Genetic and Physiological Bases of Heat-Induced Floret Sterility in Wheat

By

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Abbreviations

Symbol	Definition
AIL	Auricle interval length
BLUPs	Best linear unbiased predictors
BPA	Bioplatforms Australia
CAT	Catalase
CDPK	Calmodulin activated calcium-dependent protein kinase
cM	CentiMorgan
CS	Chinese spring
DAPI	4'-6-Diamidino-2-phenylindole-2H Cl
DAWN	Diversity among wheat geNome
DH	Doubled-haploid
GCA	General combining ability
GDP	Gross domestic product
H^2	Broad-sense heritability
HIFs	Heterogeneous inbred families
HIFST	Heat induced floret sterility tolerance
HSI	Heat susceptibility index
IWGSC	International wheat genome sequencing consortium
KASP	Kompetitive allele specific PCR
LSD	Least significance difference
Mbp	Mega base pairs
MIPS	Munich information centre for protein sequences
NILs	Near isogenic lines
BAE	Before flag leaf auricle emergence
PCA	Principal component analysis
PCR	Polymerase chain reaction
PMCs	Pollen mother cells
POTAGE	Popseq ordered *Triticum aestivum* gene expression

Symbol	Definition
QTL	Quantitative trait loci
RCB	Randomized complete block
RILs	Recombinant inbreed lines
ROS	Reactive oxygen species
sHSPs	Small heat shock proteins
SNPs	Single nucleotide polymorphisms
SOD	Superoxide dismutase
TTC	2,3,5-triphenyl tetrazolium chloride

Abstract

The global temperature is increasing at an alarming rate, which is a major concern for wheat growers due to the adverse effects of these temperatures on crop productivity. The current study focussed on the impact of brief episodes of high temperatures during the booting stage on floret fertility in wheat, using a combined approach of plant physiology and quantitative genetics.

Wheat plants were exposed to a brief heat stress (3 days, 37/27 $^{\circ}$C day/night) when the main tiller reached a particular auricle interval length (AIL) stage. Plants were then moved back into the greenhouse where they were evaluated at maturity for floret fertility and several other morphological and physiological traits.

A total of 136 durum wheats genotypes from different part of the world (including Australia) and 26 hexaploid wheat genotypes were tested (Chapter 3) to identify heat-induced floret sterility tolerance. Both heat stressed hexaploid wheat and durum genotypes showed variability for several studied traits, including floret fertility components. The result from this experiment indicating the possibility for further genetic improvement of the wheat crop through selection and cross breeding.

A total of 144 F_1-derived double haploid (DH) lines developed from crosses between hexaploid wheat cvs. Drysdale and Waagan were used to identify QTLs for tolerance to heat-induced floret sterility. A total of 29 QTL were identified. Six of the 21 wheat chromosomes, namely 1B, 2B, 3B, 4B, 4D, and 7A, showed QTL for heat-induced floret sterility tolerance with individual QTL explaining between 6 and 49 % of the phenotypic variance (Chapter 4). Both parents contributed favourable alleles for heat tolerance. A region on chromosome 2B strongly affected heat responses of floret fertility and co-located with a locus controlling resistance to the yellow rust disease, with heat tolerance being coupled with rust resistance. Tolerance QTLs for two yield components (grain size and grain number) were independent, suggesting that breeders needed to apply selection for both of these traits when breeding for hot environments. The QTL detected on 2B may provide an appropriate target for fine mapping and marker assisted selection for improving heat tolerance in wheat.

As the QTL for tolerance to heat induced floret sterility on chromosome 2B was so strong, the genotype for the tolerance locus could be confidently inferred in many of the Drysdale × Waagan DHs, allowing the locus to be placed as a single point on the genetic map (Chapter 5). Additional gene based markers in the region were designed using wheat genomics information assembled within the Diversity Among Wheat Genome (DAWN) tool, including the NRGene wheat cv. Chinese Spring genomic sequence. These markers were then scored on the DH lines. The QTL was thereby mapped to 9.1 cM interval on the genetic map, corresponding to a region of 31.5 Mb in the genome containing 203 predicted genes. Pairs of near isogenic lines (NILs; 32 lines total) were developed from eight heterogeneous inbred families for further field, physiological and molecular studies of the locus. The 2B tolerance QTL was located 21.2 cM from the centromere in a region of high recombination, which favours prospects for positionally cloning the gene.

Experiments to identify the pollen developmental stage most sensitive to heat were conducted in Chapter 6. The intolerant cv. Drysdale was most sensitive at auricle interval lengths (AILs) of 7 cm to before 13 cm. These stages corresponded to early meiosis to late uni-nucleate microspore stage of pollen development.

In Chapter 7, mechanisms of heat-induced floret sterility tolerance conferred by the chromosome 2B QTL, and its mode of expression, were investigated. Chromatin and pollen starch staining in the cvs. Drysdale and Waagan, and RIL families derived from these cvs., showed that intolerance from Drysdale was associated with failure of starch grains to accumulate starch, but mitotic divisions in the microspores were unaffected by heat treatment. Tolerance controlled by the chromosome 2B locus was expressed in a mainly dominant manner, and was sporophytic. However no major effect of the tolerance locus on female reproduction was detected. Implications of this information for breeding strategies and the identification of the underlying tolerance gene were discussed.

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.

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Million Erena

Signature Date 02/03/2018

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Dedication

To my
*Wife "**Yodit**" and daughters "**Hermela and Fenet**"*

Chapter 1: General introduction

It has been projected that the world population and the need for food and feed are expected to increase by more than 50% by the year 2050 Munck *et al.* (2008). Wheat is the first largest among all cereals and is the third largest crop in the world with over 600 million tonnes produced globally per year (Asseng *et al.*, 2011; Barlow *et al.*, 2015; Sial, 2007). However, analyses of historic data in different areas of the world revealed substantial adverse impacts of high temperatures on wheat productivity.

According to Jones *et al.* (1999), global mean temperatures will rise by 0.3 °C per decade reaching approximately 1 and 3 °C above the current temperature by years 2025 and 2100, respectively, due to global warming. Thus, because of the damaging effect of heat on plant development, global warming will have a negative effect on plant growth performance (Bita and Gerats, 2013). In Australian wheat growing regions, the booting stage coincides with rising temperature (August-September) (Wardlaw and Wrigley, 1994), and temperatures above the optimum temperature (15ºC) are frequent. Most of the wheat crops experience an average of 4 days of severe high temperatures above 35ºC and even on occasions temperatures can reach 40 ºC.

Short episodes of heat stress prior to flowering period impact on wheat productivity by causing floret sterility (Draeger and Moore, 2017; Saini and Aspinall, 1982a). Despite of the existence of its variability among wheat genotypes, there has been little progress in breeding for heat tolerance in field trials due to lack of robust selection methods; unpredictable timing, intensity and its co-occurrence with drought stress. Although considerable work has been done on heat resistance in wheat, to date, no study has explored the genetics of tolerance to heat induced floret sterility. Therefore, developing heat tolerant wheat varieties through the genetic dissection of heat tolerance is a vital and sustainable strategy for increasing or maintaining the productivity of wheat. Further, integration of genomics and pollen based microscopic studies could provide further clues about where and how these tolerance mechanisms operate. Thus this study is potentially of great practical and scientific importance.

Chapter 2 will review the literature. Chapter 3 will report on screening 26 bread wheat (*Triticum aestivum* L.), which included parents of available mapping populations and 113 durum wheat (*Triticum durum* or *Triticum turgidum* subsp. *Durum*) genotypes, to generate

baseline information on genetic variability and identify parents for existing mapping population that could be suitable for studying the genetics of heat-induced floret sterility tolerance. Chapter 4 will report on the genetic base heat induced floret sterility tolerance and other heat related traits in the Drysdale × Waagan doubled haploid mapping population after it was exposed to heat stress at booting. The chapter will also discuss whether the genetic base of fertility tolerance escaping artefact or not. Chapter 5 will report on fine mapping of major heat tolerance QTL on gene based markers using in-house NRGene data base from DAWN resource. Chapter 6 will report on association of wheat pollen developmental stage with its auricle interval (AIL) and spike length, using a detailed study of two pairs of parents contrasting for heat tolerance (ability to maintain floret fertility upon heat exposure at the booting stage). Chapter 7 will examine the biological bases and mode of expression of heat-induced floret sterility tolerance. The thesis will conclude in Chapter 8, which includes a general discussion, conclusions, summary of contributions to the knowledge, and consideration of future directions.

Chapter 2: Literature review

2.1.Production and economic importance of wheat

Wheat (*Triticum* spp.) is an important crop in terms of the harvested area, trade value and human and animal nutrition (Chakrabarti *et al.*, 2011; Pradhan *et al.*, 2012). Out of the projected world demand for cereals (56 % increase from the base year of 2000), 26% of this increase is expected from wheat (Hubert *et al.*, 2010). Since the global demand for wheat is increasing, to reach a 1050 Mt by 2020, the global production will need to increase by 1.6–2.6% annually. In other words, the global average grain yield must be increased from 2.7 t/ha to 3.8 t/ha (Houshyar and Grundmann, 2017). The production of wheat is affected or constrained by different biotic and abiotic factors. Rust (stripe, leaf and stem) (Figlan *et al.*, 2017), weeds (Shahbaz *et al.*, 2017), viral/bacterial (Murray and Brennan, 2009), insects and nematodes (Duveiller *et al.*, 2007; Smiley and Nicol, 2009) are among the biotic stresses which hamper wheat production, whereas, heat (Yang *et al.*, 2017), drought (Páscoa *et al.*, 2017), cold (Jiafeng *et al.*, 2014), nutrient deficiency and toxicity (Ohki, 1984), salinity and water logged soil (Khan and Khan, 2017; Lutts *et al.*, 2004) are considered the major abiotic stresses. Most of the wheat growing areas of the world experience drought and heat stress (Farooq *et al.*, 2014; Semenov and Shewry, 2011; Teixeira *et al.*, 2013). Pradhan *et al.* (2012) suggested that high temperature decreased wheat yield more than drought, indicating due emphasis should be given to heat stress. Developing heat tolerant wheat varieties through genetic knowledge and tools is a potentially valuable tool for increasing or maintaining the productivity of wheat.

