghanloo (2015) and the latter was extracted by Iman Lohraseb. *csSr2* primer sequences and PCR conditions were as described by Mago, R., Tabe, et al. (2011), except OneTaq DNA polymerase and OneTaq standard reaction buffer (5×) (Genesearch Pty Ltd, Arundel, Australia) were used, and PCR reactions were 25 µL volume instead of 20 µL. Reaction mix contained: 2 µL of DNA (350 ng/µL), 5 µL of 5x NEB One Taq standard reaction buffer, 1 µL of each of 2mM dNTPs, 5 µM of each forward and reverse primer, 0.125 µL of One Taq DNA Polymerase and 14.875 µL of MQ water. For restriction digestion with *Bsp*HI, reactions of 20 µL contained 15 µL of PCR mix, 2 µL of 10x CutSmart buffer

(Genesearch Pty Ltd), 2 µL of BSA (10mg/mL, acetylated) and 1 µL of *Bsp*HI (10u/ µL; Genesearch Pty Ltd). Digestion reactions were incubated at 37 ^{o}C for one hour and then subjected to electrophoresis in 2.5% agarose gels.

5.2.3 Field trials used to obtain rust resistance and pseudo black chaff scores

Scores for rust resistance and PBC were obtained from a field trial of the Drysdale x Waagan DH population at Wagga Wagga in 2013 (Table 5.2). Running and scoring of the trial was done by the group of Livinus Emebiri (EH Graham Centre for Agricultural Innovation, NSW-DPI). The trial had a complete randomized design and two replicate plots per line. The rust infection was from unintentional natural inoculation, and based on knowledge of rusts prevalent in the area at this time, it was likely to be stripe rust (Robert Park, personal communication). Rust infection levels was scored subjectively on a 1 to 9 scale, with 9 representing the most severe infection. PBC was scored as presence or absence (1 and 0, respectively) on each plot. QTL mapping was done by Hamid Shirdelmoghanloo using the 9K SNP array marker scores and procedures previously described (Shirdelmoghanloo, Taylor, et al. 2016).

5.2.4 Scoring of *Lr27* and high-temperature-induced seedling chlorosis (HTISC) under controlled conditions

Scoring for *Lr27* resistance and HTISC was done under controlled conditions by Robert Park at the Plant Breeding Institute, University of Sydney, Cobbitty (Table 5.2). For this purpose, 48 Drysdale x Waagan DH lines and single plant selections of the parental cvs. (7 for Drysdale and 8 for Waagan) were used. The 48 Drysdale x Waagan DH lines were selected to represent nearly all of the recombinants in the *QHsgw.aww-3B*/*Sr2* region from the DH population. For *Lr27*, inoculation of seedlings with the leaf rust isolate 122-2,3 [73003] was used, and infection sites scored using the codes of DL and Kolmer (1989). For HTISC, seedlings were grown in a warm growth chamber and HTISC was scored as presence or absence in each line.

5.2.5 Grain size and chlorophyll content data from controlled environment experiments involving 101 hexaploid wheat varieties

The potential relationship between *Sr2* status and grain filling and chlorophyll heat tolerance was tested using phenotypic data generated in five controlled-environment experiments (gf1-gf5) undertaken between 2010 and 2015 in The Plant Accelerator (TPA, The University of Adelaide, Waite Campus, Adelaide) (Table 5.3). A total of 101 hexaploid wheat genotypes were used in these experiments, and these were mostly Australian varieties (Table 5.1). Chlorophyll data was only collected in experiments gf3 and gf5. Experiment gf 5 was performed by me (chapter 7), while the others were performed by Iman Lohraseb and Hamid Shirdelmoghanloo. Plant growth and heat treatments were all as described in paragraph 1.2.2. The grain filling heat tolerance index was calculated using a linear regression of final single-grain weight after heat vs. in control across all genotypes in each experiment, with the tolerance index for each genotype being defined as the deviation of the value observed after heat from the value expected from the value in control based on the regression. Chlorophyll response was defined as the proportion of chlorophyll loss observed over the period of the heat treatment, as determined using SPAD readings taken on the flag leaf just before and just after the heat treatment.

Table 5.1 Hexaploid wheat genotypes used to test the association between *csSr2* marker genotype and heat tolerance for grain filling and chlorophyll retention.

Ajana	Egret*	PI626580-4	Sunvale*	Crusader*	Corack*	Opata85*
Aroona	Excalibur	PI625123-3	Sunvex	Drysdale*	EGA Bonnie Rock*	Reeves*
Avocet S	Fang	Mendos	Synthetic W7985*	EGA Eaglehawk	Emu Rock*	Scepter*
Axe*	Flanker*	Mexico-120	Tasman*	HTWYT012	Hartog*	Siete 7 Cerros
Barham	Frame*	Molineux*	Trident*	Lyallpur-73*	HTWYT007	Sokoll*
Cadoux*	Gascoigne	Najah	Vigour18	Seri M82	HTWYT028	Spitfire*
Catalina	Gladius	RAC875	Waagan*	6HRWSN125	Hydra*	Suntop*
CD87*	H45	RT414	Westonia*	Babax	Kalyansona	Tammarin Rock*
Chara	H46	RT416	WW2449	Calingiri*	Katepwa*	Vorobey
Chuan Mai 18	Halberd*	RT417	Yitpi*	Suzuky_12	Kauz	Wyalkatchem*
Cook	Janz*	RT418	Young*	Suzuky_17	King Rock*	Wyuna
Correll*	Kite	Scout*	Baxter*	Suzuky_19	Kukri*	
EGA 2248	Kord*	Stiletto*	Berkut*	Suzuky_23	Mace*	
EGA Blanco*	Krichauff*	Sunco*	Cobra*	Suzuky_24	Magenta*	
EGA Gregory*	Lincoln*	Sunstar*	Cranbrook*	Suzuky_9	Millewa*	

'*': the only genotypes assessed for chlorophyll heat tolerance

Table 5.2 Details of experiments used for PBC and rust typing.

Condition	Traits	Sowing date	Genotypes used
Field	PBC and rust resistance	11/6/2013	184 D x W DH lines and parents
Growth chambers	*Lr27_Lr31* and HTISC	23/11/2016	48 D x W DH lines, 7 selections of Drysdale and 8 selections of Waagan

Table 5.3 Details of controlled environment experiments used to assess grain filling and chlorophyll heat tolerance

Experiment name	Trait	Sowing date	No. of hexaploid wheat genotypes used	No. of replicates
gf1	Grain filling	19/7/2010	12	6
gf2	Grain filling	15/3/2011	12	10
gf3[a]	Grain filling and chlorophyll retention	23/5/2011	36	9
gf4	Grain filling	20/2/2013	60	8
gf5	Grain filling and chlorophyll retention	28/8/2015	21	4

[a]: (Shirdelmoghanloo 2015)

5.3 Results and discussion

5.3.1 New marker design

One KASP marker for each of the *GoGat, NRT2.5* and *Sr2* genes were initially designed and tested on the Waagan and Drysdale parents (Tables 4.4 and 4.5). All the markers showed no or poor amplification. Another common primer was then designed for each of the *GoGat* and *NRT2.5* markers and used with the previous allele specific primers. These primer sets amplified products but the assays did not reveal polymorphism between the parents.

One KASP assay (*KASParmarSr2*; Tables 5.4 and 5.5) was designed from a sequence located within contig 11, reported to contain the *Sr2* locus, but while this primer set amplified products, the assay did not reveal polymorphism between the parents.

Possible reasons for these failures to generate useful KASP assays include lack of polymorphism between Drysdale and Waagan even though there was a SNP between CS and Drysdale. It is possible that effective KASP markers for these genes may be obtainable if other SNPs are used as the basis for designing further assays. Another explanation could be insertion/deletion

differences such as those reported to exist between some *Sr2* resistant and susceptible varieties (Mago, Rohit et al. 2014). These authors found that the size of the region encompassed by *RKO_1* and *DOX_1* markers was 867 kb in the *Sr2* resistant cv. 'Hope" but 567kb in the susceptible cv. CS. The 300kb difference was at least in part due to extensive sequence polymorphism involving presence/absence of genes from a cluster of 17 genes encoding germin-like proteins. Consistent with this, DAWN also showed that a low number of Drysdale sequence reads mapped to the CS reference sequence in most of the scaffolds from the *Sr2* region (data not shown). The parents Drysdale and Waagan might have a similar high level of sequence divergence which may have prevented binding of some of the primers to the correct target sequence in one or both of these parents.

Table 5.4 KASP marker details[a].

		IWGSC reference genome			35K Axiom	KASP
KASP assay name	Sequence	Chromosome	Scaffold no.	Position (cM)	SNP ID	marker performance
KASParmarGoGat	CGGTGCCATAGGCCACCGAGGTGAACACAACCCACCAGGGTGCGTCTGGGCCCCCAAGCG[C/T]GTCCAGGTGGTTGTGCCCCCCTCGGGGGCACCCCTATGGTACTTCTTTGGCCCATCATGT	3B	7336	52.2	-	No amplification in both varieties
KASParmarGoGat_1	Same as KASParmarGoGat	3B	7336	52.2	-	No W/D polymorphism
KASParmarNRT2.5	GCCCGGTTTTCTTTTAAATTGGAGGCAAATTATCATATACTATCAACCGCCGTCTGCACT[T/G]GGTGGCTCAATGGGCAGGCGCACAAGTCACCGCCAATACAGTTTT	3B	3375-	52.4	-	No amplification in both varieties
KASParmarNRT2.5_1	Same as KASParmarNRT2.5	3B	3375-	52.4	-	No W/D polymorphism
KASParmarSr2	AGCTGGTGAGAAAGAGGGCCTAATAACAGAGAAGACA[C/T]TCTTGATGGGTGAGACACTAAGGGTGGGCACACATCTGGACATCGAAATTGAGCTAAATTTGA	UA	206172	NA	AX-94516789	No W/D polymorphism

[a]:KASParmarGoGat_1 and KASParmarNRT2.5_1 were the same as KASParmarGoGat and KASParmarNRT2.5 respectively, but with different common primers. 'UA': unallocated, No: monomorphic for both parents; '-`: No 35K Axiom SNP available

Table 5.5 KASP assay primers[a].

KASP Primer Name	Primer sequence (5' to 3')
KASParmarGoGatA1	GGTATTATGAGTTTATGTGATTTGATGTAT
KASParmarGoGatA2	GGTATTATGAGTTTATGTGATTTGATGTAC
KASParmarGoGatC	CTTTATGTCTCGACCATTTCT
KASParmarGoGat_1A1	Same as KASParmarGoGatA1
KASParmarGoGat_1A2	Same as KASParmarGoGatA2
KASParmarGoGat_1C	CTCATCGTCATGTCCGTGATCTC
KASParmarNRT2.5A1	TATCAACCGCCGTCTGCACTG
KASParmarNRT2.5A2	TATCAACCGCCGTCTGCACTT
KASParmarNRT2.5C	AATTTAACCAAAACTGTATT
KASParmarNRT2.5_1A1	Same as KASParmarNRT2.5A1
KASParmarNRT2.5_1A2	Same as KASParmarNRT2.5A2
KASParmarNRT2.5_1C	TGTGCGCCTGCCCATTGAG
KASParmarSr2A1	AAAGAGGGCCTAATAACAGAGAAGACAC
KASParmarSr2A2	AAAGAGGGCCTAATAACAGAGAAGACAT
KASParmarSr2C	TAGCTCAATTTCGATGTCCAGATG

[a]: 5' complementary sequence to fluor-labelled oligos: FAM: GAAGGTGACCAAGTTCATGCT, HEX: GAAGGTCGGAGTCAACGGATT were added with each A1 and A2 primers, respectively, but are not shown here; common primers were redesigned for KASParmarGoGat_1 and KASParmarNRT2.5_1 markers only.

5.3.2 Scoring of the *csSr2* marker on the Drysdale x Waagan DH lines

The *csSr2* CAPS marker assay produced one amplicon of around 337 bp before *Bsp*HI digestion from Drysdale (Fig. 5.1). After digestion of the PCR product three DNA fragments were produced, of sizes consistent with the 172, 112 and 53 bp fragments characteristic of the CS (Hope 3B) type marker allele (not shown), which is normally associated with the *Sr2* rust resistance gene (Mago, R., Brown-Guedira, et al. 2011). The *csSr2* primers failed to amplify a product from Waagan (Fig. 5.1), hence Waagan contained the *csSr2* null type marker allele that is normally associated with absence of the *Sr2* rust resistance gene (Mago, R., Brown-Guedira, et al. 2011). These results were consistent a report of *Sr2* being present in Drysdale but not Waagan (www.wheatpedigree.net).

The absence of *csSr2* amplification from Waagan enabled this marker to be scored in the D x W DH lines without *Bsp*HI digestion. A total of 184 lines were scored, but due to the presence of

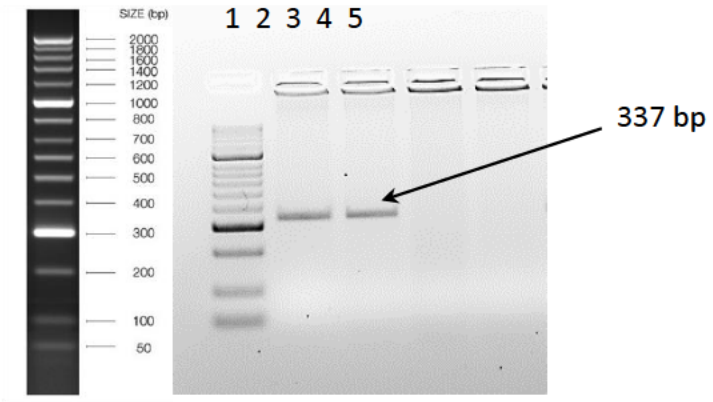


Fig. 5.1 Gel image of *csSr2* PCR amplification product of Drysdale and Waagan. Lane 1: ladder, lane 2&3: Drysdale and lane 4&5: Waagan. Only Drysdale produced 337 bp PCR product indicating presence of CS (Hope 3B) type marker allele.

clonal lines, these represented 144 unique lines. Marker haplotypes in the *QHsgw.aww-3B* region that were inconsistent with the rest of the population were observed in one sub-family derived from a particular F_1 individual (Family R, comprising 16 lines) (paragraph 4.3), therefore those lines were disregarded for further analysis. Because of poor DNA quality, line WW28450 was also removed for the analysis. This left 127 lines for analysis, among which 79 were *csSr2* positive (Fig. 5.2).

The *csSr2* marker scores were combined with the other marker data, allowing it to be genetically mapped. This placed *csSr2* in the 1.6 cM interval between markers *wsnp_Ex_rep_c67107_65584404* and *wsnp_Ex_c3005_5548573* (Fig. 5.2). Although, if line WW28454 which showed an unexpectedly high number of recombination events in the region was disregarded, it was placed in a slightly larger interval of 2.4 cM between markers *wsnp_Ex_c30368_39293103* and *wsnp_Ex_c3005_5548573*. Scores for only one line appeared inconsistent with this placement (WW28395; Fig. 5.2) may be due to a rare double recombinatoin event, or mis-scoring. These intervals were located within the region defined by the markers from the 9K SNP array that were most closely associated with the *QHsgw.aww-3B* grain filling heat tolerance effect (*wsnp_Ra_c41135_48426638* and *wsnp_BE497169B_Ta_2_1*) and as *csSr2* is extremely close to the *Sr2* locus genetically (Mago, R., Brown-Guedira, et al. 2011), this result was consistent with the hypothesis that *Sr2* is the basis of the *QHsgw.aww-3B* effects.

5.3.3 Pseudo black chaff (PBC) and rust resistance scored in the field

Analysis of the rust data from the Wagga Wagga 2013 trial of the Drysdale x Waagan DH population identified five rust resistance QTL (Table 5.6), of which one was on 3BS, with Waagan contributing the resistance allele. The marker from the 9K SNP array most closely associated with the QTL was *wsnp_Ex_c30368_39293103* (3.1cM), which was close to the *csSr2* marker locus and the *QHsgw.aww-3B* heat tolerance locus (previous section). As the resistance was from Waagan, this effect was evidently not due to *Lr27* (or *Sr2*) resistance gene that were expected to be present at this location in Drysdale, and must have been due to another rust resistance gene from Waagan. At least one stripe rust resistance gene is very closely linked to the *Sr2* locus and can be present in the absence of the *Sr2* resistance gene (Lowe et al. 2011). The rust isolate(s) present in the 2013 Wagga Wagga trial must have been avirulent on the gene from Waagan and affected less than (been more virulent on) the genes *Lr23* and *Sr2.*

Of the 127 unique D x W DH lines that were field-trialled, 37 were scored as positive for PBC in at least one of the field replicates. Initial QTL mapping indicated the presence of a single major QTL for PBC in the vicinity of the *QHsgw.aww-3B* QTL (data not shown), with the allele promoting PBC coming from Drysdale. Comparison of the PBC scores to the marker scores indicated a position for the PBC locus distal of the marker *wsnp_Ex_c3005_5548573* (Fig. 5.2). The expression of the PBC phenotype showed incomplete penetrance, as only around 50% of the lines that were expected from the marker scores to contain the PBC allele were scored positive for the trait, hence only PBC positive scores were informative. Only one line (WW28521_WW28522) was inconsistent with the designated locus position, as it was scored positive for PBC but carried Waagan marker alleles in the region. The chromosome region containing the PBC locus, distal of *wsnp_Ex_c3005_5548573*, also contained the *csSr2* marker locus. *Sr2* is unique among known wheat rust resistance genes in being associated with the PBC phenotype (McIntosh, Wellings & Park 1995). Hence these results from the PBC mapping were consistent with the idea that the Drysdale x Waagan population segregated for *Sr2*, with the resistance coming from Drysdale.

Table 5.6 Rust resistance QTL detected using data from Wagga Wagga 2013 field trial (most likely stripe rust).

Closest associated marker	Linkage group	cM Position	LOD	R^2 (%Expl. Var.)	Add. eff.	Resistance allele	P-value	1.5 LOD confidence interval
wsnp_Ex_c2337_4379619	2A	79.44	8.92	15.007	0.358	Waagan	<0.001	77.95-80.92
wsnp_RFL_Contig4483_5312236(C)	2B1	84.8	5.44	8.828	0.275	Drysdale	<0.001	76.51-84.8
wsnp_Ex_c1408_2704736(C)	2D1	0	5.13	8.034	0.262	Waagan	<0.001	0
wsnp_Ex_c104027_88843215	2D3	6.48	5.65	8.942	0.276	Drysdale	<0.001	3.6-15.2
wsnp_Ra_c41135_48426638(C)	3B1	0	4.72	7.938	0.26	Waagan	<0.001	0-11.02

5.3.4 Scoring of rust resistances and high-temperature-induced seedling chlorosis (HTISC) under controlled conditions

Mapping of *Sr2* in the Drysdale x Waagan DH population was also attempted by scoring a selected subset of 48 of the lines for HTISC and *Lr27* leaf rust resistance, under controlled conditions (tests by Robert Park, University of Sydney, Cobbitty).

HTISC was scored as positive in 22 of the tested lines. The enhanced heat induced chlorosis came from the Drysdale parent, and the locus for this trait mapped in the same marker interval as the *csSr2* marker (Fig. 5.2), i.e., between markers *wsnp_Ex_rep_c67107_65584404* and *wsnp_Ex_c3005_5548573*, or between *wsnp_Ex_c30368_39293103* and *wsnp_Ex_c3005_5548573* if line WW28454 was disregarded (Fig. 5.2). Only line WW28493 gave an HTISC score that was inconsistent with this location (positive, but carrying Waagan marker alleles). This result was therefore consistent with the ideas that the Drysdale x Waagan DH population segregated for *Sr2* locus and HTISC, and that the enhanced heat induced seedling chlorosis may be a pleiotropic effect of the rust resistance gene from Drysdale.

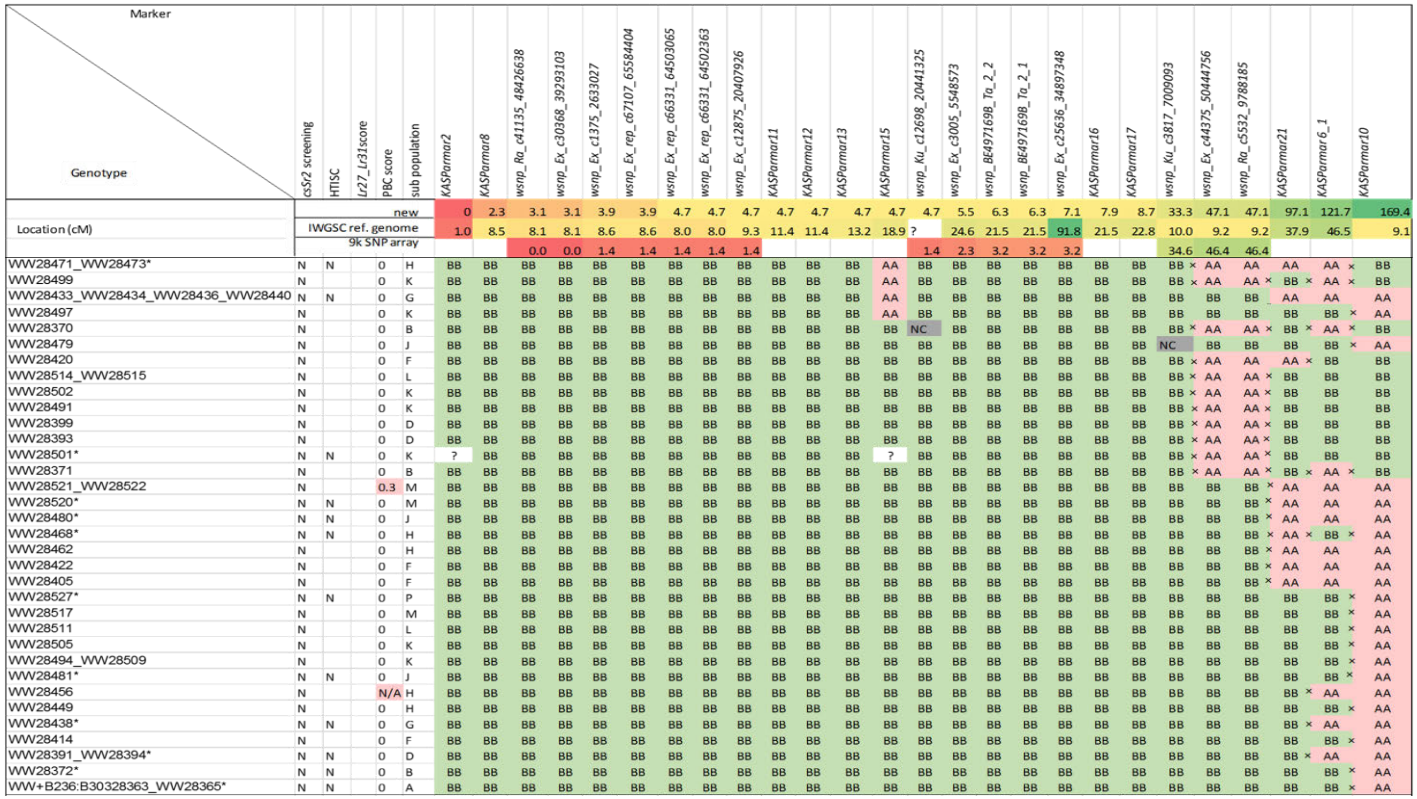
Only seven of the 48 tested lines (14.5%) were scored as positive for *Lr27* (resistant infection types of ;1-, ;1+ or ;12-). This was well below the 50% frequency of positives expected from single gene segregation, suggesting that the population also segregated for the unlinked *Lr31* gene required for expression of *Lr27*. Nonetheless, the available scores were consistent with segregation for *Lr27* on 3BS, with the resistance coming from Drysdale, and suggested a map location for *Lr27* distal of the marker *KASPamar21*. This chromosome region contained the mapped locations of all the other *Sr2* associated traits (Fig. 5.3).

Marker / Genotype	csSr2 screening	HTISC	Lr27_Lr31score	PBC score	sub population	KASParmar2	KASParmar8	wsnp_Ra_c41135_48426638	wsnp_Ex_c30368_39293103	wsnp_Ex_c1375_2633027	wsnp_Ex_rep_c67107_65584404	wsnp_Ex_rep_c66331_64503065	wsnp_Ex_rep_c66331_64502363	wsnp_Ex_c12875_20407926	KASParmar11	KASParmar12	KASParmar13	KASParmar15	wsnp_Ku_c12698_20441325	wsnp_Ex_c3005_5548573	wsnp_BE497169B_To_2_2	wsnp_BE497169B_To_2_1	wsnp_Ex_c25636_34897348	KASParmar16	KASParmar17	wsnp_Ku_c3817_7009093	wsnp_Ex_c44375_50444756	wsnp_Ra_c5532_9788185	KASParmar21	KASParmar6_1	KASParmar10
Location (cM) new						0	2.3	3.1	3.1	3.9	3.9	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	5.5	6.3	6.3	7.1	7.9	8.7	33.3	47.1	47.1	97.1	121.7	169.4
Location (cM) IWGSC ref. genome						1.0	8.5	8.1	8.1	8.6	8.6	8.0	8.0	9.3	11.4	11.4	13.2	18.9	?	24.6	21.5	21.5	91.8	21.5	22.8	10.0	9.2	9.2	37.9	46.5	9.1
Location (cM) 9k SNP array								0.0	0.0	1.4	1.4	1.4	1.4	1.4					1.4	2.3	3.2	3.2	3.2			34.6	46.4	46.4			
Drysdale185	Y					AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
Drysdale 186	Y					AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
Waagan192	N					BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
Waagan194	N					BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
WW28475*	Y	Y		0	J	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB
WW28470_WW28472	Y			0	H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28469*	Y	Y	Y	1	H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB
WW28461_WW28465	Y			0	H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB
WW28460_WW28467	Y			0	H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28447_WW28458	Y			1	H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB
WW28446	Y			0	H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28441*	Y	Y		0	G	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28432*	Y	Y	Y	1	G	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28415_WW28424_WW28429*	Y	Y		0	F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28408	Y			0.5	F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28387*	Y	Y		0.5	C	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28523	Y			1	M	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28519	Y			1	M	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28483_WW28484	Y			0	J	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28452_WW28464	Y			0	H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28388	Y			1	C	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28512	Y			0	L	AA	AA	AA	AA	AA	AA	AA	AA	AA	?	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB
WW28466*	Y	Y		0	H	?	?	?	AA	AA	AA	AA	AA	AA	?	?	?	?	AA	?	?	AA	AA	?	?	AA	AA	AA	?	AA	?
WW28362	Y			0	A	?	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28382*	Y	Y	Y	0	C	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	?	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28374	Y			0	B	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	?	AA	NC	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28403	Y			1	F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	?	AA	AA	AA	AA	AA ×	BB	BB	BB
WW28498*	Y	Y		0	K	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB
WW28496	Y			1	K	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB ×	AA ×	BB
WW28492	Y			0	K	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB
WW28487_WW28495	Y			1	K	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB ×	AA ×	BB
WW28474	Y			1	J	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB
WW28451	Y			1	H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB
WW28423*	Y	Y	Y	1	F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB
WW28404*	Y	Y	Y	1	F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB
WW28400	Y			1	F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB
WW28397*	Y	Y		1	D	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB ×	AA ×	BB
WW28396	Y			0	D	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB
WW28386	Y			1	C	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB
WW28379*	Y	Y	Y	1	B	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB
WW28375	Y			0	B	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB ×	AA ×	BB
WW28359	Y			1	A	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB

Fig. 5.2 continued..