According to Rosegrant *et al.* (2001) the world market for wheat will grow significantly between 1997 and 2020 due to projected increases in world population. Current high demand for wheat makes it the largest grain crop worldwide (Adjemian and Janzen, 2014; Lagudah *et al.*, 2001). Australia is in the top ten highest wheat producing countries (FAOSTAT) producing around 30.0 MT of grain annually (http://www.trendingtopmost.com/worlds-popular-list-top-10/2017-2018-2019-2020-2021/agriculture/biggest-wheat-producing-countries-world/). A high proportion of the Australian crop is exported to markets in Asia and the Middle East including Indonesia, Japan, South Korea, Malaysia, Vietnam and Sudan (http://www.agriculture.gov.au/ag-farm-food/crops/wheat). Thus, it is important for gross domestic product (GDP) of Australia.

2.2. Wheat biology and diversity

2.2.1. Biology of the wheat plant

Crop plants of the genus *Triticum* are annuals with winter or spring forms. Australian hexaploid ('bread') wheats (*Triticum aestivum* L.), and durum wheat (*Triticum turgidum* subsp. *durum* (Desf) Husn), are temperate cereals sown in late autum to early winter (late April to early July) (Lawes *et al.*, 2016). The endosperm provides energy for the germinating seedling until the first leaf becomes photosynthetically functional (Simmons, 1987). Temperature is a major climatic element which influences leaf appearance and extension (Kirby, 1983). Stem development coincides with the growth of leaves, tillers, roots and the inflorescence (Patrick, 1972). Genotype (mainly at the *Rht* loci) and the growing conditions determine the height of wheat plants (ranges from 30-150 cm) (Austin and Jones, 1975). Vegetative growth for winter wheat is on average 280-350 days and a shorter 120-145 days for spring wheat. The shorter vegetative period for spring wheats is due to the absence of a vernalization requirement (Austin and Jones, 1975). The beginning of the reproductive stage is marked by the appearance of double ridges on the vegetative apical meristem, the upper of which produces spikelets. The primary tiller and other early established tillers complete their development and bear seeds more frequently than tillers that created in later stages (Kirby, 1983).

2.2.2. Wheat spike anatomy and development

The characteristics of wheat spike development, such as spike length, spikelet orientation, awn length and etc., may vary from cultivar to cultivar and with the environment. The number of leaves on the main stem is correlated with morphological changes in the wheat spike. According to Gardner *et al.* (1985), there are three major morphological events during spike development. First, a change from vegetative to reproductive growth; second, lateral spikelet development; third, initiation and development of the terminal spikelet. During the reproductive stage, the spikelets and its parts differentiate and increase in size. Spikelet differentiation and development begins in the middle of the spike and proceeds toward the base and upper part of the spike. Within each spikelet, differentiation begins with the glumes, and then individual flower (floret) differentiation begins with the most basal florets and proceeds upward. Within each floret, the sequence of differentiation of its organs proceeds from the

outside inward, i.e., lemma, palea, anther and finally pistil (Bonnett, 1936). The sequence in the pistil is ovary, style then stigma (Bonnett, 1936). Reproductive development in wheat is also affected by stress at the transition period from the vegetative to the reproductive growth stage (Maas and Grieve, 1990).

2.2.3. Genome and diversity of wheat

Hexaploid wheat is the product of two hybridisation events. Firstly, hybridisation of the A genome progenitor with the B genome progenitor which eventually gave rise to durum wheat (2n=4x=28, AABB) containing the cytoplasm of the B genome donor. Secondly, hybridisation occurred between tetraploid wheat (AABB) and the D genome progenitor (Kimber and Sears, 1987). Kimber and Tsunewaki (1988) confirmed the hybrid occurred with the B genome cytoplasm to form the AABBDD hexaploid

The genome of wheat is large (~16 Gb) (Brenchley *et al.*, 2012; Consortium, 2014) compared to that of Human (3,000 Mb), rice (400 Mb) and *Arabidopsis thaliana* (130-140 Mb) (Martínez-Pérez *et al.*, 1999). Hexaploid wheat contains three closely related genomes (A, B and D) derived from three progenitor species. Gene redundancy is thus common, meaning that a triplicate homoeoallelic set is present for most genes, which complicates genetic analysis (Lee *et al.*, 2017). The *Ph1* mutation prevents the homoeologous chromosomes from pairing, allowing it to behave like a diploid at meiosis. Hexaploid wheat contains 42 chromosomes in the 2N state; in those three different genomes each contribute seven chromosomes to give a hexaploid compliment. Thus, the seven chromosomes from each of the three genomes in duplicate give rise to the 42 chromosomes. Similarly tetraploid (durum) wheat contains 28 chromosomes in the 2N state.

Genetic variability is of prime interest to the plant breeder because proper management of this diversity can produce permanent gain in the performance of the character of interest. Different authors revealed the existence of genetic variability within hexaploid and tetraploid wheat species (Bennett *et al.*, 2012; Blum *et al.*, 2001; Mason *et al.*, 2013; Moffatt *et al.*, 1990; Onyemaobi *et al.*, 2016; Shirdelmoghanloo *et al.*, 2016c; Tiwari *et al.*, 2013; Wu *et al.*, 2014; Zivy, 1987).

2.3. Impact of heat stress on wheat and the wheat industry

2.3.1. Definitions of heat stress and heat tolerance

Heat stress can be defined as a rise in temperature beyond the threshold level for a period sufficient to cause damage to plant growth and development (Singh *et al.*, 2012; Wahid *et al.*, 2007a). Whereas, heat tolerance can be defined as the ability of the plant to maintain the integrity of cellular and subcellular structures and metabolic pathways to allow continued plant growth and development and reproduction during and after exposure to heat stress (Singh *et al.*, 2012; Wahid *et al.*, 2007a). Further, maintenance of high ton per hectare yield, good physical grain characteristics (low screenings) and processing quality (dough strength, etc.) are among commercial definitions for heat tolerance in wheat.

2.3.2. Effect of heat on wheat

Heat at booting causes floret sterility (Saini and Aspinall, 1982a; Saini *et al.*, 1983), heat just after anthesis can cause grain abortion (Tashiro and Wardlaw, 1990), and heat a little later (but still early in grain filling) impacts grain size, but not grain number (Stone and Nicolas, 1995). Heat at any stage can accelerate the development of the whole plant, shortening the time for accumulating mass (including in the grain). Figure 2.3 shows changes in plant parts due to heat stress.

a. Effect of heat on wheat floret organs

Pollen sterility, drying of the stigmatic fluid, empty pockets in the endosperm and shrivelled seeds are among the effects of heat stress in wheat (Kumar and Rai, 2014). Under heat stress conditions, to cope with the heat stress, crop plants may divert resources so that limited photosynthate can be available for reproductive development. Heat accelerates senescence, potentially affecting seed set and grain filling (Siddique *et al.*, 1999). Both male and female gametophytes are sensitive to high temperature and their responses vary with genotype; however, ovules are generally less heat sensitive than pollen (Wahid *et al.*, 2007a; Willits and Peet, 1998). After heat is applied at the sensitive stage, florets become abnormal both structurally and functionally as manifested by shrivelled or/and small pollen grains (Saini and Aspinall, 1982); or grain become sterile, parthenocarpic or abortive (Tashiro and Wardlaw,

1990). In addition to this, in heat stressed ovaries, the viable pollen tube can grow haphazardly and for a short distance due to lack of attraction from the embryo sac. Ji *et al.* (2010) investigated the effect of drought on starch accumulation of both male and female gametophytes of hexaploid wheat. Based on their results, the stress applied at the young microspore stage caused irreversible starch depletion and damage to the anther of the drought sensitive wheat variety and the effect of water stress on ovary starch accumulation appeared to be reversible upon rewatering. The difference in storage carbohydrate accumulation in drought sensitive and tolerant wheat was correlated with differences in sugar profiles, cell wall invertase gene expression of fructan biosynthesis genes in the anthers and ovaries.

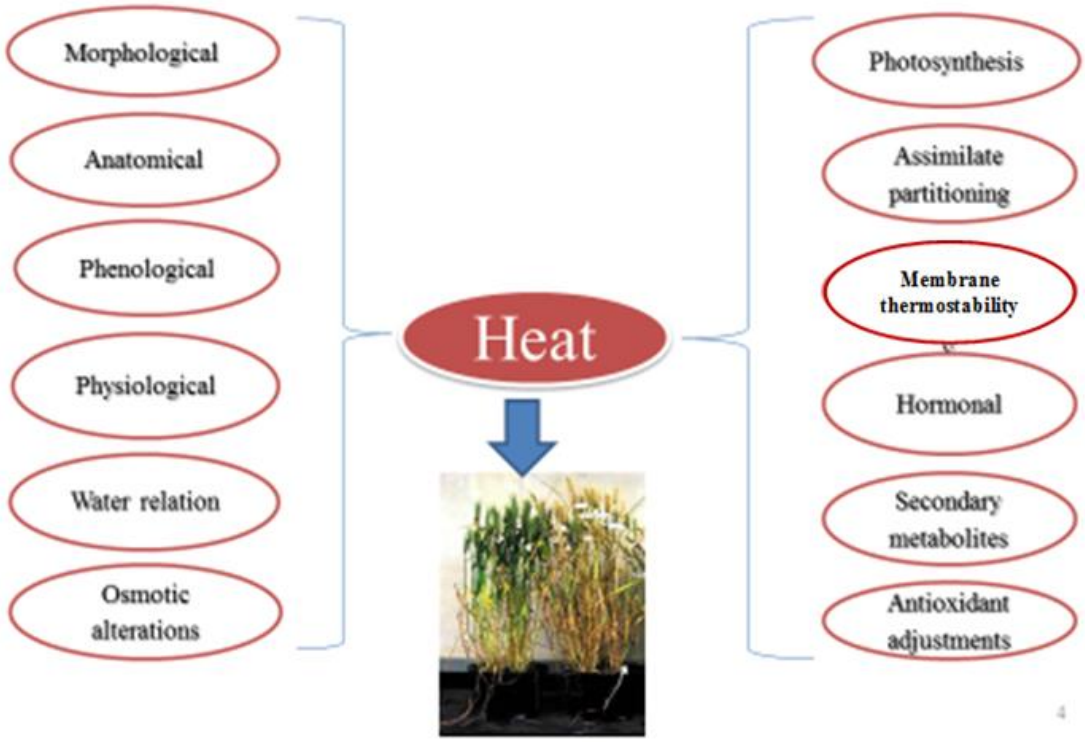

Figure 2. 1 Heat stress-induced changes in plants (Adopted from Singh et al. (2012)

The development of haploid pollen grains occurs within the anther, where the sporogenous cells are surrounded by diploid sporophytic tissue including the the tapetum. Normally, tapetal tissue plays an important role in providing nutrients to developing pollen grains, and its premature degeneration could thereby affect pollen development. With heat stress, premature degeneration of the tapetal tissue and lack of endothecium formation were observed in cowpea (Ahmed *et al.*, 1992) and common bean (Porch and Jahn, 2001), which could have been responsible for the low pollen viability. Further in cowpea, Mutters *et al.* (1989) suggested that heat injured the tapetal tissue, reducing its development and hastening its degeneration. Tapetal malfunction has been considered the cause of some forms of genetically controlled male sterility in various plant species (Dundas *et al.*, 1981; Nakashima *et al.*, 1984).

b. Developmental response of reproductive organs to heat

Saini *et al.* (1983), studied the nature of heat-induced female infertility in wheat. In the female organs, reduced development and degeneration of the nucellus, and reduction in size of the embryo sac, were observed when wheat was exposed to heat at meiosis. Accordingly, from the onset of meiosis in anthers until the conclusion of tetrad break up (beginning of microspore release to form the young microspore), and during the first pollen mitosis, was the most sensitive stage of wheat for heat induced pollen sterility, whereas from late meiosis to the beginning of megaspore selection during female embryo sac formation was determined to be the most sensitive period for female sterility (Saini and Aspinall, 1982a). In many plant species, heat has been shown to induce sterility when applied prior to anthesis (Singh *et al.*, 2012).