Marker / Genotype	cs5r2 screening	HTISC	Lr27_Lr31score	PBC score	sub population	KASPsmar2	KASPsmar8	wsnp_Ra_c41135_48426638	wsnp_Ex_c30368_39293103	wsnp_Ex_c1375_2633027	wsnp_Ex_rep_c67107_65584404	wsnp_Ex_rep_c66331_64503065	wsnp_Ex_rep_c66331_64502363	wsnp_Ex_c12875_20407926	KASPsmar11	KASPsmar12	KASPsmar13	KASPsmar15	wsnp_Ku_c12698_20441325	wsnp_Ex_c3005_5548573	wsnp_BE497169B_To_2_2	wsnp_BE497169B_To_2_1	wsnp_Ex_c25636_34897348	KASPsmar16	KASPsmar17	wsnp_Ku_c3817_7009093	wsnp_Ex_c44375_50444756	wsnp_Ra_c5532_9788185	KASPsmar21	KASPsmar6_1	KASPsmar10
				new		0	2.3	3.1	3.1	3.9	3.9	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	5.5	6.3	6.3	7.1	7.9	8.7	33.3	47.1	47.1	97.1	121.7	169.4
Location (cM)				IWGSC ref. genome		1.0	8.5	8.1	8.1	8.6	8.6	8.0	8.0	9.3	11.4	11.4	13.2	18.9	?	24.6	21.5	21.5	91.8	21.5	22.8	10.0	9.2	9.2	37.9	46.5	9.1
				9k SNP array				0.0	0.0	1.4	1.4	1.4	1.4	1.4					1.4	2.3	3.2	3.2	3.2			34.6	46.4	46.4			
WW28359	Y			1	A	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB
WW28529	Y			1	P	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB × AA	BB
WW28524	Y			0	M	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB
WW28503_WW28508	Y			0	K	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB
WW28398	Y			0	D	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB × AA	BB
WW28389*	Y	Y	Y	0	C	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB
WW28373	Y			1	B	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB × AA	BB
WW28364	Y			1	A	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB
WW28478	Y			0	J	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB × AA	AA	AA
WW28477*	Y	Y		0	J	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB × AA × BB	× AA
WW28476	Y			1	J	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB × AA	AA	AA
WW28455	Y			0.5	H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB × AA	AA	AA
WW28411	Y			0	F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB × AA × BB	× AA
WW28410	Y			0	F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB × AA × BB	× AA
WW28402	Y			1	F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB × AA	AA	AA
WW28448_WW28457_WW28506	Y			0	H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB × AA	AA	AA
WW28444_WW28445	Y			1	H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB × AA	AA	AA
WW28421	Y			0.5	F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB × AA	AA	AA
WW28417	Y			0	F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB × AA	AA	AA
WW28412_WW28426	Y			1	F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB × AA	AA	AA
WW28409	Y			0	F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB × AA	AA	AA
WW28486	Y			0	K	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	?	AA	AA	AA	AA	AA × BB	BB	BB	BB	BB ×	AA
WW28526_WW28528	Y			0	P	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB	BB × AA	AA
WW28513	Y			0	L	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB	BB ×	AA	AA
WW28500_WW28507	Y			0	K	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB	BB × AA	AA
WW28490*	Y	Y		0	K	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB	BB ×	AA	AA
WW28459	Y			1	H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB	BB × AA	AA
WW28401	Y			0.5	F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB	BB ×	AA
WW28392	Y			0	D	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB × AA	AA
WW28383_WW28385_WW28390	Y			1	C	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB	BB ×	AA
WW28360*	Y	N		1	A	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB × AA	AA
WW28418_WW28427	Y			1	F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB	BB ×	AA
WW28395	N			0	D	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB	BB ×	AA
WW28377_WW28378	Y			0	B	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB × AA	AA
WW28376	Y			0	B	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB	BB × AA	AA
WW28367_WW28368*	Y	Y		0	B	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB × AA	AA	AA	AA × BB	BB	BB
WW28463	Y			1	H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	NC	BB	BB	BB	BB	BB	BB	BB × AA
WW28439*	Y	Y		0	G	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB	BB	BB	BB × AA	AA × BB	AA
WW28413*	Y	Y		0	F	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB × AA
WW28443_WW28453*	Y	Y		1	H	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB	BB	BB	BB	BB × AA	AA	AA × BB	BB	BB	BB
WW28419*	N	N		0	F	AA	AA	AA	AA	BB	BB	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB × AA	BB	BB	AA
WW28454	Y			N/A	H	AA	AA × BB	BB	BB	BB	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB × AA	BB	BB	BB	BB	BB
WW28381_WW28384	N			0	C	AA × BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB × AA	AA
WW28435_WW28437_WW28442*	Y	Y		0	G	BB	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	?	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA × BB	BB	BB
WW28416_WW28430	Y			0	F	BB × AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	?	AA × BB	BB	BB	BB	BB × AA	AA
WW28366	N			0	A	BB	NC	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB × AA	AA × BB	BB	BB	BB
WW28407	N			0	F	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	NC	BB	BB	BB	BB × NC	AA × BB	BB	BB	BB
WW28482	N			0	J	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA
WW28504	N			0	K	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	BB	BB × AA	AA × BB	BB	BB	BB
WW28428	N			0	F	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	BB	BB × AA	AA × BB	BB	BB	BB
WW28361*	N	N		0	A	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	BB	BB × AA	AA × BB	BB	BB	BB
WW28525	N			0	P	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA
WW28518*	N	N		0	M	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA
WW28493*	N	Y		0	K	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA
WW28485_WW28488*	N	N		0	K	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA
WW28369	N			0	B	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB ×	AA
WW28471_WW28473*	N	N		0	H	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB × AA	AA	AA	AA ×	BB	

Fig. 5.2 continued..



Marker / Genotype	csSr2 screening	HTISC	Lr27_Lr31score	PBC score	sub population	KASPsmar2	KASPsmar8	wsnp_Ra_c41135_48426638	wsnp_Ex_c30368_39293103	wsnp_Ex_c1375_2633027	wsnp_Ex_rep_c67107_65584404	wsnp_Ex_rep_c66331_64503065	wsnp_Ex_rep_c66331_64502363	wsnp_Ex_c12875_20407926	KASPsmar11	KASPsmar12	KASPsmar13	KASPsmar15	wsnp_Ku_c12698_20441325	wsnp_Ex_c3005_5548573	wsnp_BE497169B_To_2_2	wsnp_BE497169B_To_2_1	wsnp_Ex_c25636_34897348	KASPsmar16	KASPsmar17	wsnp_Ku_c3817_7009093	wsnp_Ex_c44375_50444756	wsnp_Ra_c5532_9788185	KASPsmar21	KASPsmar6_1	KASPsmar10
Location (cM)	new					0	2.3	3.1	3.1	3.9	3.9	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	5.5	6.3	6.3	7.1	7.9	8.7	33.3	47.1	47.1	97.1	121.7	169.4
	IWGSC ref. genome					1.0	8.5	8.1	8.1	8.6	8.6	8.0	8.0	9.3	11.4	11.4	13.2	18.9	?	24.6	21.5	21.5	91.8	21.5	22.8	10.0	9.2	9.2	37.9	46.5	9.1
	9k SNP array							0.0	0.0	1.4	1.4	1.4	1.4	1.4					1.4	2.3	3.2	3.2	3.2			34.6	46.4	46.4			
WW28471_WW28473*	N	N		0	H	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB × AA	AA	AA	AA × BB		
WW28499	N			0	K	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB × AA	AA ×	BB × AA	× BB		
WW28433_WW28434_WW28436_WW28440	N	N		0	G	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB	BB	AA	AA		AA
WW28497	N			0	K	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA
WW28370	N			0	B	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	NC	BB	BB	BB	BB	BB	BB	BB × AA	AA ×	BB × AA	× BB		
WW28479	N			0	J	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	NC	BB	BB	BB	BB ×	AA
WW28420	N			0	F	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB × AA	AA	AA ×	BB		BB
WW28514_WW28515	N			0	L	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB × AA	AA ×	BB	BB		BB
WW28502	N			0	K	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB × AA	AA ×	BB	BB		BB
WW28491	N			0	K	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB × AA	AA ×	BB	BB		BB
WW28399	N			0	D	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB × AA	AA ×	BB	BB		BB
WW28393	N			0	D	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB × AA	AA ×	BB	BB		BB
WW28501*	N	N		0	K	?	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	?	BB	BB	BB	BB	BB	BB	BB	BB × AA	AA ×	BB	BB		BB
WW28371	N			0	B	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB × AA	AA ×	BB × AA	× BB		
WW28521_WW28522	N			0.3	M	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA		AA
WW28520*	N	N		0	M	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA		AA
WW28480*	N	N		0	J	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA		AA
WW28468*	N	N		0	H	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA ×	BB ×		AA
WW28462	N			0	H	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA		AA
WW28422	N			0	F	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA		AA
WW28405	N			0	F	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA		AA
WW28527*	N	N		0	P	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA
WW28517	N			0	M	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA
WW28511	N			0	L	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA
WW28505	N			0	K	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA
WW28494_WW28509	N			0	K	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA
WW28481*	N	N		0	J	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA
WW28456	N			N/A	H	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA
WW28449	N			0	H	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA
WW28438*	N	N		0	G	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA
WW28414	N			0	F	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA
WW28391_WW28394*	N	N		0	D	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA	AA
WW28372*	N	N		0	B	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA
WW+B236:B30328363_WW28365*	N	N		0	A	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB ×	AA

Fig. 5.2 Graphical genotyping to position loci for the *csSr2* marker, high-temperature-induced seedling chlorosis (HTISC), pseudo black chaff (PBC) and *Lr27* leaf rust resistance relative to molecular markers and the grain filling heat tolerance locus *QHsgw.aww-3B*. PBC scores are averages of two field replicates (1 for presence and 0 for absence). Asterisks indicate lines tested for HTISC and *Lr27* at Cobbitty. Red, yellow, blue and green arrows indicate the inferred direction of loci for *csSr2*, HTISC, *Lr27* rust resistance and PBC respectively, relative to critical recombination points between markers. Other details are as described in the caption of Fig. 4.1.

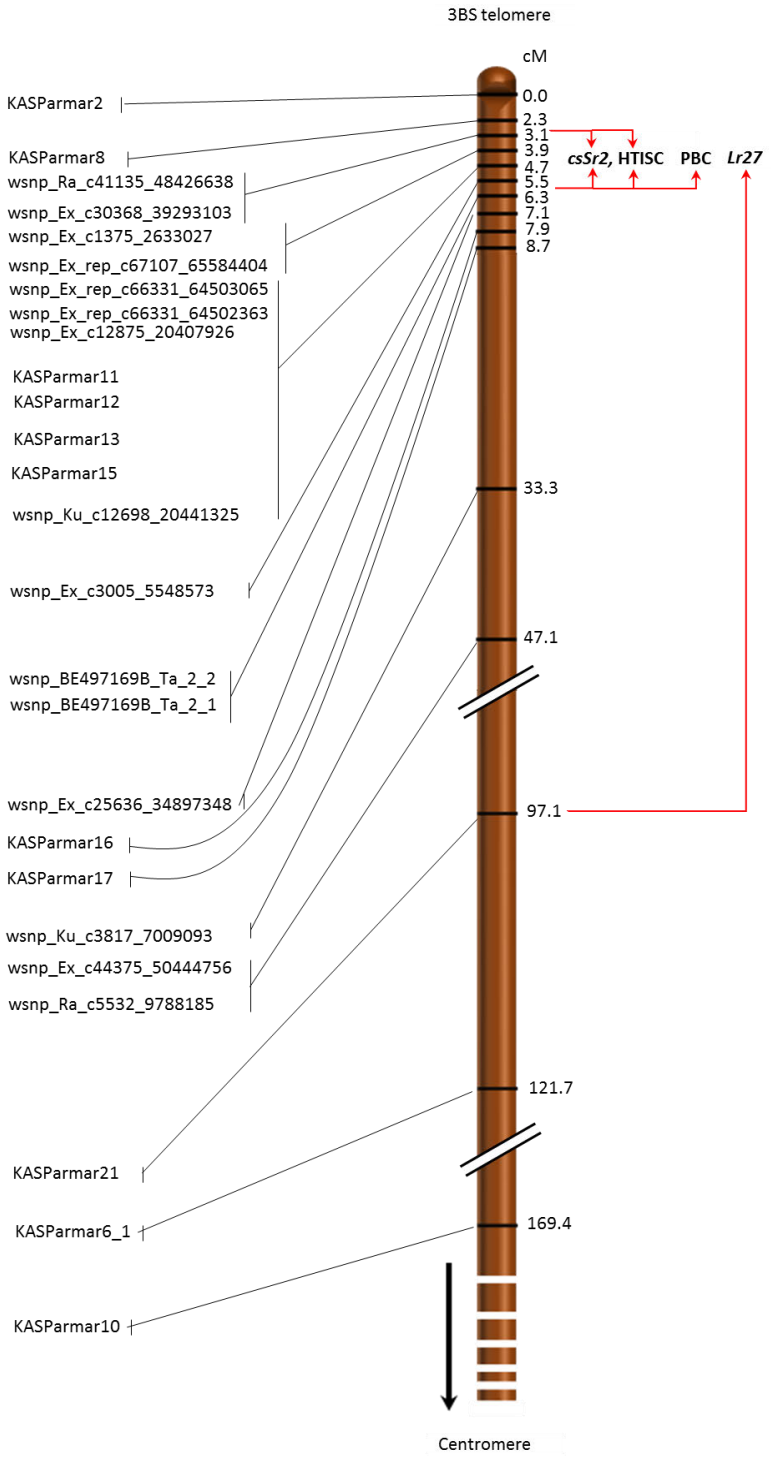


Fig. 5.3 Genetic map locations of the *csSr2* marker and loci for HTISC, *Lr27* and PBC traits on chromosome 3B. Red arrows show the mapped intervals for the trait loci.

5.3.5 Association of *csSr2* marker alleles with grain filling and chlorophyll heat tolerance obtained from greenhouse screens

The *csSr2* CAPS marker was scored on the 101 hexaploid wheat genotypes screened for heat tolerance in the greenhouse. After digestion with *BspHI*, the *csSr2* amplicons showed three different patterns on agarose gels that were consistent with the those reported by (Mago, R., Brown-Guedira, et al. 2011): No amplification product (null allele, associated with lack of *Sr2*); two fragments of 225 and 112 bp (Marquis type allele, associated with lack of *Sr2*), and three fragments of 172, 112 and 52 bp (CS (Hope 3B) type allele, associated with the presence of *Sr2*). Of the 101 genotypes, 56 had the null allele, 34 showed the Marquis type marker allele and 11 showed the CS (Hope 3B) type allele (Table 5.7).

There were 27 genotypes common to this study and that of Mago, R., Brown-Guedira, et al. (2011), and the *csSr2* scores were the same, with 2 exceptions: Scout was scored as null type in this study (consistent with wheatpedigree.net not saying it contained *Sr2*) but as CS (Hope 3B) type allele by Mago, R., Brown-Guedira, et al. (2011). Wyalkatchem was scored as Marquis type in this study, but as null type by Mago, R., Brown-Guedira, et al. (2011); the latter is consistent with the report that it contains *Sr2* (Wellings, Bariana & Park 2003). Such inconsistencies may be due to mislabelling errors, marker mis-scoring or heterogeneity for *Sr2* within these varieties.

Both in the current study and that of Mago, R., Brown-Guedira, et al. (2011), Kukri showed the Marquis type *csSr2* allele normally associated with absence of *Sr2*, although this variety was reported to contain *Sr2* (www.wheatpedigree.net) but pedigree information could also be wrong.

In this study cv. Mendos was scored as null allele but according to www.wheatpedigree.net it carries *Sr2* (Table 5.7). Eight other varieties were reported at www.wheatpedigree.net to carry *Sr2*, yet showed the Marquis-type marker allele. This inconsistency may be due to mislabelling errors, variety heterogeneity the fact that PBC is not always a clear indicator for *Sr2*, but it also raises the possibility that the *csSr2* marker is not completely diagnostic of the presence of *Sr2*.

Table 5.7 *csSr2* marker scores for hexaploid wheat genotypes used in heat tolerance screens.

Variety	***csSr2* score**
Ajana, Aroona, Avocet S, Axe, Barham, Cadoux, Catalina, CD87, Chara, Chuan Mai 18, Cook, Correll, EGA 2248, EGA Blanco, EGA Gregory, Egret, Excalibur, Fang, Flanker, Frame, Gascoigne, Gladius, H45, H46, Halberd, Janz, Kite, Kord, Krichauff, Lincoln, PI626580-4, PI625123-3, Mendos*, Mexico-120, Molineux, Najah, RAC875, RT414, RT416, RT417, RT418, Scout, Stiletto, Sunco, Sunstar, Sunvale, Sunvex, Synthetic W7985, Tasman, Trident, Vigour18, Waagan, Westonia, WW2449, Yitpi, Young	Null
Baxter*, Berkut, Cobra*, Cranbrook*, Crusader*, Drysdale*, EGA Eaglehawk*, Hartog*, HTWYT012, Lyallpur-73*, Seri M82	CS (Hope 3B) type
6HRWSN125, Babax, Calingiri, Suzuky_12, Suzuky_17, Suzuky_19, Suzuky_23, Suzuky_24, Suzuky_9, Corack*, EGA Bonnie Rock, Emu Rock*, HTWYT007, HTWYT028, Hydra, Kalyansona*, Katepwa, Kauz, King Rock, Kukri*, Mace*, Magenta, Millewa, Opata85*, Reeves, Scepter, Siete 7 Cerros, Sokoll, Spitfire*, Suntop, Tammarin Rock, Vorobey*, Wyalkatchem*, Wyuna	Marquis type

*: varieties reported at www.wheatpedigree.net to carry *Sr2*. Without *: varietal information not available or no record of *Sr2* available in www.wheatpedigree.net.

The *csSr2* marker allele scores showed subtle relationships to heat tolerance measured in greenhouse screens. The trend was for genotypes carrying the null *csSr2* marker allele to be more tolerant than those carrying either the Marquis and CS (Hope3B) type alleles, for both grain weight (higher tolerance index) and chlorophyll (less loss) (Fig. 5.4). This raised the possibility that heat tolerance was related to the haplotype at the *Sr2* locus, but that it could be uncoupled from the presence of the *Sr2* stem rust resistance gene across the two stem rust susceptible classes. However these differences between the groups were unlikely to be significant and need to be tested further.

Fig. 5.4 Heat tolerance for 1000-KW and proportion of chlorophyll change just after heat treatment, of Australian wheat varieties with Null, Marquis and CS (Hope 3B) type allele, in five experiments.

5.4 Conclusions:

- The *NRT2.5* and *GoGat* genes couldn't be tested as candidates for the gene controlling *QHsgw.aww-3B* as marker assays designed to these genes failed. Design of more marker assays for these genes may yet allow them to be mapped relative to *QHsgw.aww-3B*. Alternatively, the alleles for these genes may be identical in sequence between Drysdale and Waagan, preventing them from ever being mapped in the Drysdale x Waagan DH population.
- In the Drysdale x Waagan DH population, HTISC, PBC and *Lr27* leaf rust resistance were all mapped in coupling phase to overlapping marker intervals on the short arm of chromosome 3B, with the source of these traits being Drysdale. This was consistent with knowledge that these traits tend to occur together with the *Sr2* stem rust resistance gene, and that Drysdale carries *Sr2*.
- In the Drysdale x Waagan DH population, the grain filling and chlorophyll retention heat tolerance effects of the *QHsgw.aww-3B* locus mapped to the same marker interval as the *Sr2* locus, with the heat tolerance coming from Waagan. This was consistent with the idea that the heat susceptibility at this locus may be due to the same gene(s) and biological processes that lead to the HTISC, PBC and/or *Lr27*, *Sr2* rust resistances in Drysdale – e.g., relating to accelerated heat induced senescence. Thus, selection for these rust resistances in breeding may come with a penalty of increased heat susceptibility.
- A natural field inoculum revealed a rust resistance gene (possibly for stripe rust) from Waagan that was closely linked to the *Sr2* locus. This indicated that selection for the heat tolerance allele from Waagan at *QHsgw.aww-3B* may not always come with the penalty of increased rust susceptibility (due to the absence of *Sr2* or *Lr27*); in fact it may sometimes improve rust resistance against some field rust isolates.
- Comparing heat tolerance data from the greenhouse to scores at the *Sr2*-linked marker *csSr2* indicated that heat tolerance at *QHsgw.aww-3B* may be associated with only one of the two known non-*Sr2* haplotypes (the 'null-*csSr2'* type). Hence, in situations where breeders do not need to use *Sr2*, selection for the 'null-*csSr2'* haplotype may be required to maximize heat tolerance. However, the association was weak, so further verification is

required – e.g., by observing heat tolerance QTL segregation at *QHsgw.aww-3B* in a population segregating for the *null-csSr2* vs. *Marquis-csSr2* haplotypes.

- The Waagan allele of *QHsgw.aww-3B* may therefore be quite useful in breeding, by providing both heat tolerance (as it is a 'null-*ssSr2'* haplotype) as well as occasional protection against rust.

Chapter 6: QTL mapping of heat tolerance in Young × Reeves DH population under short term heat stress at the reproductive stage

6.1 Introduction

The wheat cultivar Young (VPM1/3*Beulah//Silverstar) was released by Australian Grain Technologies Pty Ltd in 2009 and Reeves (Bodallin//Gamenya/Inia 66) was released by Western Australian Department of Agriculture in 1989. These two varieties showed a good overall contrast for heat tolerance across a range of chamber/greenhouse assays, in the GRDC funded ACPFG heat tolerance projects UA00123 and UA00147. Erena Million F. (2015) (PhD student of ACPFG in his research project- Genetic and physiological bases of heat-induced floret sterility in wheat, unpublished), heat treated plants of various varieties at 1 or 6 cm auricle interval (AI) for three days, and found that Reeves developed a high level of floret sterility (88.5%) compared to Young (9.4%). These parents also contrasted for heat responses of single grain weight and flag leaf chlorophyll after heat treatment at 10 DAA (Shirdelmoghanloo 2015), and for responses of relative growth rate and chlorophyll loss in heat treated seedlings (Shirdelmoghanloo 2015). Young showed the least response for single grain weight (less than 3.0% loss of single grain weight due to heat while Reeves showed more than 25.0% reduction in single grain weight.

Young is an early to mid-season maturity variety and takes ~57 days to reach anthesis. It has performed best in low and medium rainfall environments of southern Australia. It also shows good tolerance to yellow leaf spot, black point, cereal cyst nematode (CCN) and acidic soils. It is classified as Australian premium white (APW) grade in the Western and Northern zones of Australia with the characteristics of high milling performance and flour quality, ideal for the production of a variety of noodle types, Middle Eastern and Indian flat breads and Chinese steamed bread.

Reeves is a mid-season, tall cultivar and takes ~60 days to reach anthesis. Reeves gives noodles with good texture, was previously classified as ASW (noodle) but was later stripped of its Western Australian noodle wheat market classification due to low falling number and became classified as a feed wheat (Australian general purpose grade; AGP).

Due to the contrasting heat tolerance of these varieties, they were crossed to make a DH population for mapping heat tolerance QTL in the laboratory of Dr Nick Collins. The contrast between the pedigrees and other quality and physical characteristics of the parents also suggests this population might be useful for genetic analysis of other types of traits. Identification of heat tolerance QTL for grain number and grain size in this one population could provide insight into how the physiology and genetics of the two types of responses may overlap. Accordingly, this chapter is focused on QTL mapping for tolerance to heat applied at two reproductive stages (6 cm AI and 10 DAA) in the Young x Reeves DH mapping population.

6.2 Materials and Methods

6.2.1 Plant materials

The mapping population of 250 F_1 derived DH lines was constructed using crosses between the cvs. Young and Reeves, using Young as the female and Reeves as the male in all of the crosses. Two plants of each parental cv. (Young-4, Young-5, Reeves-1 and Reeves-2) were used for F_1 seed production. Three F_1 plants from each of the crosses Young-4 x Reeves-1 and Young-4 x Reeves-2, in addition to two F_1 plants from the Young-5 x Reeves-2 cross, were used as donors for DH production (i.e., 8 F_1 plants total). Nick Collins did the crossing and supplied Sue Broughton at DAFWA with F_1 seed, who then used the F_1 plants to make the DHs by the anther culture technique.

6.2.2 DNA extraction

I extracted genomic DNA from the 250 DH lines and the four parental single plant parent selections, by working together with Iman Lohraseb. Leaf segments ~50 mm long was collected from two-week-old plants and DNA extracted following the protocol of Yamaya et al. (1992). DNA concentration was measured using the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies Australia Pty Ltd., Scoresby, Australia) following the manufacturer's protocol, in 96 well plates, using a PHERAstar FSX multi-mode microplate reader (BMG Labtech Pty. Ltd., Mornington, Australia) with a 485 nm excitation filter and 520 nm emission filter. The DNA quality (integrity) was tested by electrophoresis in 0.8% agarose gels and was found to be adequate.

6.2.3 DArTseq

DNA samples of DH lines and parental selections were diluted to ~50 ng/µL in TE buffer and 20 µL of each sample dispensed into fully skirted V-bottom 96 well microtiter plates (Eppendorf Twin-Tec, Eppendorf South Pacific Pty. Ltd., North Ryde, Australia) leaving wells G12 and H12 empty for negative controls. The wells were sealed with strips of 8 flat caps (65.1998.002, Sarstedt AG & Co., Nümbrecht, Germany) and shipped to Diversity Array Technology (Canberra, Australia) for DArTseq genotyping.

6.2.4 *Rht-B1*, *Vrn-A1* and *Vrn-D1* markers

Based on a previous marker analysis (Nick Collins, personal communication; and this study), cvs. Young and Reeves were known to carry functionally contrasting alleles at the dwarfing locus *Rht-B1*, and the vernalisation loci *Vrn-A1* and *Vrn-D1* (Table 6.1). I therefore scored parents and the DH lines for polymorphisms in *Rht-B1, Vrn-A1* and *Vrn-D1*. Both parents carry the tall (wild-type) allele at *Rht-D1*, hence the population was expected to segregate double-tall vs. semidwarf at *Rht-B1*. Both parents carry a spring allele at *Vrn-B1*, hence the population was expected to only contain spring types, with phenology effects potentially segregating at both the *Vrn-A1* and *Vrn-D1* loci. These *Vrn-A1* and *Vrn-D1* effects were expected to be relatively subtle (compared to a spring type vs. winter type effect).

The *Rht-B1* and *Vrn-A1* markers were scored by KASP assays using the CerealsDB (Functional Genomics Group, the University of Bristol; www.cerealsdb.uk.net/cerealgenomics/CerealsDB/kasp_download.php) primer sets wMAS000001 and wMAS000033, respectively.

The *Vrn-D1* marker was scored using a three-primer mixture (Intr1/D/F, Intr1/D/R3 and Intr1/D/R4) (Fu et al. 2005) targeting insertion/deletion variation in intron-1. Intr1/D/F and Intr1/D/R3 primers produce a 1,671 bp PCR product from the spring allele (containing the deletion) while the Intr1/D/F and Intr1/D/R4 primer pair produces a 997bp PCR product from the winter allele (not containing the deletion). The PCR program started with an initial denaturation at 94 ^{o}C for 3 min and was followed by 40 cycles of 94 ^{o}C for 10 sec, 60 ^{o}C for 10 sec, 68 ^{o}C for 2

min 30 sec and final extension at 72 °C for 10 min. PCR products were separated on 1.5% agarose gels.

Table 6.1 Phenology marker alleles present in Young and Reeves. Marker designations were based on marker assays described by Shirdelmoghanloo, Taylor, et al. (2016).