Variation in responses of ovules to heat-stress has been observed and might reflect differences in ovule developmental stages at the time of heat stress including those due to the order of floret development across the spike (Saini *et al.*, 1983). Variation of sensitivity of pollen within a spikelet could be due to developmental differences between the most advanced primary (basal-most) floret and the least advanced tertiary (upper-most) florets (Saini and Aspinall, 1982a). In addition, Saini and Aspinall (1982a) observed the existence of viable and inviable pollen grains within a single anther and proposed that this was due to asynchrony in development of pollen mother cells (PMCs) within the anther.

2.4. Stage of wheat that is sensitive to heat stress

The male reproductive organ is considered to be the most sensitive to heat stress in most crop species including wheat (Paupière *et al.*, 2014; Saini and Aspinall, 1982a, b). This phenomenon might be linked to the availability of sugars. Sink strength of anthers is thought to be high from the microspore mother cell stage to vacuolated microspore stage whereas during anthesis and grain filling period sink strength is strong for the ovary (Ji *et al.*, 2010). This could explain why the female reproductive organs are more resilient to heat stress than the male reproductive organs.

Though the critical pollen developmental stages for heat sensitive may vary with species, at or around meiosis seems to be a commonly reported sensitive stage for many crop species. Between 10 and 7 days before anthesis was considered as the most sensitive stage for tomato (Pressman *et al.*, 2002; Sugiyama *et al.*, 1965); between 9 and 7 days before anthesis (after the release of tetrads) for cowpea (Ahmed *et al.*, 1992); 4 days before anthesis (at microspore mother cell meiosis) for bell pepper (Erickson and Markhart, 2002); 7–12 days before anthesis (during microsporogenesis) for *Phaseolus vulgaris* (Porch and Jahn, 2001); at anthesis for peanuts (Prasad *et al.*, 2001) and during meiosis for barley (Sakata *et al.*, 2000).

Draeger and Moore (2017) studied a wheat cv. Chinese Spring aneuploid stock that is heat sensitive because it lacks chromoosme 5D. They identified the period from premeiotic interphase to late leptotene as the heat susceptible stage for that line. In contrast, Saini *et al.* (1984) reported that from the onset of meiosis in pollen microspores until the conclusion of tetrad break up and during the first pollen mitosis was the most sensitive stages for heat induced pollen sterility in wheat. The former stage coincided with the female sensitive stage which is from the late part of meiosis to the beginning of megaspore selection during female embryo sac formation (Saini and Aspinall, 1982a).

Investigating the nature and response to heat stress should be useful for understanding tolerance mechanisms. The exact floret developmental stage of wheat that is sensitive to heat is not clear. Studies involving exposure of wheat to heat stress at various pollen developmental stages could help to resolve this.

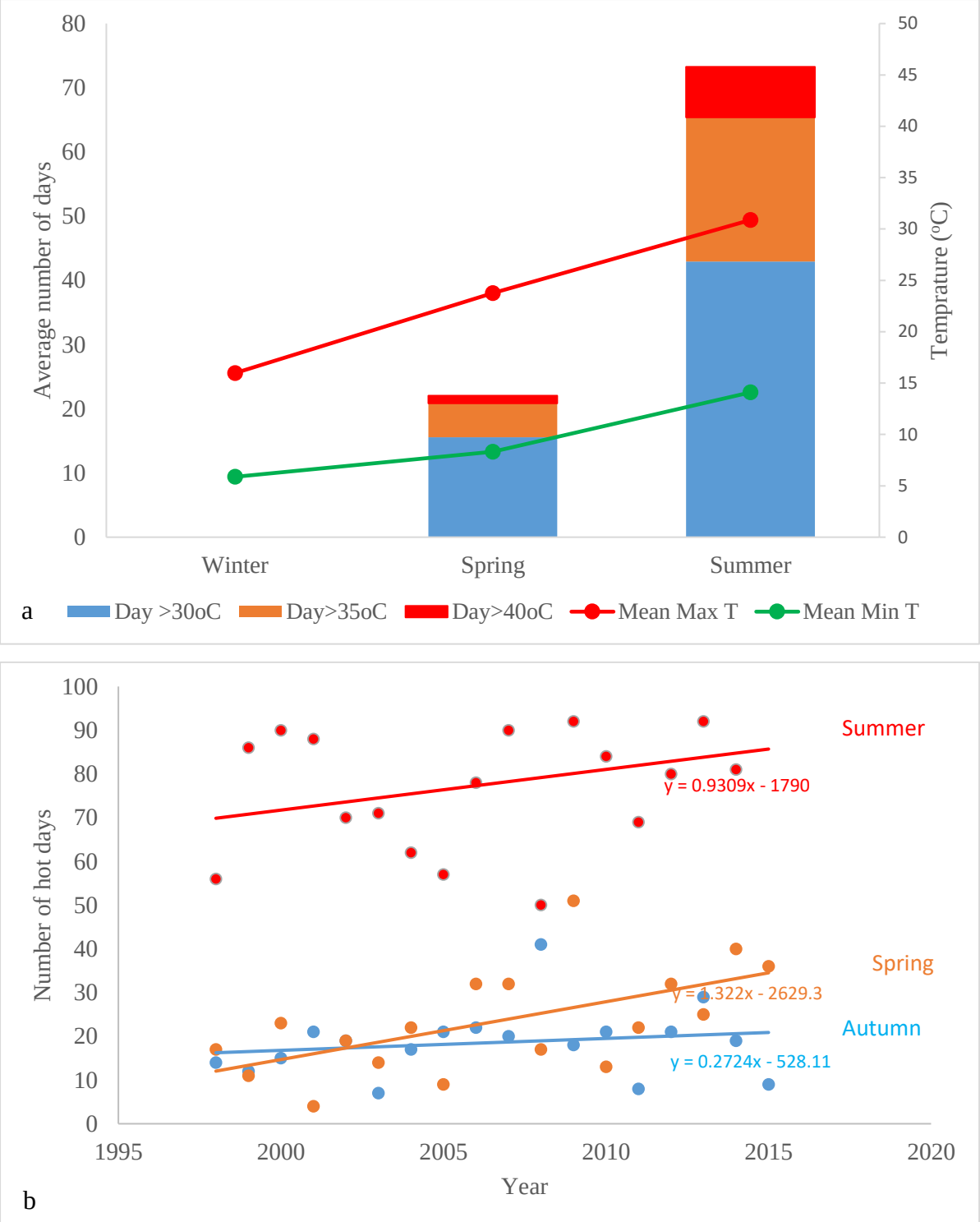

Figure 2. 2 Representation of twenty years of temperature data collected at Roseworthy, South Australia (1997-2016). Average minimum and maximum daily temperature, number of days > 30, >35 and >40 °C (a); Number of hot days per Autumn, Spring and Summer across the years 1997-2016 (b).

2.5. Fertilization and grain number determination in wheat

2.5.1. Fertilization

In the wheat floret, the female reproductive organs contain the embryo sac - an egg apparatus made up of an egg cell and two synergids at the micropylar end and a central cell with two large polar nuclei in the middle. During maturation, the anther dehisces and the pollen falls on the stigma. The grains germinate and pollen tubes enters one synergid through the filiform apparatus from the micropyle (You and Jensen, 1985). Half an hour after pollination, the two generative nuclei appear - one each in the cytoplasm of the egg and in the central cell. Oone sperm contacts the egg nucleus, while the other sperm furses with one of the polar nuclei - a process called double fertilization (GAO *et al.*, 1992; Hoshikawa, 1959). Thereafter, the fertilized egg divides and differentes, giving rise to the plant (Sprunck *et al.*, 2005).

2.5.2. Grain number determination

Number of grains per unit area is recognised as the main yield component explaining variation in wheat yield (Fischer, 2008; Slafer, 2003). This reflects the observation that grain growth is not normally limited by source strength (Borrás *et al.*, 2004) even in Mediterranean environments (Cartelle *et al.*, 2006). The number of fertile florets is determined during stem elongation (Kirby, 1988); (Fischer, 2008; Miralles and Slafer, 2007). This process can be affected by crop growth and partitioning to the growing spikes (Fischer, 1993; González *et al.*, 2003; Prystupa *et al.*, 2004). Thus, any stress during these periods can dramatically reduce grain number and hence yield (Demotes-Mainard and Jeuffroy, 2004; Fischer and Stockman, 1980; González *et al.*, 2005a; Thorne and Wood, 1987).

The wheat spikelet is an indeterminant structure, and degeneration (premature death and arrest of development) of whole florets in the apical most positions of the spikelets, and arrest of any further growth at the apex of the spikelets, occurs during (Bonnett, 1966; Fisher, 1973). This process determines the number of 'potentially fertile florets' per spikelet – referring to those florets that remain green and develop well defined floral structures (Guo and Schnurbusch, 2015). Additionally, unfavourable conditions pre-anthesis may cause some sterility (failed grain set) of potentially fertile florets. Researchers almost invariably fail to

distinguish between these two potential determinants of floret sterility. Consequently, the relative importance of various conditions that lead to one vs. the other are very poorly defined.