Variety	*Rht-B1*	*Rht-D1*	*Ppd-D1*	*Ppd-B1*	*Vrn-A1*	*Vrn-B1*	*Vrn-D1*
Young	dwarf	tall	insensitive	unknown	spring	spring	spring
Reeves	tall	tall	insensitive	unknown	heterogeneous?[a]	spring	winter

[a]: Parent plants later determined in this current study to carry only the winter allele.

6.2.5 Genetic map

The genetic map was constructed by Nick Collins and Julian Taylor (Fig. 6.1). The scores for the *Rht-B1*, *Vrn-A1* and *Vrn-D1* markers were combined with those of the DartSeq markers. In an initial analysis, 17 of the DH DNAs were found to give a high proportion of heterozygous marker calls, suggesting that the original DH 'plants' of these lines may have in fact been multiple plants (derived from multiple microspores) that were not successfully divided. These lines were excluded from further analysis. Conversely, 39 of the lines had counterparts with virtually identical marker calls, suggesting that some of the original DH plants were divisions of the same plant (derived from the same microspore). This group of lines was represented by 18 unique lines for map construction. After applying various quality filters, 4,528 polymorphic markers were retained for map construction. Markers that showed a different allele between the two selections of a parent cv. (due to residual heterogeneity in the varieties) were treated as described by Shirdelmoghanloo, Taylor, et al. (2016) so that they did not compromise the accuracy of the map. The final map contained 1,149 genetically non-redundant loci. For each redundancy group, a representative marker was chosen for map construction and reporting. The final map (Fig 6.1) contained 28 linkage groups representing all of the 21 wheat chromosomes. It had a total length of 3,499 cM, which is similar to other high-quality maps of wheat (2,937 to 4,110 cM; Akbari et al. 2006; Chalmers et al. 2001; Paillard et al. 2003; Quarrie et al. 2005).

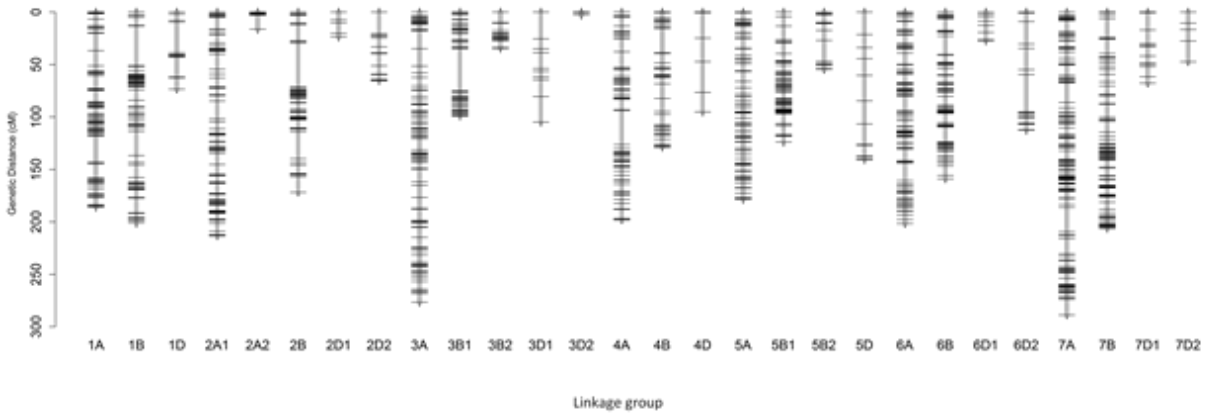


Fig. 6.1 Genetic map of the Young x Reeves DH population. Vertical lines represent each linkage group and the horizontal lines indicate the position of the markers loci on the linkage groups.

6.2.6 Plant growth conditions

Two seeds per pot were sown on 26th August, 2015 in Room 30 at The Plant Accelerator (TPA, The University of Adelaide, Waite Campus, Adelaide). After germination, plants were thinned to one per pot. The temperature control system was set at 15 / 20 °C daily minimum/maximum on a sine-wave pattern, then increased to 16 / 22 °C on 18th September. Plant growth was carried out as described by Shirdelmoghanloo, Taylor, et al. (2016). Measured greenhouse conditions are summarized in Table 6.2. The growth chamber used for heat treatments (Conviron BDW120) was set at 37/27 °C day/night temperature. Further details of growth chamber conditions were as mentioned in paragraph 2.2.2.

Table 6.2 Measured temperatures and relative humidity (%) in the greenhouse (The Plant Accelerator, Room 30) during the experiment. The stages for treatments for assessing floret fertility (6 cm AI) and grain filling (10 DAA) effects were reached during October-November and November-December, 2015 respectively.

Month	Daily avg.	Avg. daily min.	Avg. daily max.	Days >30°C	Avg. % humidity
Aug	17.9	14.9	23.5	0	61.7
Sep	19.1	14.6	29.4	0	64.3
Oct	21.6	15.8	33.7	4	60.3
Nov	22.3	15.7	31.7	3	59.5
Dec	23.3	15.6	32.5	7	60.8
Jan-16	23.8	16.1	32.8	7	62.2

6.2.7 Treatment and experimental design

The experiment employed heat treatments at two different growth stages, using a common set of plants as non-heat-treated controls. For one-third of the plants, when the AI of the main stem reached 6 cm, the main stem was tagged with colored tape and moved to the growth chamber for three days to apply heat treatment, to measure effects of heat on floret fertility on the main stem. Another third of the plants were heat treated at 10 DAA following the same method described in 2.2.3, to assess the effects of heat on final grain size. The remaining third of plants were not heat treated and served as controls for both the plants treated at 6 cm AI and at 10 DAA.

The experimental design was produced by Julian Taylor. The experiment employed a split-plot design with randomized complete blocks. There were two blocks. Each main plot comprised three plants of the same genotype. The plants in each plot represented sub-plots of one plant for each control, heat at 10 DAA and heat at 6 cm AI. Within each block, each DH had one replicate (as defined by the original DH line names; clonal lines in effect provided additional replication) and each of the four parental selections had three replicates. During heat treatment, plants were placed in arbitrary locations in the growth chamber, as there was insufficient space available to employ a proper design.

6.2.8 Trait data collection and analysis

The main stem of each plant was tagged with colored tape when it reached the target stage, to identify the tiller to score afterwards and to confirm treatment status of the plants. The traits that were measured/derived are detailed in Table 6.3.

Data were analysed following methods described in paragraph 2.2.6. Best linear unbiased predictions (BLUPs) of trait mean values for each genotype/treatment were produced by Sabela Munoz-Santa and heat tolerance indexes derived from the BLUPs for each genotype. The heat tolerance index was defined using a linear regression of the BLUPs of all the genotypes under heat vs. control conditions, and was calculated for each genotype as the difference between the observed value under heat and its expected value based on the value in the control and the overall

regression. The heat tolerance index was expressed in the original unit or transformed when required to produce a more normal frequency distribution. This tolerance index has the advantage over HSI (heat susceptibility index) (Fischer and Maurer, 1978) of being less dependent on the control values.

QTL analysis was done using GenStat 18 (www.vsni.co.uk/downloads/genstat/) for trait values (BLUPs) under control, heat and trait heat tolerance indexes. Initial 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. QTL effects were grouped into numbered QTL regions, with effects mapping within ~30 cM being assigned the same QTL number.

Table 6.3 Traits scored in the Young × Reeves DH population and parental selections.

Trait name	**Abbreviation**	**Details**	**Control plants**	**Plants treated at**	
				6 cm AI	**10 DAA**
Culm length at 10 DAA (cm)	CulmL10	Measured from the soil surface to the spike collar	yes		yes
Culm length at maturity (cm)	CulmLMat	Measured from the soil surface to the spike collar	yes	yes	yes
Peduncle length at 10 DAA (cm)	PedL10 DAA	From last stem node to the base of the spike	yes		yes
Peduncle length (cm) between 10 DAA and at maturity	PedL10ToMat	Derived from difference of the peduncle length at 10 DAA and maturity	yes		yes
Peduncle length at maturity (cm)	PedLMat	From last stem node to the base of the spike	yes	yes	yes
Spike length at maturity (cm)	SpkLMat	From the collar to the top of the spike, excluding awns	yes	yes	
Awn length at maturity (cm)	AwnL	Distance from end of awns to top of glumes of terminal spikelet	yes	yes	

Table 6.3 continued..

Trait name	Abbreviation	Details	Control plants	Plants treated at	
				6 cm AI	10 DAA
Shoot weight at maturity (g)	ShootW	Each plant was cut off at the soil surface and the shoot (stem + leaves) separated from the spike at the collar. Shoots were oven dried at 85 ^{o}C for 3 d before being weighed	yes	yes	yes
Flag leaf length (cm), at 10 DAA	FlagL	The length of the flag leaf from base of the blade to the leaf tip	yes		
Flag leaf width (cm), at 10 DAA	FlagW	The width of the flag leaf at the widest part of the flag leaf blade	yes		
Chlorophyll content at 10 DAA (SPAD units)	SPAD10 DAA	Relative chlorophyll content of the flag leaf, using a portable SPAD meter	yes		yes
Chlorophyll content at 13 DAA (SPAD units)	SPAD13 DAA	As above	yes		yes
Chlorophyll content at 27 DAA (SPAD units)	SPAD27 DAA	As above	yes		yes
Days to anthesis	Anth	Time from sowing to emergence of first anther	yes		yes
Days to Awn emergence	AwnEm	Time from sowing to when first awns were first visible past the flag leaf auricle	yes	yes	
Time from sowing to the day the target auricle interval length was reached	AIdate	When auricle interval was ~6 cm	yes	yes	
Measured length of AI on the day of treatment	AILgth	When auricle interval was ~6 cm		yes	
Days from anthesis to maturity	AnthToMat	Maturity defined as when spikes were ~ 95% yellow and seeds hard	yes		yes
Days from anthesis to flag leaf senescence	AnthToFLSen	Senescence defined as when flag leaves were 95% yellow	yes		yes
Number of developed spikelets per spike, at maturity	DevSpklt	Total spikelets minus UnderdevSpklt	(yes)*	(yes)*	yes
Total number of grains per spike, at maturity	GnNoSpk	Only those in developed spikelets were counted	(yes)*	(yes)*	yes
Grains per spikelet, at maturity	GnNoSpklt	GnSpk divided by DevSpklt	yes	yes	yes

Table 6.3 continued..

Trait name	Abbreviation	Details	Control plants	Plants treated at	
				6 cm AI	10 DAA
Number of basal under-developed spikelets per spike, at maturity	UnderdevSpklt	Under-developed spikelets defined as those having awns <50% length of majority of the other spikelets	yes	yes	yes
Number of developed spikelets per spike, at maturity	DevSpklt	Total spikelets minus UnderdevSpklt	(yes)*	(yes)*	yes
Grain weight per spike, at maturity	GnWSpk	After removing the underdeveloped spikelets, spikes were threshed and grains weighted after storage at room temperature for ~4 weeks	yes		yes
Single grain weight, at maturity	SingGW	GnWSpk divided by GnNoSpk	yes		yes
Number of grains per spike at floret positions 1+2	GnNoSpklt1&2	Recorded separately for the top, middle and bottom third of the spike, basal two floret positions per spikelet, in developed spikelets only	yes	yes	
Number of grains per spike at floret positions >2	GnNoSpklt>2	As above, but for floret positions 2 and above in the spikelets	yes	yes	

6.3 Results and Discussion

6.3.1 Greenhouse conditions

Unfortunately, while the temperature control system of the greenhouse was set at 16 / 22 °C daily minimum/maximum, much higher temperatures were reached during the sensitive developmental stages, due to late time of sowing, the resulting high outside temperatures (www.bom.gov.au), and limitations of the evaporative cooling system to limit temperatures.

Plants reached the peak heat sensitive stage for floret fertility (6 cm AI) between 30th September and 13th of November, 2015 (Fig. 6.2). The sensitive stage for this effect of heat in wheat includes three days before and after the reaching the target stage 6 cm AI (Nick Collins, personal

communication). During this period, greenhouse temperatures exceeded 25 °C for 28 days and 30 °C for six days. During this period outside temperatures were high, reaching above 30 °C for 11 days and above 35 °C for four days (Fig. 6.2) (www.bom.gov.au, Kent Town, weather station number 023090). The main sensitive period for grain filling in wheat spans approximately five days before anthesis to 20 days after anthesis and occurred in the plants from 9th of October to 17th of December (Fig. 6.2). During this sensitive period for grain filling, plants were exposed to above 25 °C for 38 days and above 30 °C for seven days.

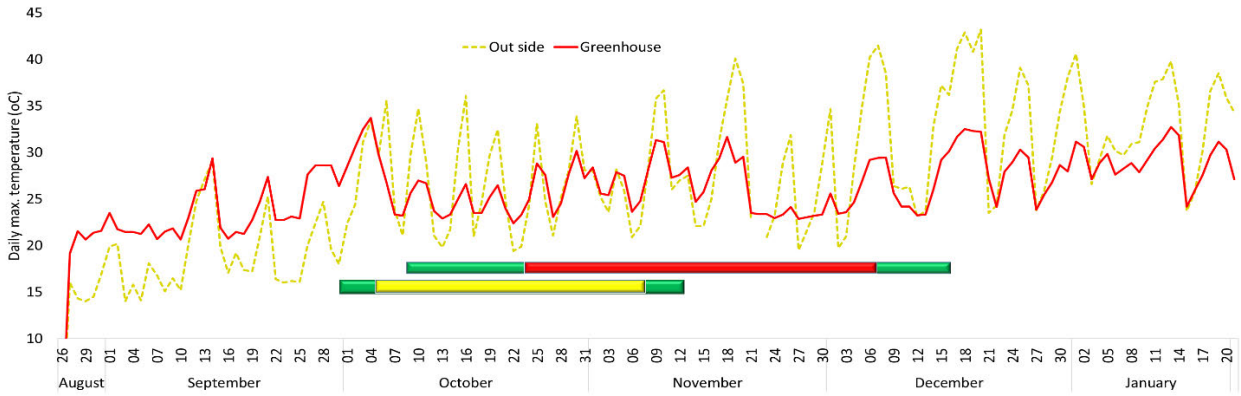


Fig. 6.2 Daily maximum temperatures of greenhouse room 30 (red line) and outside (brown line) from sowing to maturity. Yellow and red bars indicate the period when plants reached the target stage for heat treatment, for fertility (6 cm AI) and grain filling (10 DAA) effects, respectively. Green extended bars show the estimated time when plants were potentially sensitive to effects of heat on fertility or grain filling.

6.3.2 Heat responses in parental and DH lines

6.3.2.1 Responses to treatment at 6 cm AI

Duration of developmental processes

DH plants that were heat treated at 6 cm AI reached awn emergence around one day earlier than control plants, but senescence (AIToFLSen) was delayed for two days compared to the control plants (~67 vs. ~65 days). Heat also reduced final culm length by an average of ~6 cm in the DH lines and Young (Table 6.4).

Grain number

The total number of grains per spike in heat treated DH lines and Reeves was decreased by ~9 (26.26%) and ~16 (39.42%) respectively, relative to control plants. At floret position 1 and 2, heat decreased seed number per spike by ~20 (57.9%) and ~10 (36.41%) in Reeves and the DH lines, respectively. At floret positions >2 in the spikelets, grain number was decreased by 12.8% in the DH lines. The heat susceptible parent Reeves and the DH lines showed reductions in grain number per spike due to heat (BotGnNo1&2: 31.7% and 24.71%; MidGnNo1&2: 70% and 34%; TopGnNo1&2: 71% and 49%, in Reeves and the DH lines, respectively) and grain number per spikelet at floret position 1 and 2 in all three parts of the spike (BotGnNoSpklt1&2: 34% and 32.7%; MidGnNoSpklt1&2: 74% and 40%; TopGnNoSpklt1&2: 71.71 and 53.69%, in Reeves and the DH lines, respectively). BotGnNo at floret position >2 increased (doubled), and BotGnNoSpklt>2 also increased, in Reeves due to the heat treatment. Million F. Erena (personal communication) also observed significant increases in grain number in more apical florets (>2) of the spikelets in bottom and top parts of the spike, but decreases at the 1&2 floret positions in all three parts of the spike, in the Drysdale x Waagan population, with heat treatment applied at 3 cm AI. These data overall therefore suggest that heat treatment during booting can decrease fertility of the basal two floret positions in the spikelets, but increase fertility in the more apical floret positions of the spikelets. GnNoSpklt was decreased in all three genotype classes (42.8% in Reeves, 19% in Young and 33% in DH lines) and the trend was consistent with the observation of Million F. Erena showing Young being more tolerant than Reeves for this trait.

Spikelet number

Data for total number of spikelets per spike was not modelled, but the raw means showed essentially no response due to the treatment (0.42, 0.71 and 0.02 in Reeves, Young and DH lines, respectively). This was consistent with knowledge that total spikelet number is set by Zadoks stage 31 (First node detectable on the main stem) (Bennett et al. 1973; Rawson & Evans 1970) which was long before the stage for heat treatment. By contrast, the number of spikelets classified as under-developed was decreased by the heat treatment, in Reeves (12%), Young (35%) and the DH lines (35%). As a consequence, the number of spikelets per spike classified as developed

increased. Million F. Erena (personal communication) also observed that heat treatment at the booting stage decreased the number of under-developed spikelets per spike in the Drysdale x Waagan population. Therefore, it appears that heat treatment at this stage can increase the development status of spikelets located at the base of the spike, by maturity.

6.3.2.2 Responses to treatments at 10 DAA

Duration of developmental processes

Heat treatment at 10 DAA significantly shortened the period from anthesis to flag leaf senescence in Reeves and the DH lines. Reeves and the DH lines reached the flag leaf senescence stage at 45 and 48 days after anthesis, respectively, and the heat treatment reduced this interval by 11.42 and 5.59 days, respectively (Table 6.4). It is interesting to note that heat produced the opposite effect on plants when treated at 6 cm AI (delaying senescence rather than accelerating it).

Chlorophyll content

The heat treatment decreased chlorophyll content significantly in the DH lines by just after heat treatment (SPAD13 DAA), by 3.8%, and decreased it by 17.9% by two weeks after heat treatment (SPAD27 DAA) (Table 6.4).

Traits measured at maturity, including grain weight

Reeves and the DH lines took an average of 116 days to reach maturity under heat conditions which was four days earlier than under control conditions. The period from anthesis to maturity was also decreased by four days in Reeves and the DH lines.

Heat reduced single grain weight by three mg (4.7% reduction) in the DH lines (Table 6.4). On average, single grain weight was 3.5% (~2 mg) lower in the heat-treated plants than in the control plants for Reeves, and the reduction was 13% (~6 mg) in Young. Shirdelmoghanloo (2015) found that the same heat treatment protocol produced a 3.0% loss of single grain weight in Young and 25.0% reduction in Reeves. Hence, it seems that the relative tolerance of the parental lines may have been reversed in the current experiment relative to that were recorded by

Shirdelmoghanloo (2015). The reason for this difference was not known, but may have had something to do with the hot conditions experienced in the greenhouse during the current study.

Table 6.4 Trait responses for heat treatments at 6 cm AI or 10 DAA. Responses are average percent differences in heat vs. control (based on raw means), for the two parents and across all the doubled haploids (DH).

Treatments at 6 cm AI			
Trait	**Reeves**	**Young**	**DH**
Duration of developmental processes (d)			
AIToAwnEm	4.23±3.27	-16.39±6.03	-10.85±2.07***
SowToAI	-0.91±1.27	-1.52±1.31	0.32±0.29
AIToFLSen	2.52±7.15	11.33±5.91	6.96±1.42*
Traits measured at maturity			
Final organ length, or gain in organ length from the commencement of treatment (cm)			
AIToMat	2.74±2.06	0.16±0.80	1.90±0.64
AwnLMat	-8.88±3.31	1.00±4.28	1.30±1.10
CulmLMat	-4.28±5.37	-8.59±3.61*	-7.10±0.71***
PedLMat	13.18±4.96*	1.01±3.31	1.28±1.01
ShootWMat	-1.28±7.93	-3.50±6.14	0.90±1.24
SpkLMat	-2.98±2.11	0.58±2.22	2.49±0.61*
Grain and spikelet number			
GnNoSpk1&2	-56.34±7.03***	-30.56±12.17	-29.68±3.10***
GnNoSpk>2	31.35±19.67	95.65±61.11	40.49±10.68*
GnNoSpk	-37.37±5.45***	-9.59±13.35	-13.57±4.43***
GnNoSpklt1&2	-58.22±7.16***	-38.00±10.06*	-38.08±2.43***
GnNoSpklt>2	19.22±16.56	74.51±52.01	24.47±9.16
BotGnNoSpklt1&2	-29.49±8.16***	-6.03±33.29	-16.82±4.55***
BotGnNoSpklt>2	83.00±18.43**	63.89±48.99	12.15±5.57
BotGnNo1&2	-32.96±8.12***	-20.60±26.50	-27.22±3.83***
BotGnNo>2	70.81±16.70***	40.56±38.62	1.29±4.90*
MidGnNoSpklt1&2	-72.06±7.42***	-34.55±13.36*	-36.80±2.56***
MidGnNoSpklt>2	-23.01±10.97	31.50±25.47	-2.71±5.03
MidGnNo>2	-17.03±10.94	42.00±30.72	7.38±5.94
MidGnNo1&2	-70.56±8.24***	-27.05±15.35	-29.32±3.16***
TopGnNoSpklt1&2	-64.55±12.62***	-35.61±9.52*	-45.11±4.05***
TopGnNoSpklt>2	-100.00±0.00	-20.42±20.42	-10.79±9.59*
TopGnNo>2	-100.00±0.00	-16.67±26.35	-6.10±9.78**
TopGnNo1&2	-63.87±12.62***	-30.77±11.15	-38.68±4.65***
GnNoSpklt	182.50±9.23***	234.70±8.85***	210.40±2.36***
BotSpkltNo	8.06±4.31	14.39±4.65*	14.19±1.02***
MidSpkltNo	9.84±5.58	8.03±6.28	11.36±1.01***
TopSpkltNo	10.36±5.34	5.76±3.71	11.52±0.92***
DevSpkltSpk	9.26±4.75*	8.73±4.02*	11.89±0.89***

Table 6.4 continued..

Treatments at 10 DAA			
Trait	**Reeves**	**Young**	**DH**
UndDevSpkltSpik	-21.53±12.71*	-31.69±9.59**	-26.46±2.06***
DaysToMat	1.12±1.14	-0.51±0.87	0.92±0.34
LowInternMat	-16.84±6.51*	-18.31±5.19**	-12.34±1.00***
SowToAwnEm	-0.49±1.29	-2.93±1.12*	-1.55±0.31*
TotSpkltSpik	2.25±2.29	-3.82±2.83	0.52±0.44
Duration of developmental processes (d)			
SowToAnth	0.83±0.89	1.19±1.31	0.57±0.27
AnthToFLSen	-20.49±5.73*	-3.89±6.39	-5.99±1.76***
Traits measured before and after heat treatment			
CulmL10	9.89±8.01	-5.03±5.09	0.26±0.78
PedL10 DAA	5.82±6.45	-6.65±3.55	0.24±0.98
SPAD10 DAA	-1.70±2.43	1.35±2.61	0.65±0.35
SPAD13 DAA	-8.31±2.54	-1.65±1.71	-4.51±0.49***
SPAD10To13	-41.25±123.20	-432.90±315.90	-120.00±38.43***
SPAD27 DAA	-14.98±10.16	-5.31±5.51	39.38±30.97***
SPAD10To27	-416.10±231.60	164.10±243.80	-64.57±135.20***
SPAD13 DAATo27DAA	-984.20±920.90**	-157.40±145.80	142.30±140.10***
Traits measured at maturity			
AnthToMat	-7.02±3.21	-1.71±3.25	-5.63±0.89***
CulmLMat	11.17±8.63	-5.60±5.05	0.58±0.78
PedLMat	6.65±6.57**	-6.56±3.49*	0.68±1.03
ShootWMat	-2.01±6.87	-7.20±5.84	1.25±1.22
CulmL_10 DAAToMat	52.83±41.30*	-11.61±7.01	4.11±1.42
GWSpk	-2.11±8.51	-7.77±17.06	17.52±12.58
SingGW	-3.56±4.53	-13.06±8.64	-4.72±1.12***
DevSpklt	-1.97±3.33	-3.79±3.98	0.39±0.66
LowIntern10ToMat	429.70±417.20	-15.83±10.67	10.82±3.01
PedL10ToMat	22.55±11.23	-4.78±5.11	3.67±1.57
DaysToMat	-3.20±1.58	-0.34±1.53	-2.94±0.40***
GNoSpk	1.94±8.00	-0.16±17.87	18.02±9.31
GNoSpklt	4.98±7.66	-1.39±16.89	16.25±8.45
LowInternMat	15.32±10.97	-3.57±8.05	2.37±1.12
CulmLMat_PedLmat_ratior	3.66±2.81	0.75±3.42	2.27±0.73
Shoot_wt_length_ratior	13.95±7.24	3.63±4.34	3.01±0.88

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

Table 6.5 Trait raw mean ± standard error (SE) for heat treatments at 6 cm AI and 10 DAA, for the two parents and across all the doubled haploids (DH), under control and heat conditions. A common set of control plants was used for comparison to the plants treated at the different stages.

Treatment at 6 cm AI						
	Reeves		**Young**		**DH**	
Trait Genotype	**Control**	**Heat**	**Control**	**Heat**	**Control**	**Heat**
Duration of developmental processes (d)						
AIToAwnEm	5.50±0.23	5.67±0.14	4.17±0.30	3.42±0.31	4.83±0.08	3.91±0.06
SowToAI	49.33±0.53	48.83±0.44	46.42±0.40	45.67±0.40	49.35±0.32	49.41±0.32
AIToFLSen	67.83±2.54	68.55±4.25	67.25±2.00	74.00±2.93	64.86±0.68	67.04±0.66
Traits measured at maturity						
Final organ length, or gain in organ length from the commencement of treatment (cm)						
AIToMat	71.17±1.13	72.92±0.95	73.58±0.83	73.67±0.74	70.52±0.34	71.28±0.32
AwnLMat	4.90±0.18	4.46±0.21	6.11±0.18	6.08±0.20	5.30±0.05	5.22±0.05
CulmLMat	82.85±4.79	77.23±2.21	66.24±1.64	60.15±2.03	73.33±0.85	67.02±0.73
PedLMat	34.91±1.39	38.75±0.83	34.70±0.37	34.98±1.01	32.35±0.33	31.94±0.30
ShootWMat	1.58±0.09	1.50±0.07	0.97±0.04	0.92±0.04	1.21±0.02	1.17±0.02
SpkLMat	8.58±0.12	8.32±0.17	8.71±0.23	8.72±0.15	8.69±0.05	8.85±0.06
Grain and spikelet number						
GnNoSpk1&2	31.67±1.09	13.33±1.84	22.58±2.02	16.27±2.97	26.28±0.39	16.71±0.48
GnNoSpk>2	8.92±1.17	11.25±1.10	6.50±1.08	9.55±1.55	7.01±0.26	7.94±0.30
GnNoSpk	40.58±2.17	24.58±1.59	29.08±3.02	25.82±4.29	33.28±0.60	24.54±0.69
GnNoSpklt1&2	1.75±0.05	0.70±0.11	1.59±0.13	1.05±0.19	1.63±0.02	0.94±0.03
GnNoSpklt>2	0.49±0.06	0.57±0.05	0.45±0.07	0.62±0.09	0.42±0.01	0.43±0.01
BotGnNoSpklt1&2	1.83±0.07	1.19±0.13	1.37±0.19	0.84±0.19	1.59±0.03	1.07±0.03
BotGnNoSpklt>2	0.54±0.09	1.01±0.09	0.51±0.10	0.75±0.16	0.50±0.02	0.51±0.02
BotGnNo1&2	10.50±0.47	7.17±0.71	5.92±0.91	4.18±0.94	7.97±0.15	6.00±0.18
BotGnNo>2	3.08±0.54	6.25±0.63	2.25±0.46	3.64±0.79	2.55±0.10	2.85±0.11
MidGnNoSpklt1&2	1.93±0.03	0.55±0.15	1.74±0.12	1.15±0.24	1.80±0.02	1.07±0.03

Table 6.5 continued..

Treatment at 6 cm AI						
	Reeves		**Young**		**DH**	
Trait Genotype	**Control**	**Heat**	**Control**	**Heat**	**Control**	**Heat**
Grain and spikelet number						
MidGnNo>2	5.25±0.55	4.75±0.66	3.33±0.48	4.18±0.90	3.71±0.13	3.99±0.15
MidGnNo&2	11.58±0.36	3.42±0.92	8.33±0.72	5.91±1.27	9.73±0.14	6.39±0.19
TopGnNoSpklt1&2	1.52±0.11	0.43±0.11	1.64±0.10	1.13±0.18	1.49±0.02	0.69±0.03
TopGnNoSpklt>2	0.09±0.04	0.04±0.02	0.18±0.07	0.31±0.07	0.12±0.01	0.15±0.01
TopGnNo>2	0.58±0.26	0.25±0.13	0.92±0.34	1.73±0.41	0.75±0.06	1.01±0.08
TopGnNo1&2	9.58±0.68	2.75±0.74	8.33±0.51	6.18±1.03	8.58±0.16	4.33±0.18
GnNoSpklt	2.24±0.11	1.28±0.10	2.05±0.19	1.66±0.26	2.05±0.03	1.37±0.03
BotSpkltNo	5.75±0.13	6.17±0.17	4.25±0.18	4.82±0.12	4.98±0.04	5.58±0.05
MidSpkltNo	6.00±0.17	6.50±0.20	4.75±0.18	5.00±0.14	5.41±0.04	5.92±0.05
TopSpkltNo	6.33±0.19	6.92±0.23	5.08±0.08	5.36±0.15	5.69±0.04	6.25±0.05
DevSpkltSpk	18.08±0.43	19.58±0.54	14.08±0.40	15.18±0.33	16.07±0.13	17.75±0.15
UndDevSpklt	3.75±0.33	2.67±0.38	5.17±0.41	3.36±0.36	4.73±0.10	3.07±0.07
Treatment at 10 DAA						
Trait	**Reeves**		**Young**		**DH**	
	Control	**Heat**	**Control**	**Heat**	**Control**	**Heat**
Duration of developmental processes (d)						
SowToAnth	60.25±0.49	60.75±0.74	57.75±0.61	58.42±0.87	60.39±0.33	60.65±0.33
AnthToFLSen	56.92±2.49	45.50±3.98	55.92±1.86	53.58±3.62	53.76±0.67	48.17±0.72
Traits measured before and after heat treatment						
CulmL10	77.11±4.28	81.65±2.38	61.34±1.66	57.48±2.10	68.21±0.78	67.19±0.79
LowInternL10	44.25±3.20	47.60±1.75	28.87±1.60	27.18±1.54	37.77±0.63	37.55±0.64
PedL10 DAA	32.85±1.27	34.28±1.05	32.47±0.36	30.30±1.16	30.38±0.31	29.76±0.32
SPAD10 DAA	47.37±0.96	46.35±0.64	47.25±0.86	47.72±0.89	44.96±0.16	45.13±0.16
SPAD13 DAA	47.39±0.80	43.30±0.89	47.82±0.78	46.98±0.89	45.56±0.15	43.42±0.21
SPAD27 DAA	44.92±1.49	37.39±4.26	47.14±0.93	44.48±2.58	42.73±0.39	37.04±0.61
SPAD10To13	0.03±0.41	-3.05±0.65	0.57±0.42	-0.75±0.34	0.60±0.08	-1.70±0.17
SPAD10To27	-2.45±1.02	-8.96±4.01	-0.11±0.65	-3.24±2.65	-2.24±0.37	-8.09±0.60
SPAD13 DAATo27DAA	-2.48±1.02	-5.91±3.98	-0.68±0.61	-2.49±2.71	-2.84±0.36	-6.41±0.55

Table 6.5 continued..

Treatment at 10 DAA						
Trait	**Reeves**		**Young**		**DH**	
	Control	**Heat**	**Control**	**Heat**	**Control**	**Heat**
Traits measured at maturity						
AnthToMat	60.25±1.04	55.75±1.36	62.25±0.65	61.17±2.09	59.46±0.34	55.40±0.40
CulmLMat	82.85±4.79	88.35±2.49	66.24±1.64	61.77±2.37	73.33±0.85	72.64±0.86
PedLMat	34.91±1.39	36.68±1.16	34.70±0.37	32.41±1.23	32.35±0.33	31.86±0.34
ShootWMat	1.58±0.09	1.50±0.08	0.97±0.04	0.88±0.04	1.21±0.02	1.18±0.02
CulmL10 DAAToMat	5.74±0.56	6.69±0.21	4.90±0.17	4.30±0.34	5.37±0.07	5.39±0.08
GWSpk	1.73±0.07	1.64±0.11	1.02±0.13	0.74±0.11	1.22±0.03	1.17±0.02
SingGW	43.27±1.60	41.38±1.88	35.63±2.22	29.49±2.98	37.65±0.38	34.76±0.34
DevSpklt	18.25±0.35	17.82±0.42	14.08±0.40	13.42±0.38	16.09±0.13	15.96±0.13
LowIntern10ToMat	3.68±0.45	4.30±0.24	2.68±0.17	2.1±0.28	3.32±0.07	3.38±0.07
PedL10ToMat	2.06±0.14	2.40±0.14	2.23±0.05	2.11±0.11	2.06±0.03	2.03±0.03
DaysToMat	120.50±1.20	116.50±1.36	120.00±0.92	119.60±2.04	120.00±0.39	116.10±0.48
LowInternMat	47.94±3.58	51.90±1.84	31.54±1.62	29.37±1.73	40.98±0.69	40.97±0.70
CulmLMat_PedLmat_ratio	2.34±0.06	2.42±0.06	1.91±0.05	1.92±0.06	2.31±0.02	2.34±0.03
Shoot_wt_length_ratio	75.13±4.98	60.38±2.68	68.93±2.32	70.74±2.53	62.86±0.55	63.52±0.51
UnderdevSpklt	3.67±0.28	4.18±0.35	5.17±0.41	5.17±0.35	4.73±0.10	4.79±0.11

6.3.3 QTLs detected for heat tolerance and *per se* traits

The 13 QTL regions detected are summarized in Table 6.5 and described in the following sections.

QTL1

This was identified for single grain weight (SingGW) *per se* under control conditions, on chromosome 1B (58.98 cM). The positive allele was from Young and the locus contributed 6% of the phenotypic variability for this trait.

QTL 2

This was identified for awn length at maturity (AwnLMat) *per* se under heat when heat treated at 6 cm AI. The closest marker was at the 0.00 cM position on chromosome 1D. The positive allele came from Young and the locus explained 6.43% of the variability.

QTL 3

Two QTL effects were identified in this region of chromosome 2B, with peak markers at 83-87 cM, for under-developed spikelets per spike (UnderdevSpklt) *per se* under heat, and for floret fertility at the lower two floret positions in the spikelets from the middle third of spike (MidGnNoSpklt1&2). The high value allele for the UnderdevSpklt effect was contributed by Young and the effect explained 22% of the phenotypic variation. The QTL for MidGnNoSpklt1&2 explained 8.68 % of the phenotypic variance, with the tolerance allele coming from Young. The peak marker for the MidGnNoSpklt1&2 tolerance effect (*1205884|F|0--37:C>T(C)*) was located on the scaffold 57860 in the IWGSC wheat genome reference sequence. A much stronger QTL effect for heat induced floret sterility tolerance (explaining 49% of the variance) has been identified in a Drysdale x Waagan mapping population (Million F. Erena, personal communication) with the peak marker (*wsnp_JD_c3732_4781170*) located on the same scaffold 57860, suggesting an effect of the same gene was detected in the two populations. The pedigrees of the Australian varieties Waagan and Drysdale have no strong affinity to those of Young or Reeves, suggesting that variation for the same 2B floret fertility QTL may be present across a diverse range of Australian varieties. The genetic association of the tolerance effect from Young

with a greater number of underdeveloped spikelets after heat, suggests that a common process may provide both floret fertility tolerance and inhibition of development of the lower spikelets on the spike under heat.

QTL4

Markers located at 26.0 to 26.94 cM on linkage group 3B2 affected both the duration of senescence at latter tiller developmental stages *per se* (AIToFLSen, control and heat, and AIToFLSen, control), and culm length *per se* at maturity (CulmMat and LowinternMat, heat and control) (Table 6.5). The allele from Young shortened the period from target AI stage or 10DAA to flag leaf senescence, and resulted in longer culms. These effects explained between 4.5 and 6.7% of the variation in the corresponding traits.

There was also a QTL effect with a peak at 9.9 cM (which may or may not have been due to the same gene controlling the aforementioned traits) for single grain weight at maturity (SingGW), observed only under heat conditions (treatment at 10 DAA), with the positive allele coming from Young, and explaining 6.7% of the variance. As this effect was only found under heat conditions, it raises the possibility it may represent a weak effect for grain filling heat tolerance. If this is true, then the tolerance for grain filling seemed to be associated with accelerated senescence – rather than slower senescence, as found at the 3B locus described by Shirdelmoghanloo, Lohraseb, et al. (2016) in the study of the Drysdale x Waagan mapping population.

The chromosome 3B grain filling heat tolerance locus (*QHsgw.aww-3B*) identified by Shirdelmoghanloo, Taylor, et al. (2016) was located on the tip of the short arm, and explained 11-20% of the phenotypic variability. BLAST searches of the closest markers against scaffolds from the IWGSC reference sequence V4 located *QHsgw.aww-3B* at 1-21 cM. However, markers closest to the SingGW effect of QTL4 were located in scaffolds at ~105.5 cM in IWGSC reference sequence V4. Therefore, the QTL effects detected in the two populations seemed to be due to different genes, located approximately 100 cM apart on chromosome 3B.

QTL5

This region on chromosome 4A showed two QTL effects related to the magnitude of heat accelerated flag leaf senescence, with Reeves contributing the positive allele for tolerance (stability). The locus explained ~7% of the phenotypic variances. The traits concerned were the period between reaching 6 cM AI and flag leaf senescence (AIToFLSen), and the duration between anthesis and flag leaf senescence (AnthToFLSen), identified with the treatments at 6 cm AI and 10 DAA, respectively. The effects had peak markers located at 75.46 cM to 81.75 cM, respectively.

QTL6

A QTL for grain number per spikelet at floret position above two (GnNoSpklt>2) was identified under heat, on chromosome 4A, with a peak marker at 155.63 cM. It explained 7.43% of the variability and Young contributed the positive allele. As this effect was detected only under heat, it may represent a weak tolerance effect.

QTL7

Twenty five QTL effects for heat tolerance and *per* se traits were identified in this region, with peak markers at 30.92 to 39.56 cM on chromosome 4B (Table 6.5). The *Rht-B1* gene was mapped at 38.62 cM, and this population segregated for height at *Rht-B1*, hence it seems all/most of these effects were likely to be due to *Rht-B1* segregation. The Reeves allele (representing the tall marker allele), as expected, was positive for height-related and shoot weight traits *per se*. As for the QTL4 on 3B, the tall allele was also associated with a shorter period between anthesis or the target AI and complete senescence. The tall allele was associated with greater SingGW at maturity *per se*, as was the case at the *Rht-B1* and *Rht-D1* loci in the study of Shirdelmoghanloo, Cozzolino, et al. (2016). The tall allele was also associated with longer AILToAwnEm and shorter LowinternL0 *per se*, which was again probably a consequence of the height effect.

The tall allele also provided tolerance (stability) for peduncle length at maturity (PedLMat, 10DAA treatment), shoot weight at maturity (ShootWMat, 10DAA treatment), peduncle growth from 10 DAA to maturity (PedL10ToMat, 10DAA treatment) and for the duration of the period between reaching the target auricle interval and awn emergence (AIToAwnEm, 6 cm AI treatment). These

'tolerance' effects were probably the result of artefacts caused by the large effects of *Rht-B1* on development, i.e. by altering whether these organs were at a more or less sensitive stage to the heat effects, at the given target AI.

QTL8

One tolerance and sixteen *per se* QTLs effect were identified with peak markers at 166.78 to 178.54 cM on chromosome 5A. The *Vrn-A1* gene, which was known to segregate for functionally contrasting alleles in this population, was mapped at 178.54 cM. Hence it appears likely that these observed QTL effects were all due to *Vrn-A1* segregation. The winter allele from Reeves, as expected, delayed the period from sowing to flowering stage (SowToAnth, SowToAwnEm and SowToAI). The locus also showed a range of effects on spikelet number, spikelet development status, spikelet fertility and organ length/width, which also seemed consistent with developmental effects expected from *Vrn-A1* segregation.

The heat tolerance QTL was observed here for peduncle growth from 10 DAA to maturity (PedL10ToMat; 10 DAA), with tolerance (stability) coming from Young. This locus explained ~19% of the phenotypic variation for the respective traits. As for tolerance effects at *Rht-B1*, this tolerance effect might represent a staging artefact.

QTL9

QTLs for UnderdevSpklt and PedMat were identified on chromosome 5D in control plants, with closest markers at 27.64 and 14.27 cM, respectively, and explaining 5-6 % of the variability. The *Vrn-D1* gene mapped close to these QTL peaks, at 33.86 cM, so one or both of these effects may have been due to *Vrn-D1* segregation. Consistent with this notion, the spring alleles at *Vrn-A1* (QTL8) and *Vrn-D1* (QTL9), from Young, were both associated with high values for PedMat and low values for UnderdevSpklt.

QTL10

Another QTL for floret fertility tolerance was detected on chromosome 6A, at 179.09 cM (for floret positions 1&2 in the top third of the spike; TopGnNoSpklt1&2), with the tolerance allele coming from Young. It only explained 6.3% of the phenotypic variance (Table 6.6).

QTL11

This effect on linkage group 6D2 (172.42 cM) was detected for fertility *per se* only under control conditions in the floret positions above 2 (GnNoSpklt>2, whole of spike), with the high fertility allele coming from Reeves.

QTL12

This QTL for AwnLMat in control plants was located at 140.29 cM on chromosome 7A and explained 8.59% of the phenotypic variability. The Young allele contributed longer awn length.

QTL13

This QTL on linkage group 7D1 was identified for grain number per spike (GnNoSpk) and was detected only in control plants. Young contributed the positive allele. It is unclear whether this effect derives from an effect on spikelet number per spike or grains per spikelet, as a QTL effect for neither of these component traits was detected at this position.

Table 6.6 Summary of QTLs detected in the Young × Reeves DH population. Linkage group, position (cM), closest marker, LOD score, percentage of explained variation (R^2), additive effect, and high value allele (Young, Y; Reeves, R) are presented. Conditional formatting colors were used to help visualization. The traits were mapped using the BLUPS, except those marked with (*). The latter showed distributions which were not amenable to modelling, so the raw means were used; ([a]): heat and control data were combined for these traits as they were measured before heat treatment.

QTL	Linkage group	Trait	Treatment	Treatment stage	Position (cM)	Closest marker	LOD	R^2	Additive effect	Allele
QTL1	1B	SingGW	C	10 DAA	58.98	*1051996|F|0--55:A>G(C)*	3.60	6.15	0.86	Y
QTL2	1D	AwnLMatH*	H	6 cm AI	0.00	1061093|F|0--18:G>A	3.68	6.43	0.21	Y
QTL3	2B	MidGnNoSpklt1&2	T	6 cm AI	86.59	*1205884|F|0--37:C>T(C)*	4.34	8.68	0.09	Y
	2B	UnderdevSpklt*	H	6 cm AI	82.83	1091546|F|0--48:T>G	11.03	22.26	0.67	Y
QTL4	3B2	AnthToFLSen*	C	10 DAA	26.47	1674039|F|0--22:G>C(C)	4.43	6.21	2.89	R
	3B2	AlToFLSen	C	6 cm AI	26.00	*2280402|F|0--52:G>A*	4.47	6.06	1.41	R
	3B2	AlToFLSen	H	6 cm AI	26.00	*2280402|F|0--52:G>A*	4.23	5.94	1.44	R
	3B2	CulmMat*	C	10 DAA	26.94	4009891|F|0--8:G>A(C)	7.65	4.48	3.72	Y
	3B2	LowinternMat*	C	10 DAA	26.94	4009891|F|0--8:G>A(C)	8.46	5.62	3.32	Y
	3B2	SingGW*	H	10 DAA	9.90	*1120374|F|0--15:A>G*	3.88	6.71	0.80	Y
	3B2	CulmMat*	H	10 DAA	26.94	4009891|F|0--8:G>A(C)	7.35	4.88	3.95	Y
	3B2	LowinternMat*	H	10 DAA	26.94	4009891|F|0--8:G>A(C)	8.92	6.71	3.70	Y
QTL5	4A	AlToFLSen	T	6 cm AI	75.46	*1093181|F|0--18:C>A*	4.23	7.45	0.39	R
	4A	AnthToFLSen	T	10 DAA	81.10	*1107438|F|0--36:C>T(C)*	4.14	7.22	0.18	R
QTL6	4A	GnNoSpklt>2	H	6 cm AI	155.63	*5411454|F|0--16:T>A(C)*	4.27	7.43	0.05	Y
QTL7	4B	CulmL10*[a]	C	10 DAA	38.62	*2371505|F|0--24:A>C*	54.41	68.73	12.34	R
	4B	ShootWMat	C	10 DAA	30.92	*C15P31*	15.24	34.92	0.00	R
	4B	CulmMat*	C	10 DAA	38.62	2371505|F|0--24:A>C(C)	56.97	66.66	14.32	R
	4B	GWSpk*	C	10 DAA	38.62	2371505|F|0--24:A>C(C)	14.96	26.44	0.26	R
	4B	LowinternMat*	C	10 DAA	38.62	2371505|F|0--24:A>C(C)	51.39	62.25	11.03	R
	4B	PedMat*	C	10 DAA	38.62	2371505|F|0--24:A>C(C)	19.66	26.08	3.35	R
	4B	AlToAwnEm*	C	6 cm AI	39.56	4010028|F|0--43:C>G(C)	7.41	13.46	0.49	R
	4B	ShootWMat	H	10 DAA	30.92	*C15P31*	15.24	34.92	0.16	R
	4B	CulmMat*	H	10 DAA	38.62	2371505|F|0--24:A>C(C)	51.38	63.39	14.23	R
	4B	GWSpk*	H	10 DAA	38.62	2371505|F|0--24:A>C(C)	12.34	22.19	0.21	R
	4B	LowinternMat*	H	10 DAA	38.62	2371505|F|0--24:A>C(C)	46.48	58.69	10.95	R
	4B	PedMat*	H	10 DAA	30.92	C15P31	17.66	32.48	3.86	R
	4B	AlLToAwnEm*	H	6 cm AI	38.62	2371505|F|0--24:A>C(C)	15.86	27.86	0.60	R
	4B	PedLMat	T	10 DAA	39.56	*4010028|F|0--43:C>G(C)*	4.50	7.87	0.29	R
	4B	ShootWMat	T	10 DAA	38.62	*2371505|F|0--24:A>C*	15.43	0.35	0.00	R
	4B	PedL10ToMat	T	10 DAA	38.62	*2371505|F|0--24:A>C*	6.05	8.62	1.46	R
	4B	AlToAwnEm	T	6 cm AI	38.62	*2371505|F|0--24:A>C(C)*	9.67	17.45	0.08	R

Table 6.6 continued..

QTL	Linkage group	Trait	Treatment	Treatment stage	Position (cM)	Closest marker	LOD	R^2	Additive effect	Allele
	4B	LowInternL10*[a]	C	10 DAA	38.62	*2371505|F|0--24:A>C*	20.60	34.77	2.21	Y
	4B	AnthToFLSen*	C	10 DAA	39.56	4010028|F|0--43:C>G(C)	12.78	21.45	5.37	Y
	4B	AnthToMat*	C	10 DAA	38.62	2371505|F|0--24:A>C(C)	7.44	13.55	2.02	Y
	4B	AIToFLSen	C	6 cm AI	39.56	*3958247|F|0--52:C>G*	13.96	23.15	2.76	Y
	4B	AnthToFlse*	H	10 DAA	39.56	4010028|F|0--43:C>G(C)	11.98	21.54	5.89	Y
	4B	AnthToMat*	H	10 DAA	38.62	2371505|F|0--24:A>C(C)	6.37	11.52	2.22	Y
	4B	AILToMat*	H	6 cm AI	38.62	2371505|F|0--24:A>C(C)	4.34	7.64	1.59	Y
	4B	AIToFLSen	H	6 cm AI	38.62	*2371505|F|0--24:A>C*	11.84	19.94	2.63	Y
QTL8	5A	SowToAnth*	C	10 DAA	178.54	2261632|F|0--9:A>C(C)	21.70	36.69	4.14	R
	5A	SowToAIL*	C	6 cm AI	178.54	2261632|F|0--9:A>C(C)	23.69	39.40	4.26	R
	5A	SowToAwnEm*	C	6 cm AI	178.54	2261632|F|0--9:A>C(C)	22.20	37.28	4.42	R
	5A	UnderdevSpklt*	C	6 cm AI	172.42	4992467|F|0--21:G>T(C)	11.72	20.57	0.85	R
	5A	SowToAnth*	H	10 DAA	178.54	2261632|F|0--9:A>C(C)	21.38	36.31	4.09	R
	5A	GnNoSpk	H	6 cm AI	172.42	*1220783|F|0--38:C>T*	4.52	8.09	6.92	R
	5A	SowToAwnEm*	H	6 cm AI	178.54	2261632|F|0--9:A>C(C)	23.41	38.80	4.36	R
	5A	SowToAI*	H	6 cm AI	178.54	2261632|F|0--9:A>C(C)	23.20	38.71	4.19	R
	5A	UnderdevSpklt*	H	6 cm AI	177.60	1135154|F|0--9:G>A(C)	4.34	6.25	0.36	R
	5A	FlagW	C	10 DAA	178.54	*1094478|F|0--31:G>A*	15.39	27.13	0.08	Y
	5A	FlagL*	C	10 DAA	177.60	1135154|F|0--9:G>A(C)	11.75	21.18	1.91	Y
	5A	PedMat*	C	10 DAA	178.54	2261632|F|0--9:A>C(C)	17.09	21.84	3.06	Y
	5A	GnNoSpklt	C	6 cm AI	166.78	*998276|F|0--28:G>A*	5.69	10.31	0.13	Y
	5A	AwnLMat*	C	6 cm AI	168.19	1074531|F|0--11:G>A(C)	6.35	10.78	0.28	Y
	5A	PedMat*	H	10 DAA	177.60	1135154|F|0--9:G>A(C)	15.09	20.63	3.07	Y
	5A	GnNoSpklt	H	6 cm AI	172.42	*1220783|F|0--38:C>T*	6.76	12.41	0.07	Y
	5A	PedL10ToMat	T	10 DAA	172.42	*1220783|F|0--38:C>T*	11.94	19.66	2.21	Y
QTL9	5D	UnderdevSpklt*	C	6 cm AI	27.64	C20P28	3.66	5.95	0.46	R
	5D	PedMat*	C	10 DAA	14.27	C20P14	3.85	4.82	1.44	Y
QTL10	6A	TopGnNoSpklt1&2	T	6 cm AI	179.09	*3023373|F|0--13:A>T(C)*	3.70	6.35	0.12	Y
QTL11	6D2	GnNoSpklt>2	C	6 cm AI	0.00	*1001843|F|0--17:G>A*	3.84	6.67	0.03	R
QTL12	7A	AwnLMat*	C	6 cm AI	140.29	4911242|F|0--20:T>G	5.24	8.59	0.25	Y
QTL13	7D1	GnNoSpk	C	6 cm AI	61.68	*1075787|F|0--25:C>T*	3.38	5.77	1.84	Y

6.4 Conclusions

- No heat tolerance QTLs for final grain size (SingGW) were detected. The grain filling tolerance was reversed in the parents relative to what was observed by Shirdelmoghanloo (2015) (3.5% and 13% reduction in SingGW in Reeves and Young, respectively, contrasting with 25% and 3.0%, in the previous study). The high temperatures experienced by the plants in the greenhouse in the current experiment may partly explain this reversal in tolerance of the parents, and the lack of any SingGW tolerance QTL detected.
- Two floret fertility heat tolerance QTL were detected, on chromosomes 2B (QTL3) and 6A (QTL10), but they explained a relatively low amount of the phenotypic variation (8.68% and 6.35%). QTL2 was located at a similar genomic location to a floret fertility tolerance for grain number per spike (GrNoSpk), and explained 49% of the variance in the Drysdale x Waagan mapping population. The heat treatment in the current experiment had a large impact on floret fertility (e.g., 65% in Reeves and 50% in Young), hence the high temperatures experienced in the greenhouse during this experiment do not easily explain why the effect of the 2B locus was relatively weak compared to that seen in the Drysdale x Waagan population. It is possible that the two populations segregate for different pairs of alleles for this gene.
- QTL4 for SingGW *per se* under heat conditions, on chromosome 3B, was located 100 cM away from the 3B SingGW heat tolerance QTL that had been identified in the Drysdale x Waagan population, and therefore appears to represent a different gene.
- QTL5 on chromosome 4A affected tolerance for the rate of senescence from the AI target stage or from anthesis (i.e. affected the degree of heat-enhanced senescence).
- Heat tolerance QTLs for various developmental process were detected on chromosomes 4B and 5A, but it seemed likely these were due to effects of segregation at the major phenology loci *Rht-B1* and *Vrn-A1*, respectively.

Chapter 7: Genetic variation for grain filling and floret fertility response to brief heat stress treatments in 21 Australian varieties of hexaploid wheat (*Triticum aestivum* L.)

7.1 Introduction

Australia is one of the highest wheat producing countries in the world (10th highest; FAOSTAT). Across the Australian wheat belt, exposure of wheat to high temperature causes yield reduction due to accelerated leaf senescence (Asseng, Foster & Turner 2011). Heat (>20 °C) accelerates grain filling rate (by 1.32 to 1.67 mg grain^{-1} d^{-1}) but shortens grain filling duration (by 30% or more) (Stone et al. 1995; Wardlaw, Sofield & Cartwright 1980), resulting in reduced grain weight.

High temperature at around meiosis results in failed grain set due to heat induced female sterility and damage to the anthers. It can also cause complete sterility (Owen 1971; Saini & Aspinall 1982) when occurring during and after ear emergence. Moderately high temperatures (>20 °C) between spike initiation and anthesis can substantially reduce grain number per spike (Fischer, RA 1976; Warrington, Dunstone & Green 1977). Grain number per spike can decrease by 4% for every 1 °C (from 15-22 °C) increase during the 30 days before anthesis (Fischer, R 1985).

Shirdelmoghanloo, Cozzolino, et al. (2016) studied heat tolerance at grain filling stage in 37 varieties, mostly Australian, and observed that heat reduced single grain weight in 23 of the varieties. Million F Erena (PhD thesis: Genetic and Physiological Bases of Heat-Induced Floret Sterility in Wheat, unpublished) used 26 hexaploid wheat varieties (also mostly Australian) to study heat tolerance for floret fertility and observed that parental lines for seven existing mapping populations showed contrasting tolerance under heat treatment.