2.6. Mechanisms of heat stress tolerance

Effects of heat stress include a decline in photosynthesis, an increase in photorespiration, a reduction in water availability, a loss of integrity and function of the cell membrane and production of reactive oxygen species (Howarth, 2005; Singh *et al.*, 2012; Wahid *et al.*, 2007a). The extent and types of heat effects in wheat crops depend on the developmental stage at the time of heat exposure, and the intensity of the heat (Prasad *et al.*, 2017). In response to heat, wheat shows various responses at the molecular level, including production of certain miRNAs (Kumar *et al.*, 2014a), signaling molecules, transcription factors (Xue *et al.*, 2015), stress associated proteins like heat shock proteins (HSPs) (Kumar *et al.*, 2014b) and antioxidant enzymes. However, the extent to which these molecular factors protect against heat induced floret sterility in grasses, including wheat, have not been fully determined (Barnabás *et al.*, 2008; Harsant *et al.*, 2013; Hedhly, 2011; Parish *et al.*, 2012).

In another cereal crop, *Sorghum bicolor*, heat reduces pollen viability which also linked to carbohydrate metabolism (Jain *et al.*, 2007; Prasad and Djanaguiraman, 2011) In tomato, Pressman *et al.* (2002) found that high temperature decreased the number of viable pollen grains and decreased the levels of soluble sugars in pollen and the anther walls, but increased sugars in the locular fluid. Sugar is a primary metabolite that is an essential precursor for various metabolic pathways and plant nutrition (Paupière *et al.*, 2014). According to Ji *et al.* (2010), in the anther of drought tolerant wheat, the amount of fructo-oligosaccharides responsible for fructan biosynthesis (non-reducing sugar) was increased as compared to the susceptible genotype. High temperature blocks transportation of soluble sugar into locular fluids so that it can't reach the pollen (Paupière *et al.*, 2014). Heat stress applied during 3-4 days before anthesis, reduces starch concentration and alters transport and metabolism of sugar as well as its distribution over the various anther tissues. This might be linked with the alteration of invertase activities involved in carbohydrate metabolic pathways. Ji *et al.* (2010) observed reduced expression of the cell wall invertase gene *IVR1* in anthers and ovaries of drought sensitive wheat after the tetrad stage. Koonjul *et al.* (2005) observed expression of the *IVR1* gene in the tapetum and around the vascular bundle of the wheat anther. Thus, cells in

this tissues where *IVR1* gene is expressed are thought to be located to control sugar transport to the tapetum and pollen grain (Ji *et al.*, 2010). Paupière *et al.* (2014) suggested a role for the activities of invertase and abundance of soluble sugars and starch in maintaining pollen viability under high temperature.

The effect of heat stress is complex. Generally, the effect of heat in the plant cell begins with plasma membrane disruption, osmotic changes, and ionic effects (Singh *et al.*, 2012). Downstream signals and transcriptional cascades that activate stress-responsive mechanisms for reestablishment of homeostasis and protection and repair of damaged proteins and membranes might occur (Singh *et al.*, 2012; Wahid *et al.*, 2007a). Irreversible damage to cellular homeostasis and destruction of proteins and membranes (Bohnert *et al.*, 2006) leading to cell death might be due to inadequate responses at one or more steps in the gene activation processes (Bohnert *et al.*, 2006; Vinocur and Altman, 2005).

Several signalling molecules such as reactive oxygen species (ROS), Ca^{2+} and hormones reprogram gene expression (Suzuki and Mittler, 2006). Heat stress sensors are thought to be located in the thylakoid membrane (Plieth, 1999). These sensors are capable of detecting physical phase transitions, eventually leading to conformational changes in the membranes through cycles of phosphorylation and dephosphorylation. The presence of highly unsaturated lipids and temperature-sensitive photosystems make thylakoid membranes a crucial heat stress sensor (Sung *et al.*, 2003).

In the plant cell heat stress affects the plasmalemma, making it more fluid. It leads to the induction of Ca^{2+} influx and cytoskeletal reorganization, resulting in the up-regulation of mitogen-activated protein kinases (MAPK) which in turn activate various transcriptional factors via phosphorylation cascades, which then regulate the expression of genes involved in stress adaptation (Singh *et al.*, 2012). In a different pathway, calmodulin activates calcium-dependent protein kinase (CDPK) activated by Ca^{2+}. CDPK is involved in the regulation of expression of different heat shock protein (HSP) genes. Antioxidants and compatible osmolytes for osmotic adjustment may also be produced (Wahid *et al.*, 2007a).

Production of reaction oxygen species (ROS) including singlet oxygen ($^1O_2$) (Wahid *et al.*, 2007b), the superoxide free radical (O^{2-}), hydrogen peroxide (H_2O_2), and the hydroxide radical (OH^-) in the organelles (e.g., chloroplast and mitochondria) are symptoms of cellular

injury caused by high temperature. This invokes signalling as well as production of antioxidants (Bohnert *et al.*, 2006; Liu and Huang, 2000).

Under heat stress, an increase of Ca^{2+} in the cytosol may alleviate heat injury, increasing the activity of antioxidants (Gong *et al.*, 1997). Accordingly, Sairam and Tyagi (2004) proposed that in wheat genotypes, the capacity to acquire thermotolerance is associated with catalase (CAT) and superoxide dismutase (SOD) activities, higher ascorbic acid content, and less oxidative damage.

Heat stress altered patterns of gene expression (Yang *et al.*, 2006) include expression of the heat shock protein (HSP) complements and inhibition of the expression of many other genes (Yost and Lindquist, 1986). mRNAs encoding non heat-stress-induced proteins are destabilized during heat stress. Out of the five conserved families of Hsps (Hsp100, Hsp90, Hsp70, Hsp60 and sHsp), small heat shock proteins (sHsps) are found to be more prevalent in plants (Singh *et al.*, 2012). However, each major HSPs family has a unique mechanism of action of chaperonic activity. The HSPs/chaperones interact with other stress-response mechanisms (Wang *et al.*, 2004). The HSPs play a role in stress signal transduction as well as gene activation (Nollen and Morimoto, 2002), and regulating cellular redox state (Arrigo, 1998). They also interact with other stress-response mechanisms such as those involved in the production of osmolytes (Diamant *et al.*, 2001) and antioxidants (Panchuk *et al.*, 2002).

Membrane lipid saturation is considered an important element in high-temperature tolerance. In wheat, high linolenic and *trans*-3-hexaldecanoic acid concentrations are positively correlated with heat resistance (Behl *et al.*, 1996).

2.7. Breeding wheat for heat stress tolerance

Understanding heat stress response mechanisms and their genetic control could be useful for improving heat-stress tolerance. Traditional and contemporary molecular breeding protocols and transgenic approaches are among the improvement strategies that can be considered (Wahid *et al.*, 2007a). Tomato is one of the few examples of a crop species in which improvements to heat tolerance through the use of conventional breeding has been documented

(Scott *et al.*, 1995). Success in ussing genetic transformation to improve heat tolerane has also been limited, but has been reported for tobacco (Murakami *et al.*, 2000).

2.7.1 Conventional breeding approaches and its limitations

Progress in improving stress tolerance of a crop can be assisted by an understanding of the physiological mechanism and the genetic basis of stress tolerance at whole plant, cellular and molecular levels. Factors that hamper selection for heat tolerance directly in the field include: Sensitivities of specific develomental stages to the various effects of heat, variation in development rates (e.g., time to flowering) between genotypes, unpredictability of the timing and intensity of heat stress events in the field, and other uncontrollable environmental factors that may influence heat stress responses and plant performance.

Growing breeding materials in heat-prone environments and screening them for greater yield is one of the traditional approaches to select for heat tolerance. However, other biotic and abiotic stressors, specifically drought, frequently co-occur with heat, and can exacerbate its effects, making the selection process difficult. Wheat cultivars capable of maintaining high thousand-kernel weight (Reynolds *et al.*, 1994), membrane thermostability, leaf chlorophyll content during grain filling, high stomatal conductance and photosynthesis, or a cooler canopy, are considered as heat stress tolerant (Reynolds *et al.*, 1994; Singh *et al.*, 2012).

Plant breeding strategies for heat stress tolerance utilizing genetic variation from genotype collections, interspecific or intergeneric hybridization and induced mutation have produced only limited success for several reasons. Data from extensive international yield trials in more marginal environments indicate yield progress (2–3% per year) in both semi-arid and heat-stressed environments between 1979 and 1995 (Reynolds and Borlaug, 2006). This progress has been limited by a lack of tolerance genes in sexually compatible gene pools, complexity of the heat tolerance traits, a lack of understanding of high temperature tolerance genetic mechanisms, low genetic variance of yield components and a lack of efficient selection techniques. These are some of the factors that contribute to the conventional breeding limitations (Singh *et al.*, 2012).

2.7.2. Molecular breeding approaches

Heat tolerance components are controlled by different sets of genes at different developmental stages or in different tissues (Bohnert *et al.,* 2006). The use of genetic stocks with different degrees of heat tolerance, correlation and co-segregation analysis, molecular biology techniques and molecular markers to identify tolerance quantitative trait loci (QTL) are promising approaches for discovering the genetic basis of heat tolerance (Maestri *et al.*, 2002). Quantitative trait locus analysis is a powerful tool for quantitative and qualitative genetic analysis of complex traits (Roff, 1997; Shah *et al.*, 1999).

a. Molecular markers

To track variation in progeny at the DNA level, breeders exploit molecular markers. Nowadays Single Nucleotide Polymorphism (SNPs) have become popular as a basis for molecular markers because of their genome-wide abundance and amenability for high- to ultra-high-throughput detection platforms (Mammadov *et al.*, 2012). In wheat, marker-assisted selection has been applied for simple traits (Gupta *et al.,* 2010). However, to improve complex polygenic traits, high-plex genotyping platforms such as DArT should be implemented.