In the experiment described in the current chapter, 21 Australian wheat varieties were screened for heat tolerance for both grain filling and floret fertility. Young and Reeves were selected as they were parents of the mapping population described in Chapter 5. The rest of the varieties were selected to represent a selection of the Australian varieties that were most widely grown or promising for cultivation at around the time of the experiment in 2015. Most of these lines belonged to the quality categories APW (Baxter, Corack, Hydra, Kord, Magenta, Scout, Stiletto and Yitpi), APH (EGA_Gregory, Flanker, Spitfire, Suntop, Sunvale) and AH (Cobra, Emu Rock, Sceptre and Mace).

Table 7.1 Details of wheat genotypes.

Variety	Pedigree	Released by	Year of release
Baxter	INIA-66/GAMUT//COOK/4/JUPATECO/3/LERMA-ROJO-64/SONORA-64-A//(Lobell, Sibley & Ortiz-Monasterio)TIMGALEN	Dept. of Primary Industries QLD	1998
Calingiri	Chino/Kulin//Reeves	InterGrain	1997
Cobra	Westonia/Sentinel	LongReach Plant Breeders Pty Ltd.	2011
Corack	Wyalkatchem/Silverstar//Wyalkatchem	Australian Grain Technologies	2011
EGA Gregory	Pelsart/2*Batavia doubled haploid line	Dept. of Primary Industries QLD and Enterprise Grains Australia joint venture	2004
Emu Rock	Westonia/Kukri/Perenjori/Ajana	Intergrain	2011
Flanker	EGA Gregory//EGA Gregory/Lang	LongReach Plant Breeders Pty Ltd.	2014
Hydra	EGA Bonnie Rock/ Strzelecki	InterGrain	2015
Kord	Gladius'*2/4/Frame//Wild4/11A/3Sunmist	Australian Grain Technologies	2011
Mace	Wyalkatchem/ Stylet	Australian Grain Technologies	2008
Magenta	Carnamah/Tammin18	InterGrain	2007
Reeves	Bodallin//Gamenya/Inia 66	Western Australian Department of Agriculture	1989
Scepter	RAC1480/2*Mace	Australian Grain Technologies	2015
Scout	Sunstate/QH71-6//Yitpi	LongReach Plant Breeders Pty Ltd.	2009
Spitfire	Drysdale/Kukri	LongReach Plant Breeders Pty Ltd.	2011
Stiletto	Veranopolis/3*RAC177/2/3*Spear/3/Dagger	InterGrain	2000
Suntop	Sunco/2*Pastor//SUN436E	Australian Grain Technologies	2012
Sunvale	Cook*2/VPM1//3*Cook	Australian Grain Technologies	1993
Wyalkatchem	Machete/4/(W84–129*504) Gutha/3/Jacup*2//(11thISEPTON135) Iassul/H567–71	InterGrain	2001
Yitpi	C-8-MMC-8-HMM/Frame	SA Research and Development Institute	1999
Young	VPM1/3*Beulah//Silverstar	Australian Grain Technologies	2009

The findings of the experiment might allow varieties to be recommended to the breeders for use as parents for breeding programs for heat prone target environments; also varieties to

recommend to the farmers, if growing more heat tolerant varieties is desirable. The findings might also help to understand the correlation between grain filling and floret fertility heat tolerance traits.

7.2 Materials and Methods

7.2.1 Plant materials

Twenty-one Australian wheat varieties (Table 7.1) were used in this experiment to screen for heat tolerance for both grain filling and floret fertility. Seeds were sown on 28th August, 2015.

7.2.2 Experimental design, plant growth and heat stress conditions

This genotype screening was combined with the phenotyping experiment of Y x R DH mapping population. Plant growth conditions, treatment and experimental design were as described in paragraphs 6.2.6 and 6.2.7.

7.2.3 Data collection and analysis

Data for 24 traits were collected, when plants reached ~6 cm AI, at 10 DAA, after heat treatment and at maturity. Heat response was calculated as percent difference of heat vs. control. GenStat 18 (www.vsni.co.uk/downloads/genstat/) was used for analysis of variance. Detailed descriptions of the measured and derived traits are described in Table 5.2.

7.3 Results and discussion

7.3.1 Greenhouse conditions

The 6 cm AI target stage of the plants occurred between 5th October and 5th November, 2015. The sensitive stage for fertility effects of heat includes three days before and after the target stage (Nick Collins, personal communication) – some plants were exposed to one day above 30 ^{o}C during this sensitive developmental window (Fig. 7.1). Plants for the grain filling experiment reached the target stage (10 DAA) between 26th October and 28th November, 2015. The sensitive stage (five days before to 20 days after anthesis) was reached between 21st October and 18th

December, during which there were eight days above 30 °C (Fig. 7.1). The evaporative cooling system of the greenhouse was only able to cool the room by ~10 °C relative to the outside temperature. Outside temperature was very high during both sensitive stages (periods of >35 °C; Fig. 7.1) (www.bom.gov.au, Kent Town, weather station number 023090). Plants were therefore exposed to higher temperatures than intended in the greenhouse, around the sensitive stages in all plants, and during the target treatment stages in the control plants.

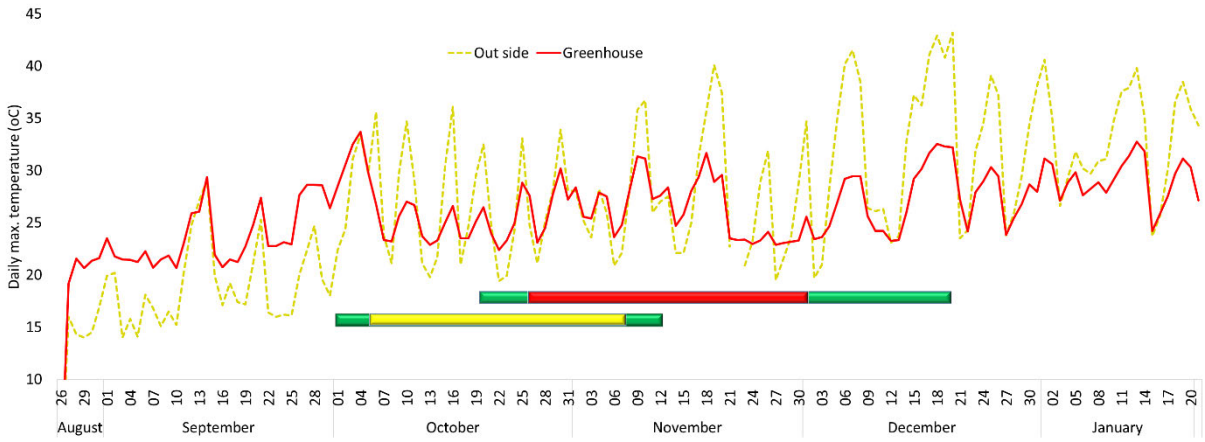


Fig. 7.1 Daily maximum temperature of greenhouse room 30 (red line) and outside (brown line) from sowing to maturity. Yellow and red bars indicate periods of reaching target stages for heat treatment for 6 cm AI (floret fertility effects) and 10 DAA (for grain filling effects), respectively. Green extended bars indicate estimated time when plants were at sensitive stages to the effects of heat on fertility or grain filling.

7.3.2 Traits established pre-heat

Significant genotypic variation for all the traits established before heat treatment (SowToAI, Sow to Anth, TotSpltSpk at 6cm AI, TotSpltSpk at 10 DAA, CulmL10 DAA, PedL10 DAA and SPAD10DAA) was observed (Table 7.2). Plants reached the target 6 cm AI stage (SowToAI) at an average of 53.97 days from sowing, with values ranging from 40 (Emu Rock) to 73 (Magenta) days. The time taken to reach anthesis after sowing (SowToAnth) averaged ~65 days. Genotypes reached this stage at variable duration, ranging from 53 to 76 days. Average total number of spikelets per spike (TotSpltSpk) was similar (~22) for plants treated at 6 cm AI or 10 DAA, supporting the notion that spikelet number was established prior to the heat treatment period. Variation among the genotypes were observed, ranging from ~14 to 31. Culm length averaged ~61 cm at 10 DAA

(CulmL10 DAA) and ranged between 38 to 81 cm. Average peduncle length at 10 DAA (PedL10 DAA) was ~28 cm and ranged from 17 to 33 cm. Average SPAD reading just prior to heat treatment (SPAD 10DAA) was 45.57, ranging between 41 and 49 between genotypes (Appendix).

Table 7.2 Mean of heat response (percent difference of heat vs. control) and P-values for genotype (G), treatment (T) and genotype x treatment (GxT) effects for plants heat treated at 6 cm AI and 10 DAA (and controls). *P*-values of <0.001, 0.01 and 0.05 are highlighted in gradients of green color.

Traits		***p*-value**		
Traits established pre-heat	**Mean of response**	**G**	**T**	**G x T**
SowToAI	0.31	<.001	0.930	0.550
Sow to Anth	0.36	<.001	0.591	0.483
TotSpltSpk (6cm AI)	0.323	<.001	0.579	0.681
TotSpltSpk (10 DAA)	-0.746	<.001	0.330	0.420
CulmL10 DAA	1.19	<.001	0.844	0.519
PedL10 DAA	3.14	<.001	0.938	0.600
SPAD10DAA	0.13	<.001	0.758	0.818
Proportion of spikelets that are underdeveloped				
PropSpltUndDev (6 cm AI)	-29.54	<.001	<.001	0.027
PropSpltUndDev (10 DAA)	22.39	<.001	0.090	0.681
Grains per spikelet				
BotGNoSpklt1&2	-32.67	<.001	<.001	0.010
MidGnNoSpklt1&2	-51.83	<.001	<.001	0.000
TopGnNoSpklt1&2	-49.48	<.001	<.001	0.000
BotGNoSpklt>2	21.06	<.001	<.001	0.730
MidGNoSpklt>2	-24.87	<.001	0.780	0.030
TopGnNoSpklt>2	-51.16	<.001	0.200	0.790
Duration of developmental processes				
AIToAwnEm (6 cm AI)	-12.49	<.001	<.001	0.070
SowToAwnEm (6cm AI)	-1.15	<.001	0.070	0.960
AIToFLSen (6 cm AI)	7.01	<.001	0.070	0.600
AnthToFLSen (10 DAA)	-3.02	<.001	0.004	0.805
AIToMat (6 cm AI)	2.84	<.001	0.050	0.450
AnthToMat (10 DAA)	-5.1	<.001	<.001	0.584
DaysToMat (6cm AI)	1.77	<.001	0.040	0.420
DaysToMat (10 DAA)	-2.46	<.001	<.001	0.524
Chlorophyll content				
SPAD13DAA	-6.67	<.001	<.001	0.047
SPAD27DAA	4.05	0.001	<.001	0.345

Table 7.2 continued..

Traits		*p*-value		
Traits established pre-heat	**Mean of response**	**G**	**T**	**G x T**
SingGW				
SingGW	-6.83	<.001	0.001	0.937
Organ final weight, final length or gain in length				
CulmLMat (6cm AI)	-5.8	<.001	<.001	0.100
CulmLMat (10 DAA)	0.8	<.001	0.960	0.587
PedLMat (6cm AI)	1.51	<.001	0.970	0.000
PedLMat (10 DAA)	1.31	<.001	0.918	0.675
SpikeLMat (6cm AI)	4.61	<.001	<.001	<.001
AwnLMat (6cm AI)	-0.48	<.001	0.390	0.930
ShootWMat (10 DAA)	-3.97	<.001	0.012	0.276
CulmL10 DAAToMat (10 DAA)	10.86	<.001	0.234	0.672
PedL10ToMat (10 DAA)	5.05	<.001	0.415	0.168

7.3.3 Proportion of spikelets that were underdeveloped

Significant treatment (T) and genotype by treatment (G x T) effects were observed for PropSpltUndDev (6 cm AI) in ANOVA for the treatment at 6 cm AI (Table 7.4). The trait value decreased in all genotypes. Wyalkatchem, Flanker, Scepter and Corack showed more than 60% decrease while Spitfire, Emu Rock and Suntop displayed less than 20% reduction in this trait under heat (Fig. 7.2). Therefore, the heat treatment somehow advanced the developmental status of these lower spikelets, by the time maturity was reached.

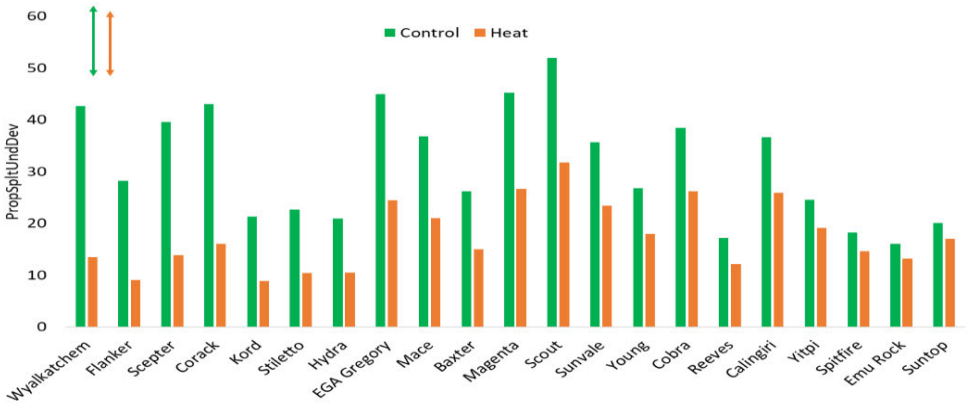


Fig. 7.2 Means of Proportion of spikelets that were underdeveloped, PropSpltUndDevat (6 cm AI) in each genotype for control (green columns) and heat-treated plants (brown columns). Genotypes are ordered by heat response (heat vs. control). The vertical arrows indicate LSD values (α = 0.05) for comparisons of means of control (green) and heat (red) across genotypes.

7.3.4 Grains per spikelet

TopGnNoSpklt1&2, MidGnNoSpklt1&2 and BotGnNoSpklt1&2

Under favorable conditions, wheat is expected to set close to two grains per spikelet at the lowest two floret positions in the spikelets (McMaster et al., 1992). In the control plants, around half the genotypes set an average of 1.8 grains or less at these positions, possibly due to the high temperatures experienced in the greenhouse (paragraph 6.3.1).

Grain number per spikelet at floret position 1&2 in the top, middle and bottom third of the spike was reduced significantly by heat treatment and genotype by treatment interaction was also observed for these traits. On average, 42, 57 and 55% reduction were observed at floret position 1&2 in top, middle and bottom part of the spike, respectively (Fig. 7.3A, B and C). Cobra, Wyalkatchem, Magenta, Suntop, Hydra, Emu Rock, Corack, Reeves and Spitfire, Wyalkatchem and Cobra showed the most reduction in seed number (70% or more) and could therefore be regarded as the most intolerant of the varieties. EGA Gregory and Scout maintained their floret fertility relatively well after heat treatment, and therefore appeared to be the most tolerant of the varieties.

TopGnNoSpklt>2, MidGnNoSpklt>2 and BotGnNoSpklt>2

Grain number per spikelet in the third and above floret positions was lowest in the top third of the spike (Fig. 7.3D, E and F). As previously observed by (Evans, Bingham & Roskams 1972), fertility was greatest in the middle of the spike for these >2 floret positions.

There were significant T or G x T effects for >2 floret positions only in some cases (Table 7.3). No genotypic differences in sterility appeared consistently over the three parts of the spike (Fig. 7.3D, E and F).

7.3.5 Duration of developmental processes

There were G effects for all these traits, but T effects for only six: AIToAwnEm and AIToMat (6 cm AI), AnthToFLSen and AnthToMat (10 DAA), DaysToMat (6cm AI and 10 DAA treatments). None of the traits showed G x T effects.

Heat accelerated awn emergence overall (AIToAwnEm; a trait measured only for the 6 cm AI treatment), and this increase was evident in 16 genotypes (Fig. 7.4A).

For the remaining traits, which described the rate at which maturity or complete flag leaf senescence was reached, the different heat treatments produced opposite effects (Table 7.3; Fig 7.4B, C, D, E, F); the heat treatment applied at 6 cm AI delayed senescence/maturity (AIToMat, DaysToMat, and AIToFLSen, although the latter was not statistically significant) while the heat treatment applied at 10 DAA acclerated senescence/maturity (AnthToFLSen, DaysToMat and AnthToMat). The overall effects were in the order of 2 to 5 days difference. These findings are consistent with observations by other authors that heat applied at grain filling shortened the grain filling duration (Stone et al. 1995; Wardlaw, Sofield & Cartwright 1980).

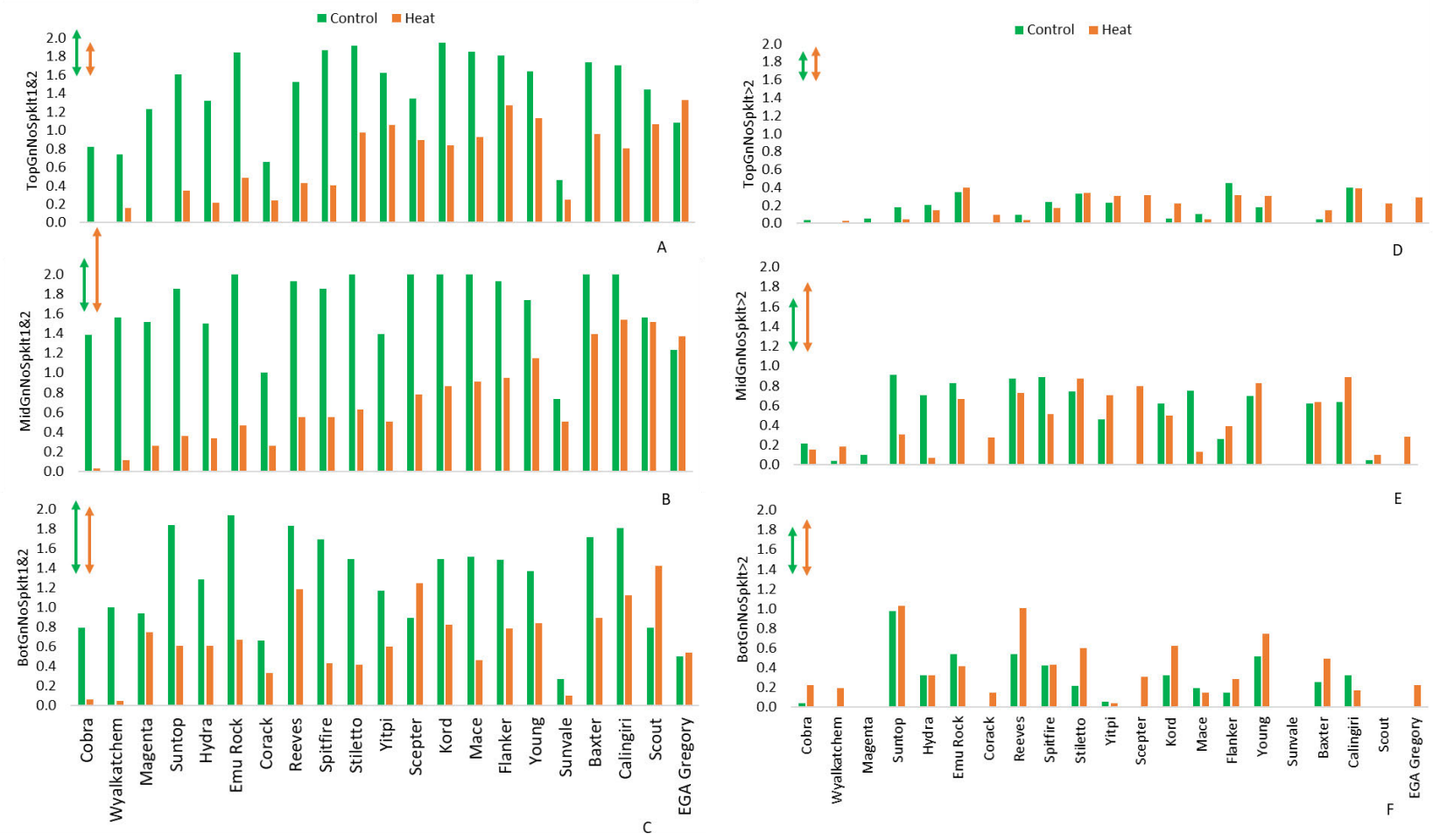


Fig. 7.3 Average grain number per spikelet in control (green) and heat-treated (brown) plants of hexaploid wheat genotypes, for the first and second floret positions in the spikelet at top (A), middle (B) and bottom (C) third of the spike. Similarly, D, E and F presents grain number per spikelet in the third and above floret positions at top, middle and bottom parts of the spike. Genotypes are ordered according to heat response of grains per spikelet at floret position 1&2 in the middle part of the spike. The vertical bars indicate the LSD values ($\alpha = 0.05$) for across genotype mean comparisons within control (green bar), or heat (brown bar).

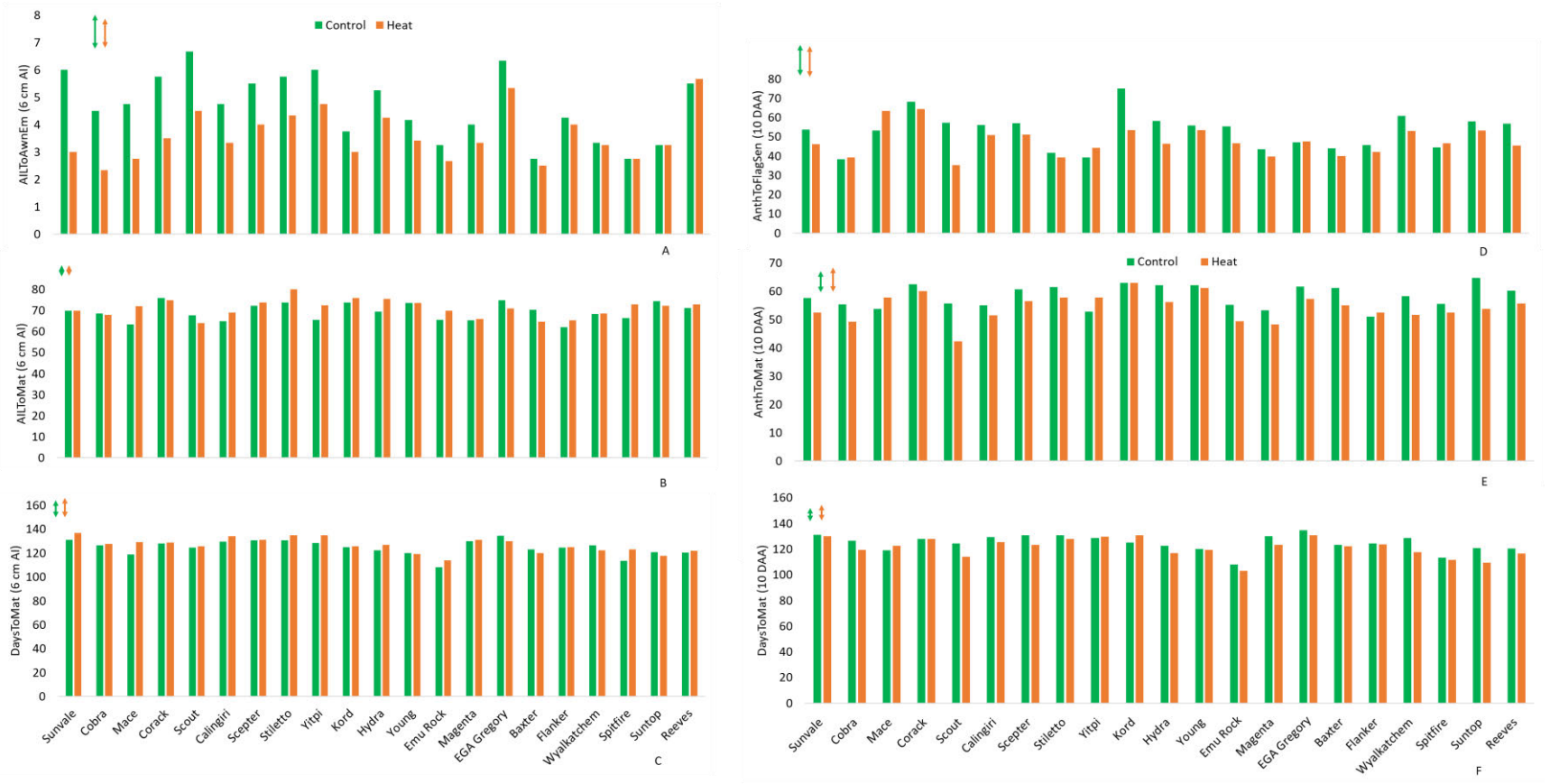


Fig. 7.4 Means of days from AIToAwnEm (6 cm AI) (A), AIToMat (6 cm AI) (B), DaysToMat (6cm AI) (C), AnthToMat (10 DAA) (D), AnthToFLSen (10 DAA) (E), and DaysToMat (10 DAA) (F) of each genotype for control (green column) and heat-treated plants (brown column). These six traits showed significant T effects but no G x T effects. The vertical arrows indicate LSD values ($\alpha = 0.05$) for comparisons of means of control (green) and heat (red) across genotypes. The genotypes are ordered according to heat response of AIToAwnEm.

7.3.6 Chlorophyll content (SPAD10 DAA, SPAD13 DAA and SPAD27 DAA)

Chlorophyll content just before heat treatment (SPAD10 DAA) differed significantly across the genotypes and averaged ~45 SPAD units. On average across all genotypes, control plants senesced (lost chlorophyll) in the two weeks after the time the heat-treated plants were removed from the heat chamber, and heat treatment reduced chlorophyll content during the heat treatment period and accelerated its loss in the two weeks after, relative to control plants (Fig. 7.5). A very similar pattern was observed by Shirdelmoghanloo, Lohraseb, et al. (2016) upon heat treating another set of wheat genotypes at 10 DAA.

In control plants, Cobra, Magenta, Baxter, Emu Rock and Yitpi showed chlorophyll decreases by 27DAA, but the other genotypes showed no appreciable senescence during this period (Fig. 7.6A). Chlorophyll decreased significantly due to the heat treatment by 13 DAA (just after heat treatment), by an average of 4 SPAD units. There were GxT effects at this time point. Yitpi and Scepter were the most stable (tolerant), while Calingiri exhibited the greatest loss (32%; Fig. 6.6A), followed by Cobra, Magenta, Sunvale, Corack and Reeves. Chlorophyll content decreased further by two weeks after heat treatment (27 DAA). GxT effects were insignificant at this point, although the means varied widely (Fig. 7.6B).

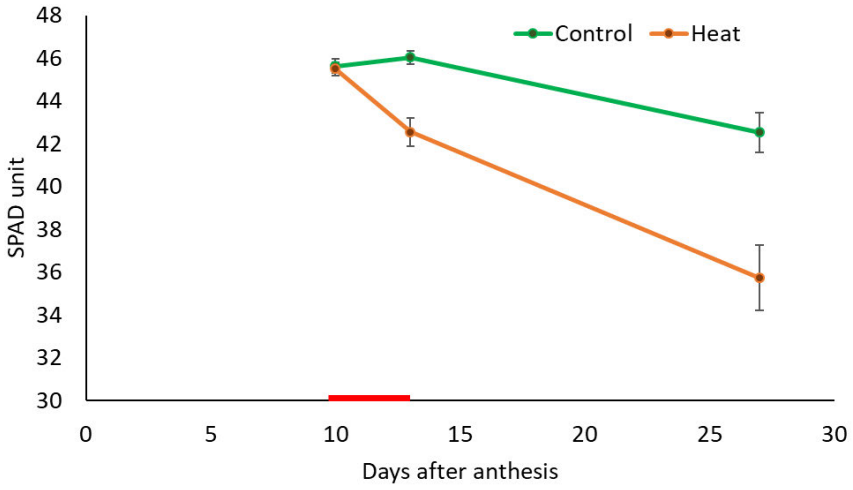


Fig. 7.5 Overall mean + SE of flag leaf chlorophyll content across all genotypes, measured at 10, 13 and 27 DAA in control (green line) and heat-treated plants (brown line). Red bar indicates time of heat treatment.