Recently a large number of SNPs have been characterized in the wheat genome mainly due to the availability of next generation sequencing technologies. The iSelect 9k genotyping array of 9,000 gene-associated SNPs is available for wheat genotyping (Cavanagh et al., 2013). Another high-density SNP genotyping array is the iSelect 90K array (Wang et al., 2014). Kompetitive Allele Specific PCR (KASP) marker assays provide a cheap and efficient means of scoring a limited numbers of markers on a large number of DNA samples, and have been developed for marker-assisted selection of several agronomically important loci (Rasheed et al., 2016). These resources and technologies provide researchers with significant capacity to undertake genetic analysis in wheat, and breeders with opportunities to maximize genetic gains.

b. Quantitative trait loci regulating heat and drought stress tolerance in wheat

Canopy temperature depression (Blum *et al.*, 1982) and low tissue electrolyte leakage (Saadalla *et al.*, 1990) have received attention as heat stress tolerance related traits. The former was associated with maintenance of higher leaf water potentials, while the latter was associated with greater grain yield and grain size. The information on genetic control of these traits could

aid selection of tolerant germplasm. Accordingly, Porter *et al.* (1995) examined genetic control of acquired high-temperature tolerance using a cell viability test based on 2,3,5-triphenyl tetrazolium chloride (TTC), revealing that only the general combining ability component effect was significant, accounting for 67% of the total genotypic variation. Moffatt *et al.* (1990) studied the genetic control of high-temperature tolerance based on chlorophyll fluorescence and revealed significant general combining ability (GCA) and maternal effects. According to Ibrahim and Quick (2001), the mean square for GCA was four times that of specific combining ability, indicating the importance of additive gene effects in acquired thermal tolerance. Yang *et al.* (2002) used heat tolerant and susceptible wheat varieties to examine inheritance of heat tolerance. Two SSR markers, *Xgwm11* (had only additive gene action), and *Xgwm293* (had both additive and dominance action), were found by QTL analysis in an F_2 population to be linked to grain filling duration under heat stress at grain filling. These results reveal that heat tolerance of wheat is controlled by several genes and theat there may be potential for exploiting additive genetic effects in improvement of acquired high-temperature tolerance.

Despite the detrimental effects of heat stress on yield and quality in wheat, no work has been reported to identify loci controlling heat tolerance of floret fertility (Table 2.1). Studies have been done in hexaploid bread wheat Ogbonnaya *et al.* (2017); Pinto *et al.* (2016); Shirdelmoghanloo *et al.* (2016c); Talukder *et al.* (2014); Mason *et al.* (2013); Tiwari *et al.* (2013); Ali *et al.* (2013); Pinto *et al.* (2010); Kumar *et al.* (2010) related to heat tolerance.

Positional cloning means the identification of a gene sequence responsible for a phenotype on the basis of its mapped genetic location on a chromosome. Positional cloning in wheat has been successful for traits with a clear phenotype such as the three vernalisation genes *VRN1, VRN2* and *VRN3* (Yan et al., 2006; Yan et al., 2004; Yan et al., 2003), the boron tolerance gene *Bo1* (Schnurbusch et al., 2007), the leaf rust resistance genes *Lr1* (Qiu et al., 2007), *Lr10* (Feuillet et al., 2003) and *Lr21* (Huang *et al.*, 2003), the powdery mildew locus *Pm3* (Yahiaoui et al., 2004), a locus controlling homologous chromosome pairing during meiosis in wheat (*Ph1*) (Griffiths et al., 2006), the *Gpc-B1* locus controlling grain quality related traits and flag leaf senescence (Uauy *et al.*, 2006) and the Q gene related to threshing and spike morphology (Faris *et al.*, 2003).

Table 2. 1 Some heat stress tolerance QTL reported in wheat

No of QTL	Traits	Condition	Reference
27	25 different morph-physiological traits including yield	Under field condition (2010 to 2013) in Sudan, Egypt and Syira.	Ogbonnaya *et al.* (2017)
2	Stay green and grain size tolerance (HSI)	3 d of heat treatment during grain filling period	Shirdelmoghanloo *et al.* (2016c)
6	Stay green	Mexico under hot-irrigated environments (2005-20013)	Pinto *et al.* (2016)
5	Thylakoid membrane damage, plasmamembrane damage, and SPAD chlorophyll content	Heat treatment at 8 days after anthesis in heat chamber for 10 d	Talukder *et al.* (2014)
25	15, e.g., kernel weight, grain filling period, flowering and maturity date, biomass, harvest index.	Field, with heat stress occurring at grain filling (2008-2010, Texas)	Mason *et al.* (2013)
7	Grain filling, thousand seed weight, grain yield and canopy temperature	Late sowing in the field, heat occurred during flowering (2007-2010, India)	Tiwari *et al.* (2013)
3	Flag leaf chlorophyll, temperature depression and single kernel weight	Heat treatment at heading in heat chamber for 8 d	Ali *et al.* (2013)
16	Grain yield and canopy temperature	Late sowing in field (2002-2006, Mexico)	Pinto *et al.* (2010)
3	Stay green	Field, average 28 ^{o}C during anthesis & 38^{o}C during grain filling (2004-2006, India)	Kumar *et al.* (2010)
2	Grain filling period	Heat during grain filling in a growth chamber	Yang *et al.* (2006)

2.8. Inheritance of abiotic stress tolerance

Heat stress tolerance is multigenic and made up of the interaction between different yield components and environmental effects. This is the reason why it is necessary to know the genetic architecture of heat-induced fertility. Generation mean analysis is a simple but useful technique for estimating gene effects for a polygenic trait, its greatest merit lying in the ability

to estimate epistatic gene effects such as additive × additive (aa), dominance × dominance (dd) and additive × dominance (ad) effects (Singh and Singh, 1992). Breeders would also like to know how much of the variation in a crop is genetic and to what extent this variation is heritable, because efficiency of selection mainly depends on additive genetic variance, influence of the environment and interaction between genotype and environment. Inheritance of heat tolerance during pod set in cowpea was shown to be governed by a single dominant gene (Marfo and Hall, 1992) in analyses of pods per peduncle and proportions of tolerant plants in F_1, F_2, and backcross populations.

2.9. Aims of the thesis

Short periods of heat stress just before flowering impact wheat yields by causing floret sterility (failed fertilization or initiation of grain growth). Genes and molecular mechanisms controlling variation in heat-induced sterility are currently not known, but such knowledge could provide breeders with tools to create new tolerant varieties. The main aims of this study were to examine the genetic basis and mechanisms associated with heat tolerance during the booting stage. Therefore, a number of experiments were conducted with the following objectives:

1. To investigate variation in tolerance to heat induced floret sterility in Australian bread wheat genotypes and exotic durums, to identify mapping populations suitable for genetic analysis of tolerance, and tolerance sources that could be used in breeding

2. To identify chromosomal regions (QTL) affecting heat-induced floret sterility in bread wheat and test whether the genetic basis of heat tolerance at booting and grain filling stages are the same or different

3. To fine map the heat-induced floret sterility tolerance QTL on 2B chromosome

4. To identify the sensitive tiller stage for heat-induced floret sterility and the corresponding stage of pollen development, in the cvs. Drysdale and Waagan

5. To investigate the biological basis and mode of expression of a major QTL for tolerance to heat-induced floret sterility on 2B chromosome

Chapter 3: Genetic variability of heat-induced floret sterility in elite hexaploid wheat (*T. aestivum* L) and durum (*Triticum turgidum* subsp. *durum* (Desf.) Husn.)

3.1. Abstract

Since there is a scarcity of information on genetic variability of tolerance to heat-induced floret sterility in wheat, 26 spring hexaploid wheats (*Triticum aestivum* L.) and 136 spring durum wheats (*Triticum turgidum* subsp. *durum* (Desf.) Husn) were screened in separate experiments for tolerance. Heat significantly reduced floret fertility in both durum and hexaploid wheats, and there was significant genetic variation for these responses, indicating a potential for genetic analysis of heat tolerance leading to the discovery of tolerance QTL. Overall, heat induced floret sterility was much higher in durum than in hexaploid wheat, even allowing for the different AIL stages used for treatment. A number of available hexaploid bi-parental mapping populations were found to have parents that contrasted for tolerance, suggesting these families would be suitable for genetic analysis to discover tolerance QTL. The screen of exotic durum accessions identified a number of consistently tolerant accessions. These have the potential to be used as tolerance donors in breeding programs to improve the tolerance of Australian durum varieties.

3.2. Introduction

The effects of heat stress on grain number is generally considered to be the main source of wheat yield losses due to heat stress. The growth period most sensitive to the effects of heat stress on grain number is from double ridge stage to anthesis. Also, the effects of heat on grain number derive from reductions in both spikelet number per spike and kernels per spike (Shpiler and Blum, 1990), To our knowledge, no QTL for heat induced floret sterility have yet been reported.

Genetic diversity for heat tolerance is needed to improve wheat's adaptation to warmer areas (Hede *et al.*, 1999). At least in Australia, durum has the reputation of being more heat sensitive to heat induced floret sterility than hexaploid wheat (personal communications from wheat breeders, via Nick Collins). There has been a report of this being the case abroad too (Fu *et al.*, 2015). Thus, sources of tolerance to heat induced floret sterility are needed to correct

this deficiency in Australian durums. Such tolerance sources may be found by screening materials from collections of overseas durum wheat.

Ali *et al.* (2010) reported variation for heat tolerance at grain filling in 16 tetraploid lines and found several that had significantly higher heat tolerance. Apart from this, information on heat tolerance of tetraploid wheat seems very limited (Fu *et al.*, 2015) and to our knowledge there seems to be no report on heat-induced floret sterility in durum wheat.

When (Shirdelmoghanloo *et al.*, 2016c) applied a 3d heat stress applied during early grain filling (10d after anthesis) they found a strong positive correlation between heat induced reductions in final grain weight and flag leaf chlorophyll. However, it is currently not known whether there is also a correlation between staygreen and floret fertility heat tolerance.

Florets of hexaploid wheat have been found to be most sensitive to heat induced sterility from the beginning of meiosis (in the anthers) to the first mitosis in the microspores (Saini and Aspinall, 1982a; Singh *et al.*, 2012). External architectural features are needed to identify tillers at this developmental stage to define the appropriate time to heat treat plants for routine tolerance screening. In rice, auricle interval length (AIL), which is the distance between the bases of the two last leaf blades, has been used for this purpose (Jagadish *et al.*, 2014; Zee and Ye, 2000; Zhou *et al.*, 2010). Unpublished data from Nick Collins' lab has suggested that hexaploid wheat and durum beome sensitive at around 6 and 3 cm on average, respectively, with some variation in sensitive stage also between genotypes within these two wheat categories.

The current work was undertaken to (a) identify sources of tolerance for heat induced floret sterility in overseas durums that could be used to improve the tolerance of Australian durums, (b) further characterize the extent of genetic diversity for heat-induced floret sterility in hexaploid wheat, including in parents of available mapping populations, and (c) characterize traits under heat and control conditions, for heritability and correlations, in both hexaploid wheat and durum.

3.3. Material and methods

3.3.1. Plant genetic materials

Two experiments were conducted to screen genotypes for tolerance to heat induced floret sterility; one each for durum and hexaploid wheat. There were 26 spring hexaploid wheat (*Triticum aestivum* L.) genotype used (Table 3.1), and 136 spring durum wheat (*Triticum turgidum* subsp. *durum* (Desf.) Husn) genotypes (Table 3.2). Most of the hexaploids were of Australian origin and most were chosen because they were parents of available mapping populations. Thirteen of the durums were of Australian origin (Table 3.2), including most of the popular Australian varieties from the last few decades, and some recent releases. The remaining 123 (overseas) durums were mainly sourced from a diverse collection assembled at the University of Bologna and seed were kindly provided Prof Roberto Tuberosa (Table 3.3). Data were not obtained from 23 of the exotic durums, either because they didn't germinate (C18643, C18644, PI164582 and PI217637) or were too late in reaching the target treatment stage (> 86 d from sowing).

Table 3. 1 Details of hexaploid wheat genotypes included in the experiment.

Variety name	Origin	Year released	Variety name	Origin	Year released
Cadoux	Australia	1992	Kukri	Australia	1999
Drysdale	Australia	2001	RAC875*	Australia	n.a.
EGA_Gregory	Australia	2004	RT#418*	Mexico	n.a.
EGA_Stampede	Australia	2007	Sokoll	Mexico	2002
Egret	Australia	1973	Sunco	Australia	1986
Excalibur	Australia	1990	Sunstar	Australia	1983
GBA_Hunter	Australia	2005	Waagan	Australia	2009
Gladius	Australia	2007	Westonia	Australia	1997
Halberd	Australia	1969	Young	Australia	2005
HTWYT_12*	Mexico	n.a.	Tasman	Australia	1993
Katepwa	Canada	1981	Janz	Australia	1989
Kite	Australia	1973	Reeves	Australia	1989
Krichauff	Australia	1998	Kauz	Mexico	1985

*Breeding line; n.a., not applicable

Table 3. 2 Details of Australian tetraploid wheat varieties and breeding lines included in the experiment.

Genotype	Type	Origin	Year Released
WID-22221	breeding line	SA	n.a.
Kamilaroi	variety	NSW	1982
Yallaroi	variety	NSW	1987
Wollaroi	variety	NSW	1993
Tamaroi	variety	SA; NSW	1998
Kalka	variety	SA	2004
Jandaroi	variety	SA; NSW	2007
Caparoi	variety	NSW	2008
Saintly	variety	SA	2008
Hyperno	variety	SA; NSW	2008
WID_801	variety = Tjilkuri	SA	2011
WID_803	variety = Yawa	SA	2012
WID_802	variety	SA	2012

n.a., not applicable

Source: Genetic Resources Information System for Wheat and Triticale (http://wheatpedigree.net/sort/show/67628)

Table 3. 3 Details of overseas tetraploid wheat genotypes included in the experiment

Genotype	Origin	Year released
500110	-	-
500132	-	-
A_Tri_14804	-	-
Adyt 02 - 505	ICARDA	-
Aghrass-1	ICARDA	-
Ainzen-1	ICARDA	-
Aldeano	Irta-Spain	-
Altar 84	Mexico	1984
Ammar-1	ICARDA	2010
Amria	Inramorocco	2005
Angre	ICARDA	-
Anouar	Inramorocco	1994
Appio	Italy	1982
Arcangelo	Italy	1983
Arcobaleno	Italy	1995
Atil x Dic line 113	-	-
Atil x Dic line 129	-	-
Atil x Dic line 133	-	-
Atil x Dic line 21	-	-
Atil_ C2000	Mexico	2001
ATLAST-1	ICARDA	-
Aus-1	ICARDA	-
Aus15216	-	-
Awali-1	ICARDA	-
Azeghar-2	ICARDA	-
Belikh-2	ICARDA	1999
Beltagy-3	-	-
Bicre	ICARDA	1990
Bigost-1	ICARDA	-
Bolenga	Irta-Spain	1997
Brachoua	ICARDA	1990

Genotype	Origin	Year released
C18643	-	-
C18644	-	-
Capeiti_8	Italy	1955
Chaba 88	ICARDA	-
Chabha 88	ICARDA	-
Cham-1	ICARDA	1984
Cil/23	-	-
CIMMYT-67/Plata 16	CIMMYT	1992
CIMMYT-73-Porto 5	CIMMYT	1993
CIMMYT 104 /Yazi 10,1	CIMMYT	1991
CIMMYT-108	CIMMYT	-
CIMMYT-247	CIMMYT	-
CIMMYT-260	CIMMYT	-
CIMMYT-47	CIMMYT	-
Colorado	USA	-
Colosseo	Italy	1995
Coulter	CANADA	1977
Daki Cyn	Mexico	-
Don pedro	Spain	1987
Duilio	ITALY	1984
Durcal	Irta-Spain	-
Durex	USA	1989
Els_6404_160_2	USA	1976
Furat-1	ICARDA	2010
Gallareta	Irta-Spain	1982
Gargano	Italy	1997
Geromtel-1	ICARDA	-
Gr/Boy	ICARDA	-
Grazia	Italy	1985
Guerou-1	ICARDA	-
Heider	Mexico	1997

Table 3.3 Continued

Genotype	Origin	Year released
Inramorocco_1804	Inramorocco	-
Haucan	ICARDA	-
Iride	Italy	1996
Italo	Italy	1993
Jabato	Irta-Spain	1989
Jawhar	Inramorocco	1994
Jennah Khetifa (Tamgurt)	-	-
Jordan	ICARDA	-
Kabir-1	ICARDA	1990
Kofa	USA	1995
Krf = Korifla	ICARDA	1987
Kronos	USA	1992
Lagonil-2	ICARDA	-
Loukos-1	Mexico	1990
Marjana	Inramorocco	1996
Marouane	Inramorocco	2003
Massara-1	ICARDA	-
Meridiano	Italy	1999
Messapia	Italy	1982
Mexicali_75	Mexico - CIMMYT	1975
Mg_7708/47	-	-
Miki-1	ICARDA	-
Mohawk	USA	1998
Mongibello	Italy	-
Moulsabil 2	ICARDA	-
Mrb17	ICARDA	1985
Nassira	Inramorocco	2003
Nile	ICARDA	1981
Omgenil-3	ICARDA	-
Omsnima-1	ICARDA	-
Ort-1 = Oronte-1	ICARDA	1990
Ouaserl-1	ICARDA	-
PI_234870	-	-
PI164582	CIMMYT	-
PI217637	CIMMYT	-
Plinio	Italy	1988
Quadalete	ICARDA	-
Quadrato	Italy	1999
Razzak	Inrat-Tunisia	1987
Renville	USA	1988
Roqueno	Spain - CIMMYT	1991
Sebatel-1	ICARDA	-
Sebatel-2	ICARDA	-
Stojocri-3	ICARDA	-
Svevo	Italy	1996
T. dicoccon (xAtil parent)	-	-
TA-10441	-	-
Telset-5	ICARDA	-
Terbol97-3	ICARDA	-
Tipai (UC-Tipai)	USA	-
Trinakria	Italy	1970
Trit_Durm_Abyssinia	Ethiopia	-
Triticum_Durum	-	-
UAD0951096	-	-
Valnova	Italy	1975
Wadalmez-1	ICARDA	-
Westbred_881	USA	1982
Yavaros 79	ICARDA	1979
Younes-1	ICARDA	-
Zeina 1	ICARDA	-

"-", information not found

3.3.2. Greenhouse conditions and treatment stages

The experiments were conducted from March to August in 2014 (hexaploid wheat), and from March to August 2015 (durum). Plants were initially grown under control conditions in a naturally lit greenhouse. For durum, the average temperature and humidity in the greenhouse was 21/18 ºC and 64/72 % day/night, respectively (Appendix table 3.1), and 19.5/16.7 ºC day/night around booting stage (heat treatment stage). Similarly, for hexaploid wheat, the average recorded temperature and humidity was 20/17 ºC and 68/76 % day/night (Appendix table 4.1).

Each plant was heat treated at a defined AIL stage of the main stem. To allow for some variation in the sensitive stage between genotypes, each class of wheat was heat treated at two different AIL stages in different replications: the durums at 1 cm and 6 cm AIL and the hexaploid wheats at 3 cm and 9 cm AIL. Black plastic pots (8 × 8 × 18 cm) were filled with a previously described coir peat based soil (Maphosa *et al.*, 2014; Shirdelmoghanloo *et al.*, 2016c). The pots were arranged in plastic tubs with drain holes to stop the pots falling over, 12 pots to a tub. Two seeds per pot were sown and a week later seedlings were thinned to one per pot. Plants were kept well-watered from the top.

3.3.3. Heat treatment

When the main stem reached the target AIL, the plants to be heat treated were transferred to a walk-in growth chamber (Conviron BDW120) set at 37°C/27°C day/night where they stayed for three days. The night temperature of 27°C was applied for 10 h between 01:00 and 04:00, then the temperature was linearly raised to 37°C for the day where it was kept level for 8 h. Then between 12:00 and 15:00 the temperature was ramped down to reach the night temperature. The heat treated plants were moved to the chamber at the beginning of the night cycle (shortly after 15:00 hrs) to provide some opportunity for heat acclimation.

Table 3. 4 Traits measured

Trait abbreviation	Description	Trait scored in
Measured pre-heat		
Day.AIL	Days from sowing to when primary tiller reached the target AIL	hexaploid and durum
AIL.PreH	Measured AIL on the day it was closest to the target length	
Ht.PreH	Primary tiller height from soil surface to base to auricle of the flag leaf on the day the target AIL was reached	
Duration of developmental processes		
Day.AwnEm	Days from sowing to when any awn first became visible above the flag leaf auricle.	hexaploid and durum
Day.Anth	Days from sowing to anthesis	
Day.AILtoAwnEm	Days from target AIL day to awn emergence	
Day.AILtoAnth	Days from target AIL day to anthesis	
Flag leaf chlorophyll content and change		
Chl.0, Chl.3, Chl.14, and Ch1.28	Chlorophyll content was measured using a self-calibrating SPAD chlorophyll meter (Model 502, Spectrum Technologies, Plainfield, IL). For each time, ten readings were taken in the middle of the flag leaf of the primary tiller and the average used. The measurements were conducted just before heat treatment (day 0), just after heat treatment (day 3) and at 14 and 28 days after the commencement of heat treatment.	durum only
RChChl.3-0, RChChl.14-3, and RChChl.28-14	Rate of change in chlorophyll (SPAD reading) between two specified time points; between Chl.3 and Chl.0 (RChChl.3-0); between Chl.14 and Chl.3 (RChChl.14-3); and between Ch1.28 and Chl.14 (RChChl.28-14).	