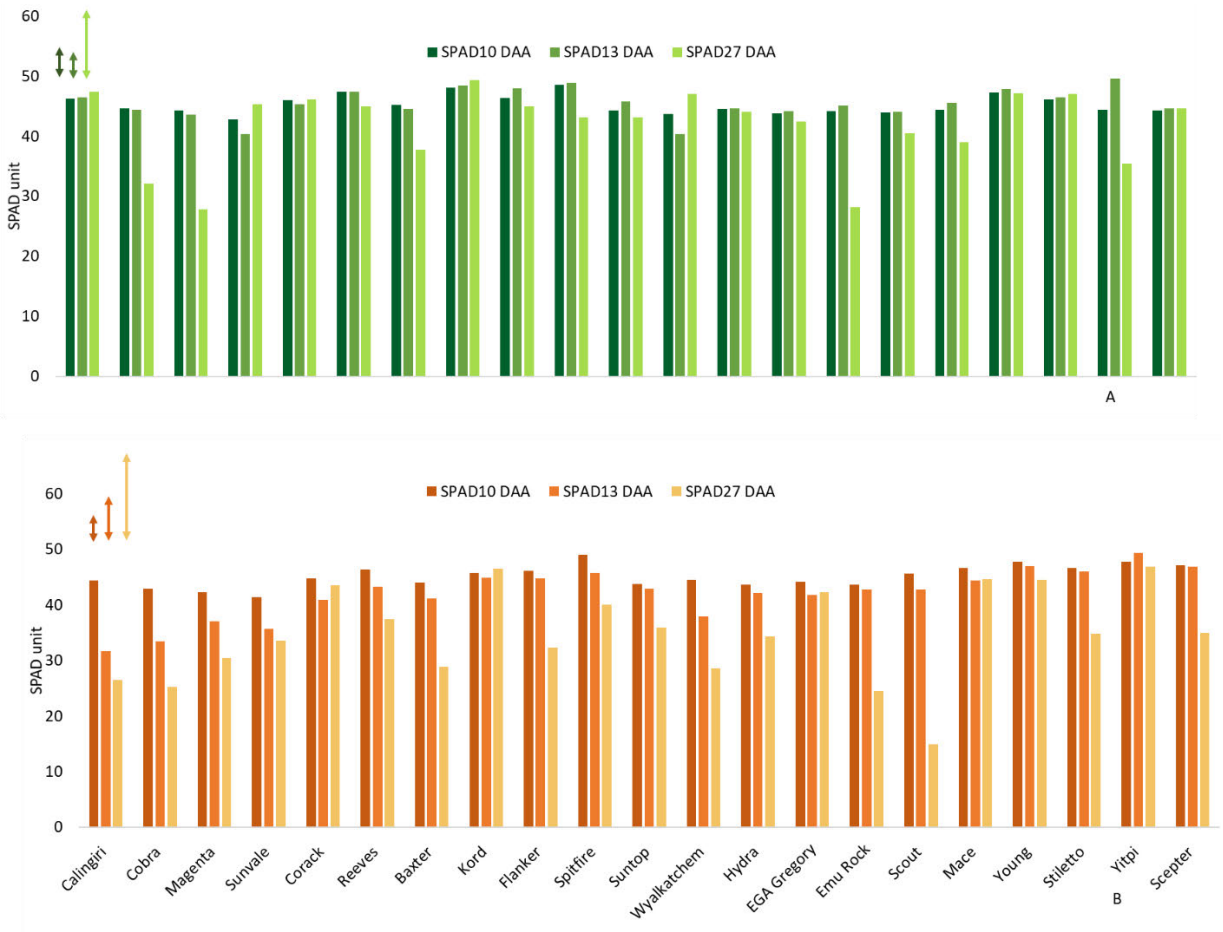


Fig. 7.6 Means of SPAD unit measured at 10, 13 and 27 DAA in each genotype for control (green columns) and heat-treated plants (brown columns). Genotypes are ordered by heat response (heat vs. control) at 13DAA. The vertical arrows indicate LSD values (α = 0.05) for comparisons of means of control (green) and heat (red) across genotypes.

7.3.7 SingGW

Significant G and T effects were observed for single grain weight. SingGW was reduced by 3.5 mg under heat treatment on average. There was no significant G x T effect but Sunvale, Young, Magenta, Suntop, Scout, Stiletto, Flanker and Calingiri appeared to be the least tolerant based on comparison of the means of heat vs. control (Fig. 7.7).

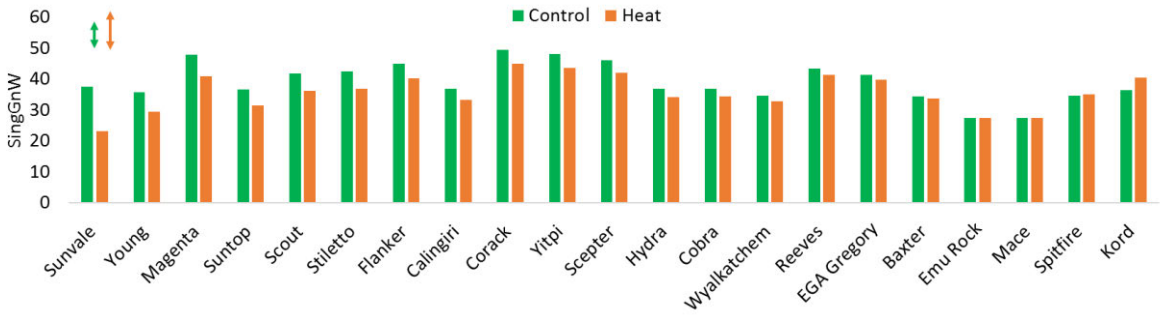


Fig. 7.7 Means single grain weight at 10 DAA of each genotype for control (green column) and heat-treated plants (brown column). The vertical arrows indicate LSD values (α = 0.05) for comparisons of means of control (green) and heat (red) across genotypes. Genotypes are ordered by heat response (heat vs. control).

7.3.8 Final organ weight and length

While all these traits showed significant G effects, only SpkLMat (6cm AI) showed both significant T and G x T effects. CulmLMat (6cm AI) and ShootWMat (10 DAA) showed only T effects but no G x T effect, whereas PedLMat (10 DAA) only showed G x T effect but no T effect.

CulmLMat (6cm AI)

Heat at 6 cm AI significantly reduced culm length, by an average of 5 cm with Yitpi, Flanker, Baxter, Spitfire, Hydra, Calingiri and Young showing the greatest responses (Fig. 7.8).

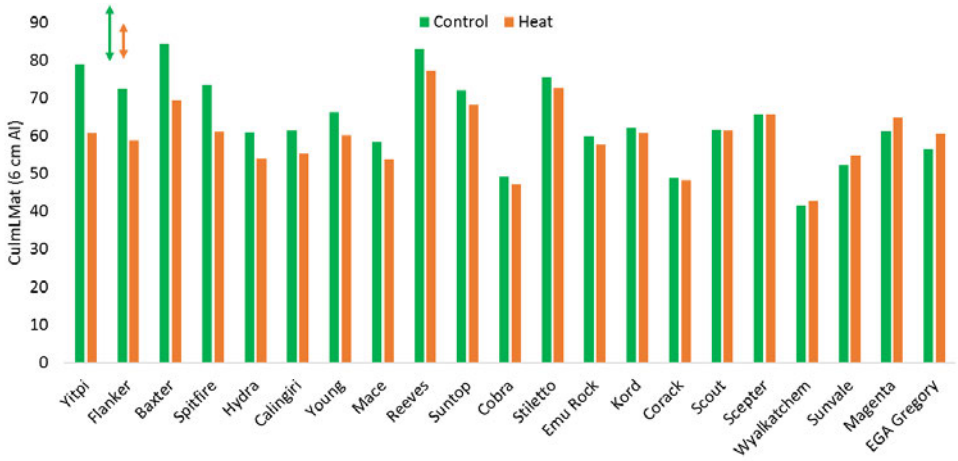


Fig. 7.8 Means of CulmLMat (6cm AI) of each genotype for control (green column) and heat-treated plants (brown column). The vertical arrows indicate LSD values (α = 0.05) for comparisons of means of control (green) and heat (red) across genotypes. Genotypes are ordered by heat response (heat vs. control).

SpkLMat (6cm AI)

SpkLMat overall was increased by heat, with Yitpi, Scout, Sunvale, Wyalkatchem, EGA Gregory and Corack responding the most (Fig. 7.9).

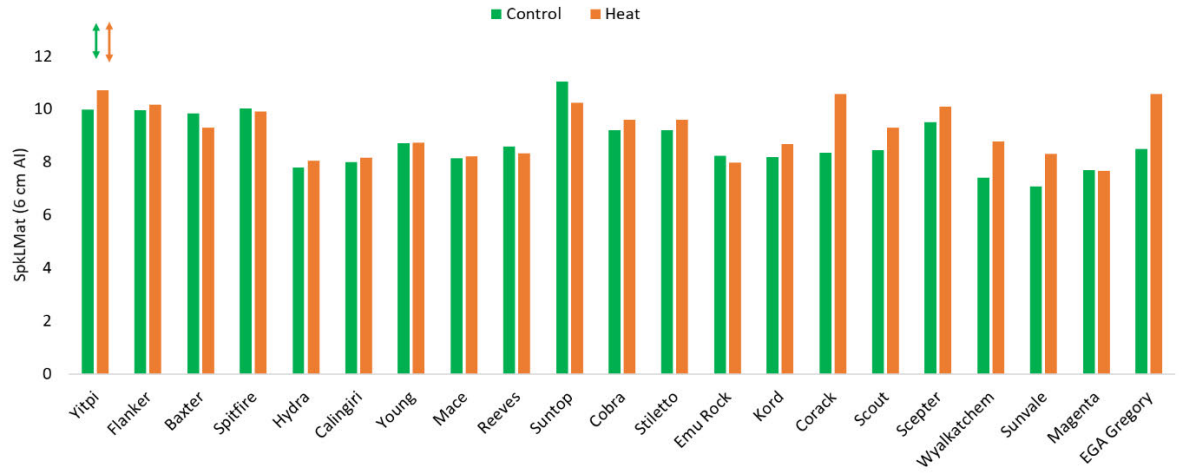


Fig. 7.9 Means of SpkLMat (6cm AI) of each genotype for control (green column) and heat-treated plants (brown column). The vertical arrows indicate LSD values (α = 0.05) for comparisons of means of control (green) and heat (red) across genotypes. Genotypes are ordered according to heat response of CulmLMat (6cm AI treatment).

ShootWMat (10 DAA)

On average, heat at 10 DAA reduced shoot weight at maturity, by 72 mg (Fig. 7.10). (Shirdelmoghanloo, Cozzolino, et al. 2016)observed a similar reduction in the Drysdale x Waagan population. Hydra showed the greatest reduction (29%). However, heat had essentially no effect on shoot weight in Magenta, Suntop, Emu Rock, and there were increases in seven varieties (Corack, Wyalkatchem, Mace, EGA Gregory, Cobra, Scepter and Scout).

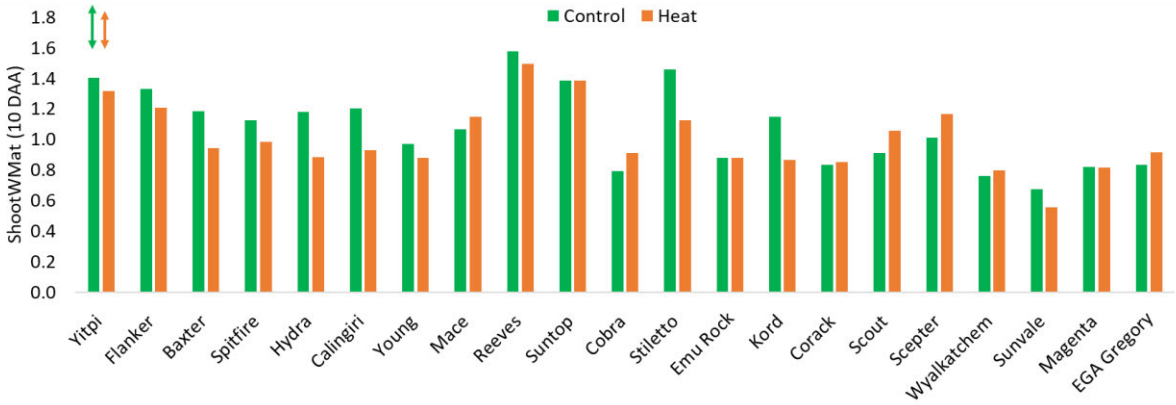


Fig. 7.10 Means of ShootWMat (10 DAA) of each genotype for control (green column) and heat-treated plants (brown column). The vertical arrows indicate LSD values (α = 0.05) for comparisons of means of control (green) and heat (red) across genotypes. Genotypes are ordered according to heat response of CulmLMat (6cm AI treatment).

PedLMat (6cm AIL)

The 6 cm AI treatment reduced peduncle length at maturity in about half of the genotypes and increased it in the other half of the varieties (Fig. 7.11).

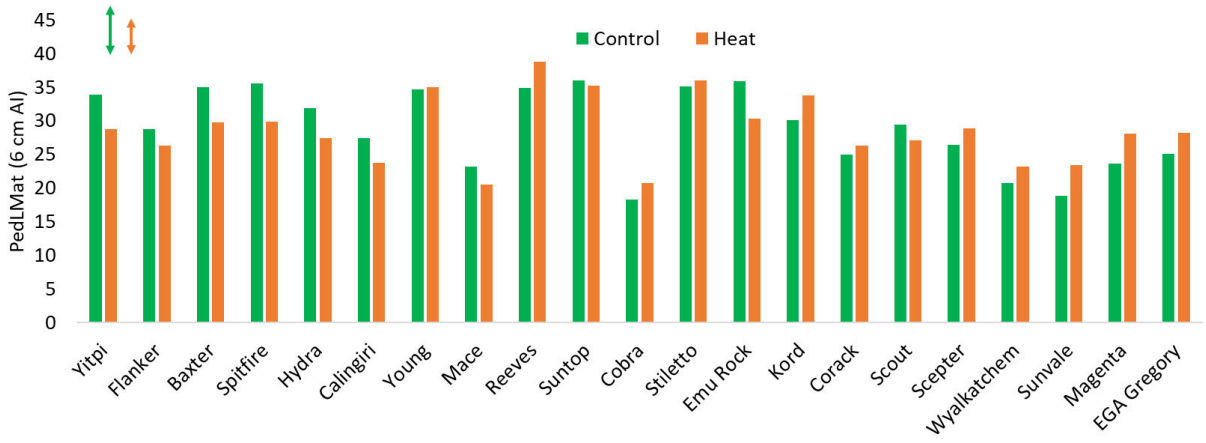


Fig. 7.11 Means of PedLMat (6cm AIL) of each genotype for control (green column) and heat-treated plants (brown column). The vertical arrows indicate LSD values (α = 0.05) for comparisons of means of control (green) and heat (red) across genotypes. Genotypes are ordered according to heat response of CulmLMat (6cm AI treatment).

7.3.9 Correlations between trait for heat tolerance

Genotypic correlations between the heat responses of six traits were investigated: SingGW (10 DAA), ave. fertility at floret positions 1&2 across the whole spike (GnNoSpklt1&2), SPAD13 DAA (10 DAA) and some representative developmental traits PropSpltUndDev (6 cm AI treatment), PedLMat (10DAA treatment) and SpkLMat (6cm AI treatment). No strong correlation (correlation coefficient > 0.5) was identified (Table 7.4). It was perhaps surprising that no correlation was found between heat accelerated chlorophyll loss (SPAD13 DAA response) and SingGW response, as Shirdelmoghanloo, Lohraseb, et al. (2016) identified strong correlations between these responses. However, lack of a significant G x T effect for SingGW in the current experiment may have precluded the possibility of detecting a genotypic correlation with this trait.

Table 7.3 Genotypic correlations (Pearsons's correlation coefficients) between average heat response (percent difference of heat vs. control) of six traits. Conditional formatting colors were used to help visualization.

	GnNoSpklt 1&2	PedLMat	PropSpltU ndDev	SingGW	SPAD13 DAA	SpikeLMat
GnNoSpklt1&2	1					
PedLMat	-0.063	1				
PropSpltUndDev	0.007	-0.217	1			
SingGW	-0.145	-0.283	-0.051	1		
SPAD13 DAA	0.0997	-0.248	0.1322	0.1566	1	
SpikeLMat	0.3554	0.4804	-0.131	-0.277	-0.032	1

7.3.10 Tolerance categorization of varieties

The varieties were categorized as intolerant, moderately tolerant or tolerant for heat effects (based on percent heat responses), for GnNoSpklt1&2 (average across whole spike), SingGW and SPAD13 DAA (Table 7.5). The lack of correlation between these tolerance traits was also evident from this table. Magenta was susceptible for both grain weight and number effects (and chlorophyll effects) and could therefore be classified as heat intolerant. Baxter and EGA Gregory (varieties both released in Queensland) were tolerant to both grain weight and grain number effects (and moderately tolerant or tolerant for chlorophyll loss) and could therefore be classified as heat tolerant. The remaining varieties showed various combinations of tolerances. Advice to growers and breeders could be based on these categorizations.

Table 7.4 Tolerance categorization of varieties (intolerant, 0; moderately tolerant, +; tolerant, ++) based on percent heat responses for three key traits. Conditional formatting colors were used to help visualization.

	GnNoSpklt1&2	SingGW	SPAD13DAA
Magenta	0	0	0
Suntop	0	+	+
Stiletto	0	+	++
Wyalkatchem	0	++	0
Spitfire	0	++	+
Cobra	0	++	++
Emu Rock	0	++	++
Mace	0	++	++
Sunvale	+	0	0
Flanker	+	+	+
Corack	+	+	+
Yitpi	+	+	++
Reeves	+	++	+
Kord	+	++	+
Hydra	+	++	++
Calingiri	++	+	0
Young	++	+	++
Scout	++	+	++
Scepter	++	+	++
Baxter	++	++	+
EGA Gregory	++	++	++

7.4 Conclusions

Wheat plants grown under good conditions are expected to show near complete fertility (set ~2 seeds per spikelet in the two lower floret positions) but in this experiment half of the genotypes under control condition set less than 1.8 seeds in these positions, perhaps reflecting the high temperatures experienced in the greenhouse.

Nonetheless, grain numbers per spikelet at floret position 1&2 and >2 were reduced significantly by the heat treatment, and genotypic differences in responses were identified, allowing identification of potentially heat tolerant and intolerant varieties.

The heat treatment at 10 DAA produced a significant reduction of single grain weight at maturity, and although there was no significant G x T effect overall, the varieities were categorized for tolerance based on the % difference between the means of the heat and control plants. Perhaps the high temperatures in the greenhouse interfered with the ability to discriminate the genotypes for SingGW tolerance.

The tolerance categorizations (Table 7.5) potentially provide a basis for choice/avoidance of varieties by breeders and growers, where heat tolerance is desired. However, it should be cautioned that these categorizations are so far based on a single experiment, and hence need verification. Tolerance for floret fertility and grain weight were not correlated. However, cvs. Baxter and EGA Gregory were classified as tolerant for both, potentially offering good options for the Northern growing region.

Heat applied at the 6 cm AI stage prolonged senescence, but heat applied at 10 DAA accelerated it. However, G x T effects were only observed for flag leaf chlorophyll just after the heat treatment (SPAD13 DAA). No correlation was identified between responses of SingGW and SPAD13 DAA, in contrast to previous studies.

Heat significantly reduced culm length (CulmLMat) and peduncle length (PedLMat) but increased the spike length at maturity (SpkLMat; for the treatment at 6 cm AI), but G x T effects were only detected for the latter.

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Appendix: 1 Mean ± SE of traits measured under heat and control condition for 6 cm AI and 10 DAA

6 cm AI

Trait	Baxter		Calingiri		Cobra		Corack	
	Control	Heat	Control	Heat	Control	Heat	Control	Heat
AIToAwnEm	2.75±0.47	2.50±0.28	4.75±0.47	3.33±0.88	4.5±0.64	2.33±0.33	5.75±0.47	3..005±0.28
AIToFLSen	53.00±3.80	46.25±3.86	66.00±3.89	73.33±2.02	53.75±8.14	54.67±4.33	81.75±7.52	80.05±8.37
AIToMat	70.25±3.68	64.75±3.96	65.00±3.02	69.00±1.52	68.67±3.33	68.00±4.16	76.00±0.91	75.00±1.29
AwnLMat	6.00±0.43	5.65±0.43	5.10±0.25	5.36±0.37	3.17±0.37	0.00±0.00	5.55±0.28	5.67±0.32
BotGNoSpklt1&2	1.71±0.18	0.89±0.12	1.80±0.07	1.12±0.24	0.78±0.29	0.06±0.03	0.66±0.2	0.33±0.12
BotGNoSpklt>2	0.25±0.15	0.49±0.17	0.32±0.19	0.16±0.16	0.03±0.03	0.21±0.07	0.00±0.00	0.14±0.08
BotGnNo1&2	9.00±1.00	5.50±0.64	7.25±0.85	5.66±2.02	4.75±1.88	0.50±0.28	2.50±0.86	2.25±0.85
BotSpkltNo	5.25±0.25	6.25±0.47	4.00±0.40	6.25±0.47	5.5.0±0.64	7.25±0.75	3.75±0.47	6.25±0.47
BotGnNo>2	1.25±0.75	3.25±1.31	1.50±0.95	1.00±1.00	0.25±0.25	1.75±0.62	0.00±0.00	1.00±0.57
CulmLMat	84.2±1.46	69.38±2.28	61.4±2.76	55.33±3.93	49.25±3.40	47.02±3.27	48.9±3.33	48.17±2.73
DevSpkltSpk	17.00±0.70	19.75±0.94	12.75±1.31	15.00±2.08	17.75±1.49	22.25±2.09	13.00±1.41	20.00±1.58
GNoSpike1&2	30.75±0.47	21.00±4.24	23.75±3.32	17.00±2.08	18.75±5.12	0.75±0.47	10.00±2.04	5.75±2.62
GNoSpike>2	4.75±1.79	8.50±2.78	6.50±2.17	8.33±4.91	2.00±2.00	3.00±1.08	0.00±0.00	3.75±2.13
GnNoSpk	35.5±1.84	29.5±3.32	30.25±5.48	25.33±6.56	20.75±6.42	3.75±1.49	10.00±2.04	9.50±3.12
GnNoSpklt	2.11±0.18	1.51±0.21	2.30±0.25	1.64±0.23	1.09±0.29	0.15±0.06	0.78±0.15	0.44±0.13
GnNoSpklt1&2	1.81±0.07	1.08±0.25	1.83±0.09	1.16±0.16	1.00±0.24	0.03±0.01	0.78±0.15	0.27±0.11
GnNoSpklt>2	0.29±0.11	0.42±0.12	0.46±0.15	0.48±0.27	0.09±0.09	0.12±0.04	0.00±0.00	0.17±0.08
MidGNoSpklt1&2	2.00±0.00	1.39±0.36	2.00±0.00	1.53±0.17	1.38±0.4	0.03±0.03	1.00±0.1	0.26±0.17
MidGNoSpklt>2	0.61±0.21	0.63±0.21	0.63±0.21	0.88±0.48	0.21±0.21	0.15±0.07	0.00±0.00	0.27±0.16
MidGnNo>2	3.25±1.10	4.25±1.43	3.00±1.08	5.00±2.88	1.50±1.50	1.25±0.62	0.00±0.00	2.00±1.35
MidGnNo1&2	11.00±0.57	9.25±2.28	8.50±0.95	7.66±1.20	8.75±2.78	0.25±0.25	4.50±0.64	1.75±1.18
MidSpkltNo	5.50±0.28	6.75±0.25	4.25±0.47	5.00±0.57	6.00±0.40	7.25±0.75	4.50±0.50	6.50±0.64
PedLMat	35.02±1.42	29.77±0.96	27.48±3.42	23.70±4.02	18.33±1.87	20.75±1.63	25.02±2.61	26.27±1.19
ShootWMat	1.18±0.02	0.89±0.11	1.20±0.07	1.10±0.14	0.79±0.14	1.01±0.15	0.83±0.07	0.80±0.06
SowToAI	5.03±1.78	55.25±0.62	64.5±3.12	65.00±3.21	61.25±3.35	58.33±0.66	52.00±1.00	53.75±0.75
SpikeLMat	9.82±0.02	9.30±0.42	8.00±0.22	8.16±0.08	9.20±0.33	9.60±0.54	8.35±0.33	10.57±0.21
TopGnNoSpklt1&2	1.73±0.17	0.95±0.30	1.70±0.23	0.80±0.47	0.82±0.16	0.00±0.00	0.65±0.21	0.23±0.10
TopGnNoSpklt>2	0.04±0.04	0.14±0.10	0.40±0.24	0.38±0.20	0.03±0.03	0.00±0.00	0.00±0.00	0.09±0.06
TopGnNo>2	0.25±0.25	1.00±0.70	2.00±1.22	0.00±2.08	0.25±0.25	0.00±0.00	0.00±0.00	0.75±0.47
TopGnNo1&2	10.75±0.75	6.25±1.70	8.00±1.68	3.66±1.66	5.25±1.31	0.00±0.00	3.00±1.08	1.75±0.85
TopspkltNo	6.25±0.25	6.75±0.25	4.50±0.50	5.33±0.66	6.25±0.47	7.75±0.62	4.75±0.47	7.25±0.47
UndDevSpkltSpk	6.00±0.00	3.50±0.86	7.50±1.65	5.33±2.33	11.25±2.01	8.00±2.41	9.75±1.03	3.75±1.10

Appendix:1 continued…

Trait	EGA Gregory		Emu Rock		Flanker		Hydra		Kord	
	Control	Heat	Control	Heat	Control	Heat	Control	Heat	Control	Heat
AIToAwnEm	6.33±0.33	5.33±1.76	3.25±0.94	2.66±0.33	4.25±0.47	4.00±0.57	5.25±1.25	0.00±0.00	3.75±0.85	3.00±0.00
AIToFLSen	60.33±0.88	69.00±5.29	65.75±13.6	75.33±2.84	56.75±5.8	55.75±5.79	68.00±5.13	64.25±2.46	85.75±5.58	80.33±3.84
AIToMat	75.00±2.08	71.00±4.00	65.5±7.17	70.00±2.64	62.00±4.79	65.25±6.47	69.5±3.88	75.50±0.64	73.75±1.65	76.00±1.00
AwnLMat	5.80±0.11	5.46±0.18	6.47±0.73	6.80±0.45	6.97±0.26	6.05±0.14	6.72±0.65	6.62±0.25	5.90±0.33	6.16±0.42
BotGNoSpklt1&2	0.50±0.50	0.54±0.40	1.93±0.06	0.66±0.54	1.48±0.13	0.78±0.46	1.28±0.43	0.61±0.20	1.48±0.30	0.82±0.19
BotGNoSpklt>2	0.08±0.08	0.22±0.22	0.53±0.14	0.41±0.41	0.14±0.14	0.28±0.20	0.32±0.19	0.32±0.14	0.25±0.14	0.62±0.11
BotGnNo1&2	2.00±2.00	3.33±2.40	7.75±0.85	2.66±2.18	8.75±1.65	5.50±3.22	6.50±2.39	3.50±1.32	6.50±1.19	4.33±0.88
BotSpkltNo	4.00±0.00	6.00±0.57	4.00±0.40	4.00±0.00	5.75±0.62	7.25±0.25	5.00±0.40	5.50±0.64	4.50±0.28	5.33±0.33
BotGnNo>2	0.33±0.33	1.33±1.33	2.25±0.75	1.66±1.66	1.00±1.00	2.00±1.41	1.75±1.03	2.00±0.91	1.00±0.57	3.33±0.66
CulmLMat	56.5±4.07	60.57±6.45	59.85±4.13	57.6±4.52	72.45±3.34	58.70±2.60	60.93±1.48	54.02±2.12	62.00±3.17	60.77±0.27
DevSpkltSpk	13.00±0.57	18.67±1.85	13.25±0.85	13.00±0.57	18.75±1.88	23.00±1.08	16.00±0.81	17.25±1.49	14.75±0.85	1.007±0.57
GNoSpike1&2	12.33±3.75	21.67±8.95	25.50±1.84	7.00±4.50	33.00±4.02	22.75±5.79	22.00±3.97	7.25±4.38	26.75±0.62	14.00±2.08
GNoSpike>2	0.33±0.33	5.33±4.84	7.75±1.79	6.33±5.84	6.25±4.00	7.50±4.44	6.75±2.86	3.50±2.02	4.25±1.43	7.66±2.02
GnNoSpk	12.67±4.05	2.07±12.74	33.25±3.52	13.33±10.35	39.25±7.95	30.25±10.14	28.75±6.66	10.75±6.3	31.00±1.58	21.67±1.45
GnNoSpklt	0.99±0.35	1.34±0.61	2.48±0.11	1.03±0.79	2.04±0.21	1.33±0.44	1.78±0.37	0.00±0.00	2.12±0.17	1.28±0.11
GnNoSpklt1&2	0.96±0.32	1.08±0.41	1.92±0.05	0.54±0.34	1.75±0.06	1.00±0.25	1.37±0.23	0.37±0.20	0.00±0.00	0.83±0.15
GnNoSpklt>2	0.02±0.02	0.26±0.24	0.56±0.10	0.48±0.44	0.28±0.17	0.33±0.19	0.41±0.16	0.17±0.09	0.30±0.10	0.44±0.11
MidGNoSpklt1&2	1.23±0.43	1.37±0.49	2.00±0.00	0.46±0.14	1.92±0.07	0.94±0.37	1.50±0.37	0.33±0.27	2.00±0.00	0.86±0.36
MidGNoSpklt>2	0.00±0.00	0.28±0.28	0.82±0.11	0.66±0.54	0.26±0.20	0.39±0.22	0.70±0.23	0.07±0.07	0.61±0.18	0.50±0.28
MidGnNo>2	0.00±0.00	2.00±2.00	3.75±0.62	2.66±2.18	2.00±1.68	3.00±1.78	3.75±1.31	0.50±0.50	3.00±0.91	3.00±1.73
MidGnNo1&2	5.33±1.76	9.33±3.71	9.00±0.57	2.00±0.57	12.5±1.25	7.25±3.03	8.00±2.12	2.25±1.93	10.00±0.81	4.66±1.66
MidSpkltNo	4.33±0.33	6.33±0.66	4.50±0.28	4.33±0.33	6.50±0.64	7.75±0.47	5.25±0.25	5.75±0.47	50.00±0.40	5.66±0.33
PedLMat	25.07±4.33	28.2±2.53	35.93±1.88	30.35±0.86	28.8±0.65	26.3±2.04	31.93±1.47	27.48±1.37	30.15±2.07	33.77±0.38
ShootWMat	0.83±0.04	1.17±0.08	0.87±0.09	0.93±0.07	1.33±0.15	1.03±0.17	1.18±0.08	1.04±0.06	1.14±0.12	1.23±0.11
SowToAI	59.67±3.33	59.00±2.64	42.75±0.94	43.00±1.15	62.5±0.28	60.00±1.87	5.003±2.04	51.25±2.35	51.5±1.5	49.67±0.33
SpikeLMat	8.50±0.28	0.00±0.98	8.22±0.25	7.96±0.26	9.95±0.49	10.15±0.13	7.77±0.34	8.05±0.52	8.17±0.17	8.66±0.18
TopGnNoSpklt1&2	1.08±0.14	1.32±0.46	1.83±0.09	0.48±0.36	1.8±0.10	1.26±0.14	1.31±0.14	0.21±0.21	1.95±0.05	0.83±0.16
TopGnNoSpklt>2	0.00±0.00	0.28±0.21	0.35±0.12	0.40±0.40	0.45±0.17	0.31±0.18	0.20±0.12	0.14±0.14	0.05±0.05	0.22±0.05
TopGnNo>2	0.00±0.00	2.00±1.52	1.75±0.62	2.00±2.00	3.25±1.43	2.50±1.44	1.25±0.75	1.00±1.00	0.25±0.25	1.33±0.33
TopGnNo1&2	5.00±0.57	9.00±3.51	8.75±0.75	2.33±1.85	11.75±1.37	10.00±0.70	7.50±0.64	1.50±1.50	10.25±0.62	5.00±1.00
TopspkltNo	4.66±0.33	6.33±0.66	4.75±0.25	4.66±0.33	6.50±0.64	800±0.400	5.75±0.25	6.00±0.40	5.25±0.25	6.00±0.00
UndDevSpkltSpk	10.67±0.88	6.00±1.52	2.50±0.50	2.00±0.57	7.25±1.49	2.25±0.85	4.25±0.62	2.00±0.7	4.00±0.70	1.66±0.33

Appendix:1 continued…

Trait	Mace		Magenta		Reeves		Scepter		Scout	
	Control	Heat	Control	Heat	Control	Heat	Control	Heat	Control	Heat
AIToAwnEm	4.75±0.75	2.75±0.25	4.00±0.40	3.33±0.33	5.5±0.23	5.66±0.14	5.50±0.28	4.00±0.00	6.66±0.88	4.5±0.28
AIToFLSen	6.003±9.61	77.00±1.47	55.5±2.32	68.33±0.88	67.83±2.53	68.55±4.25	68.5±6.51	71.75±8.64	69.33±9.38	54.00±2.38
AIToMat	63.5±4.78	7.002±1.29	65.25±0.47	66±0.57	71.17±1.12	72.92±0.94	72.25±1.75	73.75±1.88	67.67±1.33	64.00±1.52
AwnLMat	4.35±0.10	4.27±0.22	6.85±0.35	6.53±0.49	4.90±0.18	4.45±0.21	5.10±0.10	5.30±0.18	4.72±0.36	4.65±0.43
BotGNoSpklt1&2	1.51±0.23	0.45±0.17	0.93±0.25	0.74±0.13	1.82±0.07	1.18±0.12	0.88±0.29	1.24±0.28	0.78±0.32	1.42±0.37
BotGNoSpklt>2	0.18±0.12	0.14±0.09	0.00±0.00	0.00±0.00	0.53±0.09	1.00±0.08	0.00±0.00	0.30±0.15	0.00±0.00	0.00±0.00
BotGnNo1&2	6.50±1.19	2.50±1.04	4.25±1.10	4.33±0.33	10.50±0.46	7.16±0.70	4.00±1.47	8.25±2.32	3.50±1.55	7.25±2.05
BotSpkltNo	4.25±0.25	5.50±0.28	4.50±0.28	6.00±0.57	5.75±0.13	6.16±0.16	4.50±0.28	6.50±0.64	4.00±0.40	5.25±0.47
BotGnNo>2	0.75±0.47	0.75±0.47	0.00±0.00	0.00±0.00	3.08±0.54	6.25±0.62	0.00±0.00	2.25±1.31	0.00±0.00	0.00±0.00
CulmLMat	58.40±2.83	53.77±0.98	61.33±2.72	64.7±2.95	82.85±4.79	77.23±2.20	65.75±2.80	65.62±2.67	61.57±2.29	61.38±2.81
DevSpkltSpk	13.75±0.47	17.00±0.70	14.50±0.64	19.33±1.76	18.08±0.43	19.58±0.54	15.25±0.75	20.50±1.70	12.00±1.22	17.25±1.37
GNoSpike1&2	23.67±2.33	12.75±2.78	18.00±3.24	6.00±1.15	31.67±1.08	13.33±1.84	18.67±0.33	20.25±6.03	16.00±4.30	22.50±3.70
GNoSpike>2	4.75±1.25	1.75±0.85	0.75±0.47	0.00±0.00	8.91±1.17	11.25±1.1	0.25±0.25	10.00±3.10	0.00±0.00	2.00±1.22
GnNoSpk	28.67±4.05	14.5±2.06	18.75±3.66	6.00±1.15	40.58±2.16	24.58±1.59	18.67±0.33	30.25±8.93	16.25±4.51	24.50±4.87
GnNoSpklt	2.13±0.25	0.86±0.15	1.28±0.23	0.31±0.06	2.24±0.10	1.27±0.09	1.28±0.07	1.45±0.35	1.27±0.24	1.43±0.24
GnNoSpklt1&2	1.82±0.11	0.76±0.18	1.23±0.20	0.31±0.06	1.75±0.05	0.7±0.10	1.44±0.17	0.97±0.24	1.26±0.23	1.31±0.19
GnNoSpklt>2	0.37±0.12	0.10±0.04	0.05±0.03	0.00±0.00	0.49±0.06	0.57±0.05	0.00±0.00	0.47±0.11	0.01±0.01	0.11±0.06
MidGNoSpklt1&2	2.00±0.00	0.90±0.39	1.51±0.23	0.25±0.16	1.93±0.03	0.55±0.14	2.00±0.00	0.78±0.35	1.56±0.21	1.51±0.25
MidGNoSpklt>2	0.75±0.14	0.13±0.04	0.10±0.05	0.00±0.00	0.87±0.08	0.73±0.10	0.00±0.00	0.79±0.19	0.05±0.05	0.10±0.10
MidGnNo>2	3.50±0.64	0.75±0.25	0.5±0.28	0.00±0.00	5.25±0.55	4.75±0.66	0.25±0.25	5.50±1.70	0.25±0.25	0.50±0.50
MidGnNo1&2	8.66±0.66	5.00±2.04	7.25±1.31	1.66±1.20	11.58±0.35	3.41±0.91	10.00±0.00	5.75±2.81	6.50±1.44	8.50±1.32
MidSpkltNo	4.33±0.33	5.75±0.25	4.75±0.25	6.33±0.66	6.00±0.17	6.05±0.19	5.00±0.00	6.75±0.62	4.00±0.40	5.75±0.47
PedLMat	23.18±0.75	20.52±1.38	23.67±1.84	0.00±0.00	34.91±1.38	38.75±0.82	26.43±1.13	28.88±1.14	29.43±2.88	27.07±3.11
ShootWMat	1.06±0.07	1.01±0.08	0.82±0.01	0.92±0.00	1.57±0.08	1.49±0.06	1.01±0.09	1.13±0.18	0.91±0.09	0.91±0.08
SowToAI	55.5±0.86	57.25±1.03	64.75±0.25	65.33±0.88	49.33±0.52	48.83±0.44	58.5±0.86	57.25±0.25	56.00±1.52	61.00±0.70
SpikeLMat	8.12±0.14	8.20±0.19	7.70±0.17	7.66±0.17	8.58±0.12	8.31±0.17	9.50±0.20	10.10±0.34	8.45±0.34	9.30±0.25
TopGnNoSpklt1&2	1.85±0.09	0.92±0.20	1.22±0.19	0.00±0.00	1.52±0.11	0.42±0.11	1.34±0.22	0.89±0.22	1.43±0.21	1.06±0.09
TopGnNoSpklt>2	0.10±0.05	0.04±0.04	0.05±0.05	0.00±0.00	0.09±0.04	0.03±0.01	0.00±0.00	0.31±0.06	0.00±0.00	0.22±0.16
TopGnNo>2	0.50±0.28	0.25±0.25	0.25±0.25	0.00±0.00	0.58±0.26	0.25±0.13	0.00±0.00	2.25±0.47	0.00±0.00	0.00±2.38
TopGnNo1&2	9.25±0.47	5.25±1.10	6.50±1.19	0.00±0.00	9.58±0.67	2.75±0.74	7.50±1.55	6.25±1.43	6.00±1.47	6.75±1.03
TopspkltNo	5.00±0.00	5.75±0.25	5.25±0.25	7.00±0.57	6.33±0.18	6.91±0.22	5.50±0.28	7.25±0.47	4.00±0.40	6.25±0.47
UndDevSpkltSpk	8.00±0.40	4.50±0.50	12.00±0.81	7.00±1.52	3.75±0.32	2.66±0.37	10.00±0.81	3.25±1.49	13.00±1.29	8.00±1.08

Appendix:1 continued…

Trait	Spitfire		Stiletto		Suntop		Sunvale	
	Control	Heat	Control	Heat	Control	Heat	Control	Heat
AIToAwnEm	2.75±0.25	2.75±0.25	5.75±0.62	4.33±0.66	3.25±0.62	3.25±0.25	6.00±0.00	3.00±0.00
AIToFLSen	55.5±6.93	59.00±8.32	54.00±4.77	67.00±11.02	67.75±6.92	67.33±2.18	66.00±4.50	61.00±5.00
AIToMat	66.5±3.32	73.00±1.22	73.75±1.97	80.00±4.58	74.5±2.59	72.25±3.42	70.00±1.15	70.00±0.00
AwnLMat	5.25±0.55	5.70±0.17	7.60±0.67	7.96±0.32	7.27±0.33	7.12±0.12	3.76±0.63	3.80±1.40
BotGNoSpklt1&2	1.68±0.14	0.43±0.17	1.49±0.10	0.41±0.15	1.83±0.05	0.60±0.07	0.26±0.26	0.10±0.10
BotGNoSpklt>2	0.42±0.24	0.00±0.00	0.21±0.15	0.59±0.13	0.97±0.20	1.02±0.34	0.00±0.00	0.00±0.00
BotGnNo1&2	9.50±1.75	2.75±1.1	8.25±0.85	2.66±0.88	8.25±0.47	3.00±0.40	1.33±1.33	0.50±0.50
BotSpkltNo	5.50±0.64	6.00±0.70	5.50±0.28	6.66±0.33	4.50±0.28	5.00±0.40	5.00±0.00	6.00±1.00
BotGnNo>2	2.75±1.60	2.50±0.95	1.25±0.94	4.00±1.00	4.25±0.75	5.25±1.70	0.00±0.00	0.00±0.00
CulmLMat	73.57±1.86	60.98±1.44	75.53±3.95	72.63±1.03	72.03±2.52	68.12±2.36	52.33±2.97	54.70±5.20
DevSpkltSpk	17.0.±1.58	19.00±2.48	17.00±0.57	20.33±1.2	15.00±0.70	16.00±0.91	15.67±0.33	20.00±3.00
GNoSpike1&2	30.75±3.03	9.00±3.67	30.75±1.37	13.33±3.84	26.5±2.98	6.75±0.25	7.66±3.52	5.00±5.00
GNoSpike>2	9.50±3.52	6.75±2.17	7.25±2.13	12.33±2.9	10.00±1.08	7.25±2.39	0.00±0.00	0.00±0.00
GnNoSpk	40.25±6.54	15.75±5.67	38.00±2.16	25.67±1.45	36.5±3.27	14.00±2.19	7.66±3.52	5.00±5.00
GnNoSpklt	2.33±0.21	0.82±0.28	0.00±0.00	1.27±0.10	2.42±0.12	0.87±0.12	0.48±0.21	0.29±0.29
GnNoSpklt1&2	1.81±0.07	0.46±0.18	1.80±0.03	0.67±0.22	1.75±0.13	0.42±0.03	0.48±0.21	0.29±0.29
GnNoSpklt>2	0.52±0.16	0.36±0.11	0.42±0.12	0.59±0.12	0.66±0.06	0.44±0.14	0.00±0.00	0.00±0.00
MidGNoSpklt1&2	1.85±0.14	0.54±0.13	2.00±0.00	0.62±0.34	1.85±0.09	0.35±0.06	0.73±0.4	0.50±0.50
MidGNoSpklt>2	0.88±0.16	0.51±0.13	0.74±0.14	0.87±0.31	0.90±0.05	0.03±0.12	0.00±0.00	0.00±0.00
MidGnNo>2	5.25±1.25	3.00±0.70	4.00±0.7.	6.00±2.30	4.75±0.25	1.75±0.75	0.00±0.00	0.00±0.00
MidGnNo1&2	10.50±0.50	3.50±0.95	11.00±0.57	4.00±2.08	9.75±0.85	2.00±0.40	3.66±2.02	3.00±3.00
MidSpkltNo	5.75±0.47	6.25±0.94	5.50±0.28	6.66±0.33	5.25±0.25	5.50±0.28	5.00±0.00	7.00±1.00
PedLMat	35.6±0.42	29.9±1.75	35.12±2.99	35.97±0.83	36.00±1.40	35.27±2.03	18.83±1.87	23.45±2.15
ShootWMat	1.12±0.11	1.03±0.12	1.46±0.04	1.40±0.03	1.38±0.08	1.47±0.07	0.67±0.05	0.74±0.11
SowToAI	47.00±1.29	50.25±1.88	57.00±1.47	56.33±1.85	46.25±0.94	45.5±0.28	64.33±1.20	67.00±2.00
SpikeLMat	10.02±0.5	9.90±0.47	9.20±0.39	9.60±0.20	11.03±0.20	10.22±0.36	7.06±0.53	8.30±0.40
TopGnNoSpklt1&2	1.86±0.09	0.39±0.29	1.91±0.04	0.97±0.18	1.60±0.28	0.34±0.15	0.45±0.19	0.25±0.25
TopGnNoSpklt>2	0.23±0.15	0.17±0.13	0.33±0.11	0.34±0.12	0.18±0.06	0.04±0.04	0.00±0.00	0.00±0.00
TopGnNo>2	1.50±0.95	1.25±0.94	2.00±0.70	2.33±0.88	1.00±0.40	0.25±0.25	0.00±0.00	0.00±0.00
TopGnNo1&2	10.75±1.10	2.75±2.09	11.50±0.28	6.66±0.88	8.50±1.70	1.75±0.75	2.66±1.20	1.50±1.50
TopspkltNo	5.75±0.47	6.75±0.85	6.00±0.00	7.00±0.57	5.25±0.25	5.50±0.28	5.66±0.33	7.00±1.00
UndDevSpkltSpk	3.75±1.43	3.00±0.70	5.00±0.70	2.33±0.88	3.75±0.62	3.25±0.62	8.66±0.33	6.00±2.00

Appendix:1 continued…

Trait	Wyalkatchem		Yitpi		Young	
	Control	Heat	Control	Heat	Control	Heat
AIToAwnEm	3.33±0.33	3.25±0.47	6.00±1.08	4.75±0.25	4.16±0.29	3.41±0.31
AIToFLSen	68.25±2.86	76.67±13.09	52.00±1.82	54.25±1.7	67.25±2.00	74.00±2.92
AIToMat	68.33±2.18	68.67±3.84	65.500±3.61	72.5±3.79	73.58±0.83	73.67±0.74
AwnLMat	4.77±0.47	4.92±0.38	7.75±0.44	6.62±0.37	6.10±0.18	6.08±0.20
BotGNoSpklt1&2	1.00±0.29	0.05±0.05	1.16±0.41	0.60±0.31	1.36±0.18	0.84±0.18
BotGNoSpklt>2	0.00±0.00	0.19±0.13	0.05±0.05	0.03±0.03	0.51±0.09	0.74±0.15
BotGnNo1&2	4.50±2.17	0.25±0.25	6.25±2.25	3.75±2.05	5.91±0.90	4.18±0.94
BotSpkltNo	4.00±0.70	6.25±0.47	6.00±0.70	7.00±0.70	4.25±0.17	4.81±0.12
BotGnNo>2	0.00±0.00	1.25±0.94	0.25±0.25	0.25±0.25	2.25±0.46	3.63±0.78
CulmLMat	41.55±1.93	42.67±1.84	78.9±3.95	60.62±2.37	66.24±1.64	60.15±2.02
DevSpkltSpk	13.25±2.17	20.00±1.78	19.50±2.25	21.25±1.88	14.08±0.39	15.18±0.32
GNoSpike1&2	16.25±6.14	2.25±1.03	26.00±1.87	15.00±4.18	22.58±2.01	16.27±2.97
GNoSpike>2	0.25±0.25	3.00±2.67	4.50±2.59	7.25±2.72	6.50±1.08	9.54±1.54
GnNoSpk	16.5±6.38	5.25±2.78	30.5±4.21	22.25±6.89	29.08±3.01	25.82±4.29
GnNoSpklt	1.12±0.25	0.24±0.11	1.65±0.30	1.06±0.32	2.04±0.19	1.66±0.26
GnNoSpklt1&2	1.11±0.23	0.10±0.04	1.40±0.19	0.72±0.20	1.59±0.12	1.04±0.18
GnNoSpklt>2	0.01±0.01	0.13±0.11	0.25±0.14	0.34±0.12	0.45±0.07	0.61±0.09
MidGNoSpklt1&2	1.56±0.30	0.11±0.06	1.39±0.31	0.50±0.31	1.74±0.12	1.14±0.24
MidGNoSpklt>2	0.04±0.04	0.18±0.18	0.45±0.26	0.70±0.17	0.69±0.09	0.82±0.16
MidGnNo>2	0.25±0.25	1.50±1.50	2.75±1.60	4.75±1.10	3.33±0.48	4.18±0.90
MidGnNo1&2	7.50±2.02	0.75±0.47	8.25±1.43	3.50±2.17	8.33±0.72	5.9±1.26
MidSpkltNo	4.50±0.64	6.75±0.75	0.00±0.00	7.00±0.70	4.75±0.17	5.00±0.13
PedLMat	20.73±1.69	23.15±1.58	33.87±2.26	28.73±0.85	34.7±0.37	34.98±1.00
ShootWMat	0.76±0.10	0.88±0.08	1.40±0.05	1.32±0.05	0.97±0.04	0.92±0.04
SowToAI	58.33±4.05	56.00±4.34	63.00±1.58	62.5±0.64	46.42±0.39	45.67±0.39
SpikeLMat	7.40±0.28	8.77±0.57	9.97±0.24	10.7±0.68	8.70±0.23	8.71±0.15
TopGnNoSpklt1&2	0.73±0.32	0.15±0.09	1.62±0.14	1.05±0.21	1.63±0.09	1.13±0.18
TopGnNoSpklt>2	0.00±0.00	0.03±0.03	0.22±0.13	0.30±0.20	0.17±0.06	0.3±0.06
TopGnNo>2	0.00±0.00	0.25±0.25	1.50±0.95	2.25±1.43	0.91±0.33	1.72±0.40
TopGnNo1&2	4.25±2.59	1.25±0.75	11.05±1.93	7.75±1.88	8.33±0.51	6.18±1.02
TopspkltNo	0.00±0.00	7.00±0.57	7.00±0.70	7.25±0.47	5.08±0.08	5.36±0.15
UndDevSpkltSpk	10.00±2.34	3.00±0.40	6.25±1.88	5.00±1.73	5.16±0.40	3.36±0.36

Appendix:1 continued…

10 DAA

Trait	Baxter		Calingiri		Cobra		Corack	
	Control	Heat	Control	Heat	Control	Heat	Control	Heat
1000GnW(mg)	34.4 ± 1.08	33.76±3.19	36.87 ± 2.43	33.13±1.52	34.25 ± 6.01	34.32±6.83	49.33 ± 3.9	44.81±1.32
AnthToFLSen	44.00 ± 3.80	40.00±3.44	56.00 ± 3.55	51.00±6.74	38.33 ± 11.55	39.25±7.99	68.25 ± 6.42	64.50±4.40
AnthToMat	61.25 ± 3.68	55.00±1.58	55.00 ± 2.67	51.50±1.84	55.33 ± 3.93	49.25±4.36	62.5 ± 0.50	60.00±0.81
CulmL10 DAAToMat	5.77 ± 0.22	6.26±0.84	4.40 ± 0.26	4.10±0.28	3.53 ± 0.63	3.52±0.43	2.87 ± 0.33	3.25±0.41
CulmL10	78.42 ± 1.47	69.87±7.12	57.00 ± 2.59	54.17±2.03	48.93 ± 1.04	47.17±1.70	46.03 ± 3.02	49.70±2.61
CulmLMat	84.2 ± 1.46	76.13±7.95	61.40 ± 2.76	58.28±2.21	49.25 ± 3.40	50.7±1.60	48.9 ± 3.33	52.95±2.88
CulmLMat_PedLMat_ratio	2.41 ± 0.06	2.63±0.14	2.3 0± 0.19	2.20±0.11	2.63 ± 0.05	2.77±0.29	1.99 ± 0.16	1.79±0.06
DaysToMat	123.2 ± 6.6	122.2±3.72	129.5 ± 5.79	125.5±2.21	126.7 ± 2.40	119.5±4.62	128.00 ± 1.08	128.00±0.91
DevSpklt	17.00 ± 0.70	15.05±1.04	12.75 ± 1.31	11.75±0.47	17.75 ± 1.49	16.50±2.10	13 .00± 1.41	12.75±0.854
GnNoSpk	35.5 ± 1.84	19.75±5.54	30.25 ± 5.48	25.5±3.06	20.75 ± 6.42	21.75±6.12	10.00± 2.04	12.25±2.25
GnNoSpklt	2.11 ± 0.18	1.28±0.35	2.30 ± 0.25	2.15±0.18	1.09 ± 0.29	1.23±0.21	0.78 ± 0.15	0.95±0.15
GnWSpk	1.21 ± 0.03	0.69±0.23	1.08 ± 0.16	0.83±0.08	0.72 ± 0.28	0.70±0.22	0.47 ± 0.05	0.54±0.09
LowIntern10ToMat	3.62 ± 0.27	4.46±0.70	2.67 ± 0.18	2.37±0.33	1.95 ± 0.75	2.67±0.44	1.22 ± 0.18	1.30±0.40
LowInternL10	45.55 ± 0.51	42.60±6.11	31.25 ± 1.28	29.12±0.85	29.85 ± 1.65	29.02±1.49	22.65 ± 1.82	21.93±1.2
LowInternMat	49.17 ± 0.69	47.07±6.77	33.92 ± 1.36	31.50±1.13	29.53 ± 2.65	31.70±1.74	23.88 ± 1.99	23.23±1.58
PedL10 DAA	32.88 ± 1.32	28.50±1.95	25.75 ± 3.22	25.05±2.05	19.05 ± 0.15	18.15±2.22	23.38 ± 2.38	27.77±1.94
PedL10ToMat	2.15 ± 0.10	1.72±0.13	1.72 ± 0.20	1.72±0.13	1.15 ± 0.05	0.85±0.13	1.65 ± 0.24	1.95±0.17
PedLMat	35.02 ± 1.42	30.23±1.99	27.48 ± 3.42	26.77±2.14	18.33 ± 1.87	19.00±2.30	25.02 ± 2.61	29.73±2.07
Shootw_length_ratio	71.23 ± 2.14	89.67±6.58	51.59 ± 4.04	63.28±5.21	66.77 ± 10.05	56.5±3.29	59.92 ± 7.24	66.1±10.78
ShootWMat	1.18 ± 0.02	0.94±0.13	1.2 0± 0.07	0.93±0.05	0.79 ± 0.14	0.91±0.07	0.83 ± 0.07	0.85±0.11
SingGW(g)	34.4 ± 1.08	33.76±3.19	36.87 ± 2.43	33.13±1.52	36.87 ± 2.43	34.32±6.83	49.33 ± 3.90	44.81±1.32
SowToAnth	62.00 ± 1.78	67.25±3.50	74.50 ± 3.52	74.00±1.58	71.33 ± 3.48	70.25±2.86	65.50 ± 1.19	68.00±1.35
SPAD13DAA	44.52 ± 0.91	41.15±2.03	46.45 ± 1.02	31.65±5.34	44.4 ± 2.06	33.48±8.62	45.35 ± 1.48	40.95±1.55
SPAD27DAA	37.7 ± 0.60	28.82±6.10	47.43 ± 0.84	26.45±6.58	32.07 ± 10.73	25.25±10.71	46.08 ± 1.41	43.55±1.52
SPAD10 DAA	45.17 ± 0.9	44.08±1.31	46.25 ± 0.91	44.42±0.99	44.57 ± 1.22	42.88±1.97	46.03 ± 0.94	44.8±0.48
SPAD10To13	-0.65 ± 0.09	-2.92±1.19	0.20 ± 0.29	12.78±5.55	-0.16 ± 1.23	-9.40±7.65	-0.67 ± 0.61	-3.85±1.35
SPAD10To27	-7.47 ± 0.38	-15.25±5.75	-1.17 ± 0.65	-17.98±7.38	0.00 ± 17.15	-17.62±10.15	0.05 ± 1.94	-1.25±1.21
SPAD13DAATo27DAA	-6.82 ± 0.35	-12.32±6.30	0.97 ± 0.73	-5.20±5.01	-12.33 ± 8.71	-8.22±5.43	0.72 ± 2.53	2.60±0.41
UnderdevSpklt	6.00 ± 0.00	8.00±0.70	7.50 ± 1.65	8.75±0.94	11.25 ± 2.01	12.00±2.19	9.75 ± 1.03	12.50±0.5