Table 3.4 Continued…

Traits abbreviation	Description	Traits scored in
Final organ length, or change in length in the period including heat treatment and up to maturity		
AwnL.Mat	Length of longest awn at maturity	hexaploid and durum
SpkL.Mat	Length of spike from the bottom to top most glume at maturity	
AIL.Mat	AIL at maturity	
PedL.Mat	Peduncle length at maturity	
Ht.Mat	Plant height from the soil surface to the top of the spike (excluding awns) at maturity	
AIL.GroPostH	Change in AIL from the day AIL reached the target, up to maturity, calculated as AIL.Mat - AIL.PreH	
PlHt.GroPostH	Change in plant height from the day AIL reached the target, up to maturity, calculated as Ht.Mat - Ht.PreH	
Spikelet number and development of basal spikelets		
ProUndsplt	Proportion of spikelets that were underdeveloped. Underdeveloped spikelets were defined as spikelets having awn length less than 50% that of the spikelets from the middle of the spike.	hexaploid and durum
UndvSplt.Spk	Number of under developed spikelets per spike. Underdeveloped spikelets were defined as spikelets having awn length less than 50% that of the spikelets from the middle of the spike.	
NoDevSplt.Top, NoDevSplt.Mid and NoDevSplt.Bot	Number of developed spikelets in top, middle and bottom third of the spike.	
NoDevSplt.Spk	Number of developed spikelets per spike (NoDevSplt.Top + NoDevSplt.Mid + NoDevSplt.Bot)	
NoSplt.Spk	Total number of spikelets per spike (NoDevSplt.Spk + UndvSplt.Spk)	

Table 3.4 Continued…

Traits abbreviation	Description	Traits scored in
Floret fertility (excluding bottom under-developed spikelets)		
GrNoSplt.1&2.Top, GrNoSplt.1&2.Mid and GrNoSplt.1&2.Bot	Grain number per spikelet in bottom two floret positions, in the top, middle and bottom thirds of spike	hexaploid and durum
GrNoSplt.>2.Top, GrNoSplt.>2.Mid and GrNoSplt.>2.Bot	Grain number per spikelet in floret positions 3 and above in the spikelets, in the top, middle and bottom thirds of spike	
GrNoSplt.Spk	Average grain number per spikelet across the whole spike	
GrNo.Spk	Total grain number per spike	

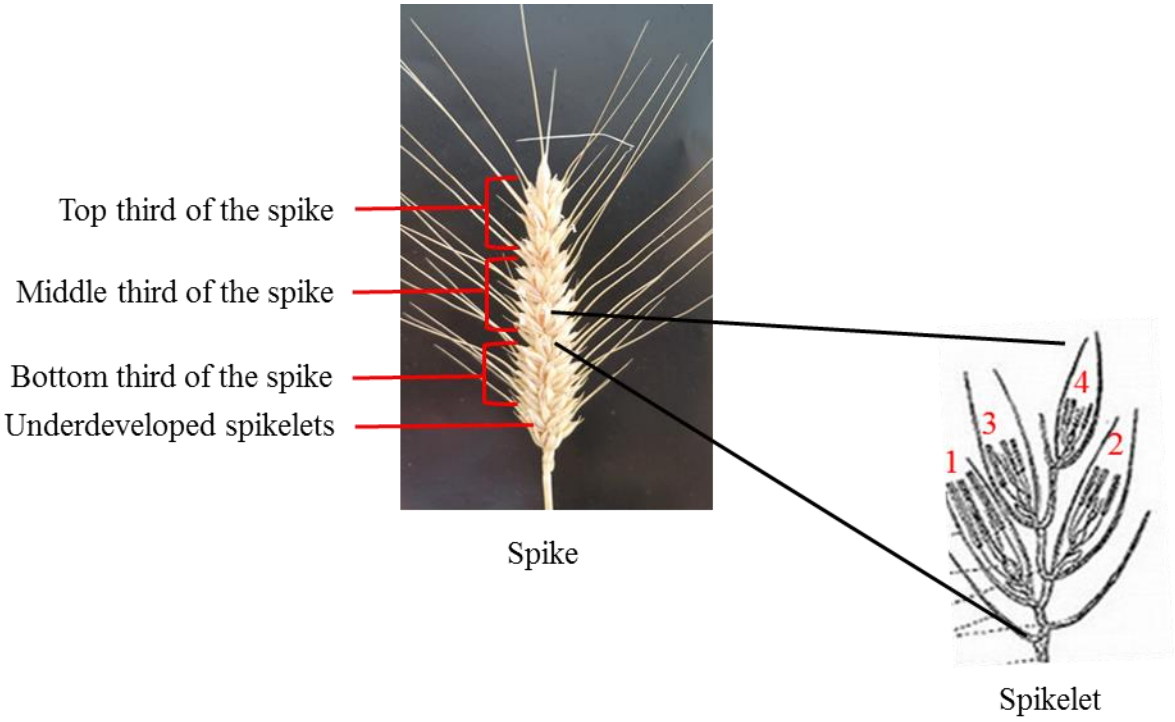

Figure 3. 1 Parts of the spike relevant to the traits scored under the floret fertility sub title of Table 3.1. The thirds of the spike were defined by dividing the number of developed spikelets by three. Florets are numbered in the spikelet picture on the right.

3.3.4. Experimental design and data analysis

The experiments were arranged in a randomized block split-plot design with genotypes assigned as main plots and the second factor, treatment (heat and control), as sub-plots within each main plot. Thus, for any given genotype, the control plant and the plant assigned to the heat treatment were neighbours within each main plot replicate. Each of the experiments had four replications (blocks).

The main stem (primary tiller) was used to determine the stage of heat treatment, and all trait measurements were made on the main stem. For the hexaploid wheat experiment, heat stress was applied at 3 cm AIL stage in two replications and at 9 cm in the other two replications. For durum, there were two replications each for 1 and 6 cm AIL.

Each of the experiments was analysed separately using a spatial mixed model that accounted for genetic and non-genetic sources of variation, using GenStat version 16th.

a. Heat susceptibility index

Heat susceptibility index (HSI) for the traits for each of the tested materials was calculated as:

$$HSI = [(1-Y/Yp)/D]$$

where Y =measured trait at 37/27°C, Yp = =measured trait in control, D = stress intensity = 1- X/Xp, X = mean of Y of all genotypes, and Xp = mean of Yp of all genotypes (Fischer and Maurer, 1978). Genotypes were categorized as tolerant and susceptible according to (Khanna-Chopra and Viswanathan, 1999). Genotypes having HSI ≤ 0.5 were considered to be highly tolerant, HSI > 0.500 to ≤ 1.000 moderately tolerant and those having HSI > 1.0 were regarded as susceptible.

b. Heritability

Broad-sense heritability (H^2) was calculated using the formula of Allard (1960), and represents the proportion of trait variance that is due to all genetic factors including dominance and gene-gene interactions.

c. Correlation analysis

Each trait was analysed separately using linear mixed models, and a best linear unbiased predictor (BLUP) generated for each genotype. Prior to undertaking principal component (PC) analysis, the BLUPs were standardized to a mean of 0 and variance of 1, as per Wold *et al.* (1987), using GenStat version 16th (Payne *et al.*, 2009; Payne, 2009).

3.4. Results and discussion

The two experiments (hexaploid wheat and durum) were conducted in different seasons. Results from the analysis of variance, using linear mixed models and best linear unbiased predictors, are given in Table 3.6 and 3.6. To assess the effect of staging (different auricle interval length), data from heat treated plants were subjected to anlysis of variance as shown in Table 3.7. Broad sense heritability estimations to show the extent to which the observed variation was due to genetic effects are presented in Table 3.8. The results of correlation analysis tables are also presented from Table 3.9 to Table 3.12.

All fertility traits in durum (and for both heat treatment stages), and most of the fertility traits for the later stage of heat treatment in hexaploid wheat, exhibited genotype by treatment interaction effects (Table 3.6). Similarly, all fertility traits in durum, and fertility traits for the first and secondary floret positions of the spikelets in hexaploid wheat, showed treatment effects (Table 3.5). Detailed accounts of each trait are now presented and discussed under each trait category.

Table 3. 5 Trait heat responses (percent difference of heat vs. control) and trait means in control plants in durum and hexaploid wheat.