Appendix:1 continued…

Trait	EGA Gregory		Emu Rock		Flanker		Hydra	
	Control	Heat	Control	Heat	Control	Heat	Control	Heat
1000GnW(mg)	41.29 ± 2.76	39.74±3.73	27.43 ± 3.36	27.35±3.37	44.85 ± 0.19	40.08±3.94	36.8 ± 2.85	34.13±4.39
AnthToFLSen	47.00 ± 1.52	47.67±3.84	55.5 ± 13.16	46.75±12.36	45.75 ± 5.15	42.25±6.53	58.33 ± 5.23	46.5±5.73
AnthToMat	61.67 ± 2.60	57.33±1.85	55.25 ± 6.75	49.5±5.36	51.00 ± 3.80	52.5±2.32	62.25 ± 1.65	56.25±0.47
CulmL10 DAAToMat	4.16 ± 0.48	3.76±0.47	4.20 ± 0.42	4.47±0.18	5.42 ± 0.51	5.40±0.67	3.66 ± 0.14	4.30±0.36
CulmL10	52.33 ± 3.59	52.20±6.58	55.65 ± 3.76	51.22±4.70	67.03 ± 2.89	67.62±4.81	54.02 ± 3.39	54.87±0.59
CulmLMat	56.50 ± 4.07	55.97±7.03	59.85 ± 4.13	55.7±4.80	72.45 ± 3.34	73.03±5.39	60.93 ± 1.48	59.17±0.84
CulmLMat_PedLMat_ratio	2.33 ± 0.22	2.33±0.12	1.66 ± 0.08	1.77±0.19	2.36 ± 0.04	2.28±0.02	1.91 ± 0.04	1.86±0.05
DaysToMat	134.7 ± 2.33	130.7±1.20	108.2 ± 6.78	103.2±4.58	124.5 ± 4.94	123.8±2.86	122.5 ± 2.98	116.8±0.75
DevSpklt	13.00 ± 0.57	12.00±0.57	13.25 ± 0.85	12.5±0.64	18.75 ± 1.88	19.00±1.47	16.00 ± 0.81	13.50±0.64
GnNoSpk	9.50 ± 4.27	13.67±5.36	33.75 ± 3.90	28.75±7.43	39.25 ± 7.95	40.25±6.57	28.75 ± 6.66	24.75±4.80
GnNoSpklt	0.99 ± 0.35	1.15±0.45	2.52 ± 0.14	2.23±0.52	2.04 ± 0.21	2.08±0.20	1.78 ± 0.37	1.80±0.26
GnWSpk	0.39 ± 0.17	0.00±0.58	0.92 ± 0.14	0.72±0.17	1.76 ± 0.36	1.65±0.35	1.03 ± 0.24	0.78±0.04
LowIntern10ToMat	2.46 ± 0.17	2.20±0.15	1.80 ± 0.45	2.36±0.14	3.43 ± 0.58	3.37±0.53	1.83 ± 0.26	2.3±0.30
LowInternL10	28.97 ± 0.60	29.33±2.61	22.12 ± 2.98	17.33±2.94	36.9 ± 0.23	37.77±2.97	26.78 ± 0.5	24.93±1.39
LowInternMat	0.00 ± 0.75	31.53±2.72	23.93 ± 3.33	19.70±2.80	40.33 ± 0.39	41.15±3.33	0.00 ± 0.34	27.23±1.61
PedL10 DAA	23.37 ± 3.99	22.87±4.13	33.52 ± 1.72	31.43±2.86	27.27 ± 0.48	29.85±1.85	27.25 ± 2.98	29.93±0.8
PedL10ToMat	1.70 ± 0.34	1.56±0.32	2.4 0± 0.18	2.13±0.38	1.53 ± 0.27	2.02±0.21	1.83 ± 0.23	2.00±0.05
PedLMat	25.07 ± 4.33	24.43±4.46	35.93 ± 1.88	33.57±3.23	28.8 ± 0.65	31.88±2.06	31.93 ± 1.47	31.93±0.78
Shootw_length_ratio	68.37 ± 7.71	60.74±5.88	69.89 ± 5.87	66.96±5.72	55.55 ± 3.58	60.46±1.98	52.06 ± 4.00	61.5±3.05
ShootWMat	0.83 ± 0.04	0.91±0.02	0.87 ± 0.09	0.87±0.16	1.33 ± 0.15	1.20±0.08	1.18 ± 0.08	0.88±0.09
SingGW(g)	41.29 ± 2.76	39.74±3.73	27.43 ± 3.36	27.35±3.37	44.85 ± 0.19	40.08±3.94	36.8 ± 2.85	34.13±4.39
SowToAnth	73.00 ± 4.00	73.33±2.33	53.00 ± 0.70	53.75±0.85	73.5 ± 1.19	71.25±1.25	60.25 ± 1.97	60.5±0.95
SPAD13DAA	44.17 ± 0.66	41.83±1.48	45.12 ± 0.89	42.83±1.27	48.02 ± 1.06	44.82±0.89	44.6 ± 0.95	42.17±0.92
SPAD27DAA	42.43 ± 0.93	42.23±3.42	28.15 ± 8.12	24.45±7.80	44.98 ± 2.04	32.30±9.68	44.05 ± 1.27	34.35±8.81
SPAD10 DAA	43.83 ± 0.2	44.2±0.92	44.12 ± 1.19	43.60±1.12	46.38 ± 1.02	46.08±0.98	44.55 ± 1.09	43.65±0.96
SPAD10To13	0.33 ± 0.49	-2.36±0.75	0.33 ± 0.49	-0.63±1.70	1.65 ± 0.15	-1.25±1.75	0.05 ± 0.33	-1.47±0.20
SPAD10To27	-1.4 0± 1.10	-1.96±2.56	-15.97 ± 9.22	-13.33±6.34	-1.40 ± 3.01	-13.77±9.71	-0.50 ± 0.23	-9.3±9.65
SPAD13DAATo27DAA	-1.73 ± 1.39	0.40±2.56	-16.97 ± 9.01	-18.38±7.14	-3.05 ± 3.07	-12.52±9.15	-0.55 ± 0.48	-7.82±9.58
UnderdevSpklt	10.67 ± 0.88	12.33±1.20	2.50 ± 0.50	3.00±0.70	7.25 ± 1.49	7.25±1.60	4.25 ± 0.62	4.50±0.64

Appendix:1 continued…

Trait	Kord		Mace		Magenta		Reeves	
	Control	Heat	Control	Heat	Control	Heat	Control	Heat
1000GnW(mg)	36.44 ± 1.33	40.42±1.39	27.41 ± 2.82	27.41±1.38	47.86 ± 1.13	40.9±5.82	43.27 ± 1.59	41.38±1.88
AnthToFLSen	75.00 ± 6.67	53.50±5.60	53.25 ± 9.22	63.50±2.25	43.50 ± 3.12	39.75±6.01	56.92 ± 2.48	45.05±3.98
AnthToMat	63.00 ± 1.73	63.00±1.78	53.75 ± 4.49	57.75±0.75	53.25 ± 1.88	48.25±5.6	60.25 ± 1.03	55.75±1.35
CulmL10 DAAToMat	3.62 ± 0.29	3.33±0.43	4.32 ± 0.14	4.05±0.32	4.90 ± 1.26	5.22±0.96	5.73 ± 0.56	6.69±0.21
CulmL10	58.38 ± 2.92	53.13±1.12	54.08 ± 2.72	56.38±2.26	56.43 ± 1.88	58.3±3.30	77.11 ± 4.28	81.65±2.38
CulmLMat	62.00 ± 3.17	56.47±1.39	58.4 ± 2.83	60.42±2.51	61.33 ± 2.72	63.52±4.00	82.85 ± 4.79	88.35±2.48
CulmLMat_PedLMat_ratio	2.06 ± 0.07	2.35±0.26	2.51 ± 0.05	2.91±0.18	2.55 ± 0.12	2.58±0.09	2.34 ± 0.06	2.41±0.06
DaysToMat	125.2 ± 2.28	130.8±1.03	119 ± 4.02	122.5±1.19	130 ± 0.40	123.2±5.46	120.5 ± 1.19	116.5±1.36
DevSpklt	14.75 ± 0.85	13.25±0.47	13.75 ± 0.47	13.25±1.43	14.50 ± 0.64	15.33±0.33	18.25 ± 0.35	17.82±0.42
GnNoSpk	31.00 ± 1.58	19.75±2.56	30.00 ± 3.16	31.00±6.60	18.75 ± 3.66	18.75±4.21	40.67 ± 2.15	39.75±1.75
GnNoSpklt	2.12 ± 0.17	1.48±0.18	2.17 ± 0.18	2.26±0.25	1.28 ± 0.23	1.38±0.29	2.22 ± 0.10	2.25±0.09
GnWSpk	1.12 ± 0.04	0.78±0.08	0.83 ± 0.15	0.83±0.15	0.88 ± 0.15	0.79±0.21	1.73 ± 0.07	1.64±0.10
LowIntern10ToMat	1.90 ± 0.30	2.05±0.65	2.87 ± 0.21	3.06±0.41	2.26 ± 0.52	3.50±0.89	3.68 ± 0.45	4.3±0.23
LowInternL10	29.95 ± 1.38	29.70±1.20	32.35 ± 2.07	36.00±3.25	33.07 ± 1.66	35.38±2.39	44.25 ± 3.20	47.6±1.74
LowInternMat	31.85 ± 1.64	31.75±1.85	35.22 ± 2.16	39.07±3.48	35.33 ± 1.86	38.88±2.81	47.94 ± 3.58	51.9±1.84
PedL10 DAA	28.43 ± 1.94	24.55±0.95	21.73 ± 0.72	20.97±0.48	22.27 ± 1.69	22.92±1.67	32.85 ± 1.26	34.28±1.05
PedL10ToMat	1.72 ± 0.16	1.45±0.05	1.45 ± 0.11	1.13±0.18	1.40 ± 0.15	1.72±0.14	2.05 ± 0.14	2.4±0.13
PedLMat	30.15 ± 2.07	26.00±0.90	23.17 ± 0.75	22.10±0.58	23.67 ± 1.84	24.65±1.68	34.91 ± 1.38	36.68±1.16
Shootw_length_ratio	55.09 ± 3.59	74.10±6.01	55.24 ± 3.67	52.74±1.93	75.13 ± 4.98	78.44±4.40	75.13 ± 4.98	60.38±2.67
ShootWMat	1.14 ± 0.12	0.86±0.10	1.06 ± 0.07	1.14±0.03	0.82 ± 0.01	0.81±0.05	1.57 ± 0.08	1.49±0.07
SingGW(g)	36.44 ± 1.33	40.42±1.39	27.41 ± 2.82	27.41±1.38	47.86 ± 1.13	40.90±5.82	43.27 ± 1.59	41.38±1.88
SowToAnth	62.25 ± 2.59	67.75±2.42	65.25 ± 0.62	64.75±0.85	76.75 ± 1.54	75.00±1.35	60.25 ± 0.49	60.75±0.74
SPAD13DAA	48.45 ± 1.5	44.95±0.58	45.6 ± 1.34	44.45±0.59	43.55 ± 1.71	37.12±1.11	47.39 ± 0.80	43.3±0.89
SPAD27DAA	49.35 ± 1.95	46.55±0.63	39.00 ± 8.31	44.68±0.38	27.85 ± 8.38	30.45±8.30	44.92 ± 1.48	37.39±4.25
SPAD10 DAA	48.05 ± 1.29	45.82±1.11	44.40 ± 1.51	46.6±1.38	44.32 ± 1.28	42.32±1.48	47.37 ± 0.96	46.35±0.64
SPAD10To13	0.40 ± 0.48	-0.87±0.92	1.20 ± 0.26	-2.15±1.17	-0.77 ± 0.55	-5.20±1.77	0.02 ± 0.41	-3.05±0.64
SPAD10To27	1.30 ± 0.96	0.72±0.77	-5.40 ± 8.21	-1.92±1.46	-16.48 ± 9.19	-11.88±8.61	-2.45 ± 1.01	-8.95±40
SPAD13DAATo27DAA	0.90 ± 0.56	1.60±0.89	-6.60 ± 8.15	0.22±0.49	-15.7 ± 9.33	-6.67±7.33	-2.47 ± 1.01	-5.9±3.97
UnderdevSpklt	4.00 ± 0.70	5.00±1.08	8.00 ± 0.40	8.00±1.63	12.00 ± 0.81	10.67±0.66	3.66 ± 0.28	4.18±0.35

Appendix:1 continued…

Trait	Scepter		Scout		Spitfire		Stiletto	
	Control	Heat	Control	Heat	Control	Heat	Control	Heat
1000GnW(mg)	45.98 ± 4.12	41.94±4.31	41.66 ± 2.15	36.06±3.03	34.63 ± 2.01	35.09±2.62	42.36 ± 0.52	36.82±6.01
AnthToFLSen	57.00 ± 5.75	51.25±10.77	57.33 ± 9.02	35.25±5.25	44.5 ± 6.71	46.75±6.86	41.75 ± 5.07	39.25±5.45
AnthToMat	60.75 ± 1.43	56.5±5.36	55.67 ± 1.45	42.25±4.95	55.5 ± 3.17	52.5±2.06	61.50 ± 2.1	57.75±3.44
CulmL10 DAAToMat	4.55 ± 0.53	5.42±0.40	4.23 ± 0.57	5.60±0.20	5.32 ± 0.35	5.32±0.21	5.77 ± 0.33	5.70±0.26
CulmL10	61.20 ± 2.70	65.85±1.05	56.45 ± 1.58	68.4±3.92	68.25 ± 1.66	66.05±2.48	69.75 ± 3.66	67.65±3.69
CulmLMat	65.75 ± 2.80	71.28±1.32	61.57 ± 2.29	74.00±3.95	73.57 ± 1.86	71.38±2.51	75.53 ± 3.95	73.57±5.46
CulmLMat_PedLMat_ratio	2.50 ± 0.15	2.50±0.09	2.12 ± 0.18	2.31±0.10.00	2.06 ± 0.06	2.02±0.03	2.17 ± 0.16	2.22±0.12
DaysToMat	130.8 ± 1.49	123.5±5.69	124.5 ± 0.86	114.2±4.53	113.5 ± 2.17	111.5±2.63	130.8 ± 0.62	127.8±2.35
DevSpklt	15.25 ± 0.75	16.25±0.62	12.00 ± 1.22	14.75±1.54	17.00 ± 1.58	14.5±0.64	17.00 ± 0.57	14.75±1.25
GnNoSpk	22.50 ± 3.84	29.25±5.86	16.25 ± 4.51	24.75±6.65	40.25 ± 6.54	37.25±3.11	38.00 ± 2.16	28.25±8.81
GnNoSpklt	1.46 ± 0.18	1.79±0.33	1.27 ± 0.24	1.59±0.28	2.33 ± 0.21	2.56±0.17	2.23 ± 0.10	1.78±0.53
GnWSpk	0.99 ± 0.06	1.17±0.22	0.70 ± 0.22	0.87±0.20	1.38 ± 0.21	1.28±0.06	1.61 ± 0.10	0.92±0.31
LowIntern10ToMat	3.00 ± 0.60	3.55±0.33	2.40 ± 0.55	3.90±0.17	3.12 ± 0.29	2.80±0.20	3.80 ± 0.10	3.50±0.11
LowInternL10	36.33 ± 2.86	39.08±0.43	31.4 0± 2.06	39.23±3.63	34.85 ± 1.86	33.67±2.15	36.60 ± 1.17	36.63±1.61
LowInternMat	39.32 ± 3.15	42.62±0.46	32.13 ± 2.24	43.13±3.46	37.98 ± 2.07	36.47±2.21	40.40 ± 1.23	40.13±1.70
PedL10 DAA	24.88 ± 1.01	26.77±1.48	25.05 ± 3.14	29.10±2.30	33.4 ± 0.34	33.73±0.93	33.15 ± 2.77	31.23±3.77
PedL10ToMat	1.55 ± 0.13	1.87±0.10	1.83 ± 0.29	1.90±0.20	2.20 ± 0.12	2.40±0.05	1.97 ± 0.22	2.20±0.20
PedLMat	26.43 ± 1.13	28.65±1.54	29.43 ± 2.88	31.00±2.50	35.60 ± 0.42	36.13±0.99	35.12 ± 2.99	33.43±3.97
Shootw_length_ratio	66.16 ± 4.28	64.12±9.00	68.76 ± 6.36	70.39±2.7	67.83 ± 8.08	72.83±2.52	51.78 ± 2.75	69.39±7.88
ShootWMat	1.01 ± 0.09	1.16±0.14	0.91 ± 0.09	1.05±0.08	1.12 ± 0.11	0.98±0.06	1.46 ± 0.04	1.12±0.15
SingGW(g)	45.98 ± 4.12	41.94±4.31	41.66 ± 2.15	36.06±3.03	34.63 ± 2.01	35.09±2.62	42.36 ± 0.52	36.82±6.01
SowToAnth	70.00 ± 1.68	67.00±1.47	68.00 ± 1.52	72.00±1.78	58.00 ± 1.41	59.00±0.57	69.25 ± 1.6	70.00±1.41
SPAD13DAA	44.60 ± 0.48	46.92±0.71	44.10 ± 0.27	42.75±2.02	48.90 ± 1.16	45.75±0.63	46.47 ± 1.01	46.05±1.42
SPAD27DAA	44.62 ± 1.36	35.00±10.51	40.52 ± 2.73	14.98±9.38	43.17 ± 2.53	40.08±3.52	47.08 ± 0.9	34.88±10.39
SPAD10 DAA	44.30 ± 1.25	47.18±0.54	43.95 ± 0.45	45.65±0.54	48.50 ± 0.93	49.05±0.58	46.17 ± 1.32	46.6±0.83
SPAD10To13	0.30 ± 0.90	-0.25±0.29	0.15 ± 0.47	-2.90±1.95	0.40 ± 0.77	-3.30±0.91	0.30 ± 0.68	-0.55±2.23
SPAD10To27	0.32 ± 0.67	-12.17±10.29	-3.42 ± 2.60	-30.68±9.53	-5.32 ± 2.18	-8.97±3.92	0.90 ± 0.57	-11.73±9.60
SPAD13DAATo27DAA	0.02 ± 1.24	-11.92±10.3	-3.57 ± 2.47	-27.77±9.02	-5.72 ± 1.69	-5.67±3.94	0.60 ± 0.72	-11.17±11.81
UnderdevSpklt	10.00 ± 0.81	7.50±0.64	13.00 ± 1.29	10.00±1.58	3.75 ± 1.43	4.25±0.25	5.00 ± 0.70	8.00±2.70

Appendix:1 continued…

Trait	Suntop		Sunvale		Wyalkatchem		Yitpi		Young	
	Control	Heat	Control	Heat	Control	Heat	Control	Heat	Control	Heat
1000GnW(mg)	36.52 ± 2.92	31.48±4.53	37.37 ± 1.07	23.08±5.12	34.54 ± 5.56	32.74±4.77	47.94 ± 2.98	43.58±2.57	35.63 ± 2.22	29.49±2.97
AnthToFLSen	58.00 ± 6.64	53.25±12.41	53.67 ± 3.84	46.25±9.49	60.75 ± 4.36	53.00±12.15	39.25 ± 2.05	44.25±2.49	55.92 ± 1.85	53.58±3.61
AnthToMat	64.75 ± 2.13	53.75±8.97	57.67 ± 0.66	52.5±3.88	58.25 ± 1.54	51.75±5.43	52.75 ± 3.27	57.75±2.35	62.25 ± 0.65	61.17±2.09
CulmL10 DAAToMat	5.97 ± 0.24	5.45±0.20	3.73 ± 0.16	3.27±0.35	2.70 ± 0.33	3.32±0.48	5.05 ± 0.36	5.60±0.48	4.90 ± 0.16	4.30±0.34
CulmL10	66.05 ± 2.47	64.3±1.35	48.60 ± 2.80	47.25±3.24	38.85 ± 3.7	41.70±3.14	73.85 ± 3.63	68.5±2.58	61.34 ± 1.65	57.48±2.09
CulmLMat	72.03 ± 2.52	69.75±1.35	52.33 ± 2.97	50.53±3.45	41.55 ± 1.93	45.02±3.59	78.9 ± 3.95	74.10±3.00	66.24 ± 1.64	61.77±2.37
CulmLMat_PedLMat_ratio	2.00 ± 0.02	2.00±0.02	2.80 ± 0.14	2.48±0.06	2.02 ± 0.10	2.19±0.12	2.30 ± 0.23	2.28±0.14	1.91 ± 0.04	1.91±0.06
DaysToMat	120.8 ± 1.65	109.5±8.64	131.00 ± 3.34	130±5.01	128.5 ± 2.46	117.5±2.39	128.5 ± 5.12	129.8±1.60	120.00 ± 0.92	119.6±2.03
DevSpklt	15.00 ± 0.70	15.00±0.70	15.67 ± 0.33	15.75±1.10	11.75 ± 1.10	11.5±1.50	19.50 ± 2.25	15.5±1.75	14.08 ± 0.39	13.42±0.37
GnNoSpk	36.5 ± 3.27	35.00±3.85	5.75 ± 3.14	19.75±6.67	16.25 ± 6.14	10.75±3.01	30.50 ± 4.21	35.75±9.19	29.08 ± 3.01	25.00±3.81
GnNoSpklt	2.42 ± 0.12	2.31±0.14	0.48 ± 0.21	1.19±0.31	1.32 ± 0.43	0.87±0.19	1.65 ± 0.30	2.19±0.34	2.04 ± 0.19	1.80±0.25
GnWSpk	1.31 ± 0.09	1.05±0.07	0.21 ± 0.11	0.38±0.08	0.58 ± 0.25	0.36±0.10	1.43 ± 0.14	1.56±0.39	1.01 ± 0.13	0.73±0.11
LowIntern10ToMat	3.47 ± 0.29	3.12±0.18	2.50 ± 0.26	2.07±0.36	1.45 ± 0.37	2.13±0.21	3.06 ± 0.61	3.60±0.55	2.67 ± 0.17	2.10±0.28
LowInternL10	32.55 ± 1.18	31.8±1.08	31.00 ± 1.21	28.07±2.24	19.38 ± 0.92	23.07±1.32	42.27 ± 6.82	37.77±2.80	28.87 ± 1.6	27.18±1.53
LowInternMat	36.02 ± 1.24	34.92±1.08	33.50 ± 1.47	30.15±2.30	20.83 ± 1.19	25.2±1.32	45.33 ± 7.43	41.38±3.28	31.54 ± 1.61	29.37±1.72
PedL10 DAA	33.5 ± 1.36	32.5±0.31	17.60 ± 1.83	19.18±1.24	19.48 ± 1.59	21.67±1.57	31.7 ± 2.06	30.73±1.49	32.47 ± 0.35	30.3±1.16
PedL10ToMat	2.5 ± 0.07	2.32±0.04	1.23 ± 0.17	1.20±0.14	1.25 ± 0.100	1.60±0.20	0.00 ± 0.35	2.00±0.18	2.22 ± 0.05	2.10±0.10
PedLMat	36.00 ± 1.40	34.83±0.34	36.00 ± 1.40	20.38±1.35	20.73 ± 1.69	23.27±1.77	20.73 ± 1.69	32.72±1.64	34.7 ± 0.37	32.41±1.22
Shootw_length_ratio	52.25 ± 1.44	50.66±2.33	79.60 ± 10.43	90.57±7.14	56.50 ± 5.21	57.07±4.26	56.5 ± 3.28	56.41±1.73	68.93 ± 2.32	70.74±2.53
ShootWMat	1.38 ± 0.08	1.38±0.05	0.67 ± 0.05	0.55±0.01	0.76 ± 0.10	0.79±0.08	1.40 ± 0.05	1.31±0.07	0.97 ± 0.04	0.88±0.03
SingGW(g)	36.52 ± 2.92	31.48±4.53	37.37 ± 1.07	23.08±5.12	34.54 ± 5.56	32.74±4.77	47.94 ± 2.98	43.58±2.57	35.63 ± 2.22	29.49±2.97
SowToAnth	56.00 ± 0.57	55.75±0.47	76.67 ± 0.88	77.50±1.89	70.25 ± 3.44	65.75±5.66	75.75 ± 1.97	72.00±1.47	57.75 ± 0.60	58.42±0.86
SPAD13DAA	45.75 ± 0.57	42.88±0.96	40.40 ± 3.56	35.75±4.95	40.40 ± 3.56	37.92±3.49	49.58 ± 1.21	49.32±1.55	47.82 ± 0.77	46.98±0.89
SPAD27DAA	43.1 ± 1.93	35.98±6.38	45.27 ± 0.69	33.6±10.26	47.00 ± 1.22	28.65±10.51	35.45 ± 8.6	46.9±1.92	47.14 ± 0.93	44.48±2.58
SPAD10 DAA	44.3 ± 0.44	43.8±1.19	42.83 ± 0.92	41.38±1.66	43.65 ± 1.35	44.55±1.18	44.45 ± 4.60	47.80±1.46	47.25 ± 0.86	47.72±0.88
SPAD10To13	1.45 ± 0.80	-0.92±0.34	-2.43 ± 2.64	-5.62±3.34	-0.50 ± 0.35	-6.62±2.95	5.12 ± 4.53	1.52±0.36	0.56 ± 0.42	-0.75±0.33
SPAD10To27	-1.20 ± 1.71	-7.82±5.45	2.43 ± 1.29	-7.77±8.66	3.35 ± 1.09	-15.9±10.59	-9.00 ± 11.61	-0.90±1.78	-0.10 ± 0.64	-3.24±2.65
SPAD13DAATo27DAA	-2.65 ± 2.06	-6.90±5.79	4.86 ± 3.85	-2.15±5.32	3.85 ± 0.79	-9.27±8.15	-14.12 ± 9.67	-2.42±2.14	-0.67 ± 0.61	-2.49±2.70
UnderdevSpklt	3.75 ± 0.62	4.50±0.28	8.66 ± 0.33	8.00±1.41	10.00 ± 2.34	11.25±3.54	6.25 ± 1.88	9.75±2.17	5.16 ± 0.40	5.160±0.34