Studied traits	Durum		Mean for control	Hexaploid wheat		Mean for control
	1 cm AIL	6 cm AIL		3 cm AIL	9 cm AIL	
Traits measured pre-heat						
Day.AIL	-1.36	-0.49	61.3	0.5	-0.9	56.7
AIL.PreH	-9.68	-3.77	3.6	-12.5	0.0	5.95
Ht.PreH	-1.38	-.85	37.9	-1.1	0.9	40.9
Chl.0	0.51	0.38	35.2	-	-.	-
Duration of developmental processes						
Day.AwnEm	-1.31**	-1.46**	63.0	-4.09**	-2.65**	61.7
Day.Anth	-5.39***	-5.42***	75.4	-5.60	-6.10***	71.6
Day.AILtoAwnEm	-25.2	-39.9	2.6	-17.1***	-44.5*	6.1
Day.AILtoAnth	-21.2***	-26.4***	14.8	-20.4***	-42.9***	14.6
Final organ length, or gain in organ length from the commencement of treatment						
AwnL.Mat	-4.08***	-3.47***	12.2	-7.08*	-4.72	5.6
SpkL.Mat	-0.36*	-1.18	6.3	3.69	0.68	8.7
AIL.Mat	-11.4***	-15.9***	16.1	-19.8***	-21.8***	18.9
PedL.Mat	-10.7***	-9.54***	28.9	-3.95	-6.48	29.4
Ht.Mat	-8.66***	-8.81***	70.8	-6.97***	-7.92***	76.4
AIL.GroPostH	-12.0***	-22.4***	12.0	-8.97***	-47.8***	12.4
PlHt.GroPostH	-8.50***	-18.7***	34.0	-3.60***	-25.5***	33.8
Flag leaf chlorophyll content and change						
Ch1.3	4.83***	1.0	39.0	-	-	-
Ch1.14	6.67***	2.54***	41.7	-	-	-
Ch1.28	7.16***	1.94**	41.7	-	-	-
RChChl.3-0	38.0***	-9.81	1.2	-	-	-
RChChl.14-3	20.9*	16.9*	0.2	-	-	-
RChChl.28-14	81.3	75.6	0.0	-	-	-
Number of developed and under-developed spikelets per spike						
NoSplt.Spk	0.03	0.10	17.5	-2.8	-1.8	21.6
ProUndsplt	-7.39	1.03	8.8	-22.0***	-12.4*	20.4
NoDevSplt_Top	-0.16	0.89	5.7	3.4*	1.6	6.05
NoDevSplt_Mid	0.77	0.76	5.3	3.6	1.7	5.7
NoDevSplt_Bot	1.76	0.01	5.0	1.9	1.8	5.4
NoDevSplt_Spk	0.82	0.15	16.0	2.4	1.7	17.2
UndvSplt_Spk	1.76	-1.94	1.6	-22.7***	-15.9*	4.4
Grain number per spikelet						
GrNoSplt. 1&2.Top	-66.9***	-44.6***	1.8	-33.9***	-38.1***	1.5
GrNoSplt.1&2.Mid	-65.3***	-39.6***	1.8	-23.7***	-29.3***	1.8
GrNoSplt.1&2.Bot	-75.3***	-55.9***	1.8	-18.8***	-27.9***	1.8
GrNoSplt.>2.Top	-73.0***	-75.4***	0.5	0.0	-50.0	0.2
GrNoSplt.>2.Mid	-72.4***	-68.6***	0.9	-14.3	-25.0	0.75
GrNoSplt.>2.Bot	-67.9***	-74.9***	0.7	-18.3	-25.0*	0.62
GrNoSplt.Spk	-69.5***	-51.8***	2.5	-26.3***	-29.5***	2.22

*, ** and ***indicate significant difference between control and heat-treated plants at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. - indicates traits not recorded.

Table 3. 6 *P*-values for genotype × treatment (G × T) effects for traits, in durum and hexaploid wheat genotypes (each for two AIL heat treatment stages). Green highlighted figures showed significant effects ($p < 0.05$).

	Durum		Hexaploid wheat	
Trait	1 cm AIL	6 cm AIL	3 cm AIL	9 cm AIL
Traits measured pre-heat				
Day.AIL	n.a.	n.a.	n.a.	n.a.
AIL.PreH	n.a.	n.a.	n.a.	n.a.
Ht.PreH	n.a.	n.a.	n.a.	n.a.
Chl.0	n.a.	n.a.	-	-
Duration of developmental processes				
Day.AwnEm	1.000	0.002	0.881	0.246
Day.Anth	0.455	<0.001	0.559	0.016
Day.AILtoAwnEm	0.841	0.062	0.999	0.596
Day.AILtoAnth	0.221	<0.001	0.970	0.475
Flag leaf chlorophyll content and change				
Ch1.3	0.057	<0.001	-.	-.
Ch1.14	0.476	<0.001	-	-
Ch1.28	0.530	<0.001	-	-
RChChl.3-0	0.015	<0.001	-	-
RChChl.14-3	0.539	0.015	-	-
RChChl.28-14	<0.001	0.128	-	-
Final organ length, or gain in organ length from the commencement of treatment				
AwnL.Mat	0.002	0.635	0.677	0.501
SpkL.Mat	0.026	0.362	<0.001	<0.001
AIL.Mat	<0.001	<0.001	0.010	0.045
PedL.Mat	<0.001	0.124	0.766	0.653
Ht.Mat	<0.001	0.306	0.932	0.560
AIL.GroPostH	0.174	0.004	0.014	0.575
PlHt.GroPostH	<0.001	0.023	0.405	0.651
Spikelet number and development of basal spikelets				
NoSplt.Spk	0.042	0.023	0.587	0.725
ProUndsplt	0.041	0.980	0.001	0.322
NoDevSplt_Spk	0.029	0.503	0.013	0.398
NoDevSplt_Top	0.028	0.037	0.016	0.294
NoDevSplt_Mid	0.384	0.002	0.100	0.545
NoDevSplt_Bot	0.012	0.649	0.068	0.558
UndvSplt_Spk	0.163	0.755	0.012	0.264
Grain number per spikelet				
GrNoSplt. 1&2.Top	<0.001	<0.001	0.063	0.002
GrNoSplt.1&2.Mid	<0.001	<0.001	0.019	<0.001
GrNoSplt.1&2.Bot	<0.001	<0.001	0.058	<0.001
GrNoSplt.>2.Top	<0.001	<0.001	0.003	0.206
GrNoSplt.>2.Mid	<0.001	<0.001	0.094	0.008
GrNoSplt.>2.Bot	<0.001	<0.001	0.631	0.014
GrNoSplt.Spk	<0.001	<0.001	0.005	<0.001
GrNo.Spk	<0.001	<0.001	0.001	<0.001

n.a., not applicable for the traits measured prior to heat treatment; - Chlorophyll traits not recorded on hexaploid wheat genotypes.

3.4.1. Traits measured pre-heat

The analysis of variance in both experiments showed the genotypic effects were highly significant (except for AIL.PreH in durum) indicating the existence of variability across both the hexaploid wheat and durum genotype sets. In both experiments, traits measured before heat treatment showed no significant treatment effects, as expected.

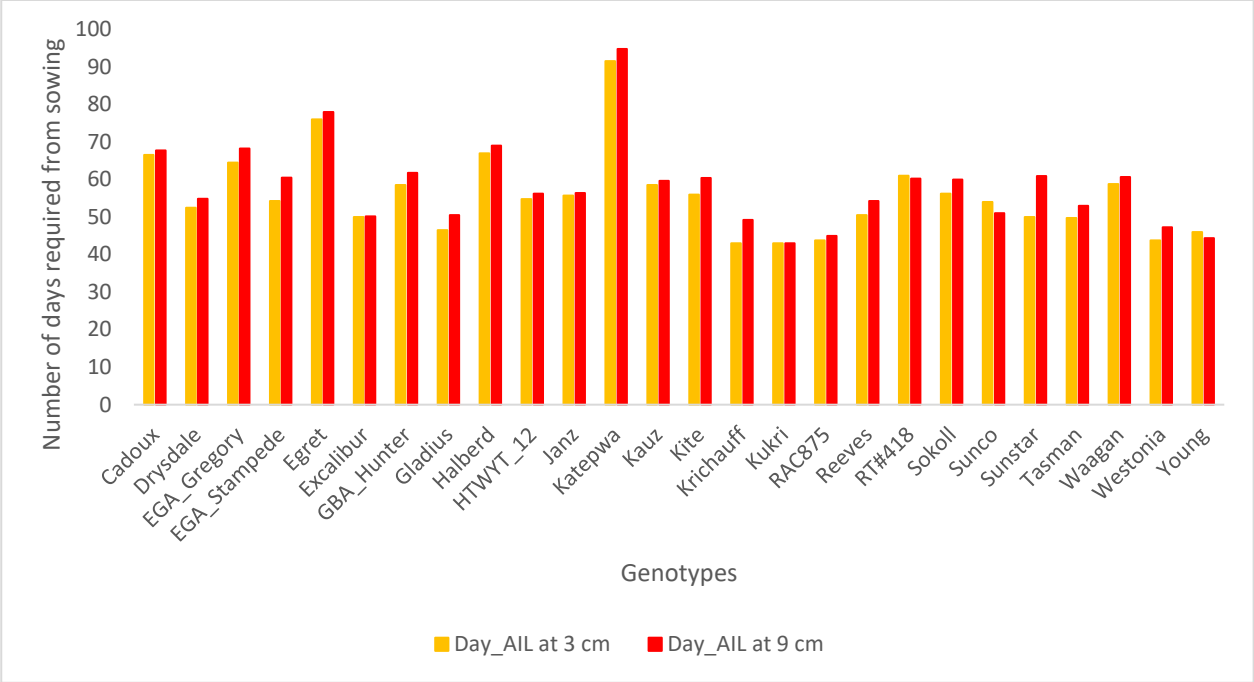

Figure 3. 2 Number of days required to reach at 3 or 9 cm AIL from date of sowing for each tested hexaploid wheat genotype

In hexaploid wheat, the number of days from sowing to the date at which 3 or 9 cm AIL stage was reached (Day.AIL) varied significantly between genotypes. This ranged from 43 to 91 d for the earlier stage (3 cm AIL) and 45 and 95 d for the later stage (9 cm AIL) (Figure 3.2). On average, the 9 cm stage was reached 2.5 d after the 3-cm stage.

In durum, Day.AIL ranged from 46 to 87 d for the 1 cm AIL stage and from 48 to 89 d for the 6 cm AIL stage (Appendix table 3.2). On average, 2 d separated the 1 cm stage and the 6 cm stage.

Flag leaf chlorophyll content before the heat treatment (Chl.0) varied almost twofold within the tested durum genotypes, from 27.2 to 43.5 SPAD units (Table 3.4).

3.4.2. Traits related to duration of developmental processes

For this trait category, in both durum and hexaploid wheat genotypes, all the trait/stage combinations (except for Day.AILtoAnth in hexaploid wheat for the 9 cm AIL treatment) exhibited genotype effects. Likewise, in durum wheat, except for Day.AILtoAwnEm, all traits exhibited treatment effects. However, none of them, except Day.Anth, showed genotype by treatment interaction effects.

Under control conditions, Day.Anth ranged from 58 d to 117 d in hexaploid wheat, and from 53 to 105 d in durum. Similarly, Day.AwnEm ranged from 46 to 88 d in hexaploid wheat and 47 to 100 d in durum under control conditions. In durum, only 7% (9) of genotypes on average had awns emerged at the 1 cm AIL stage and 30% of genotypes (37) had awn emerged by the 6 cm AIL stage. However, as mentioned, Day.AILtoAwnEm showed no treatement effect. By contrast, in the hexaploids 96% of the genotypes on average had awn emerged after the earliest treatment stage (3 cm AIL), so it was not surprising that Day.AILtoAwnEm showed heat treatment effect in the hexaploids

In hexaploid wheat, heat applied at either stage significantly shortened the duration of Day.AILtoAnth. The average reduction was 20.4% (~3 d) and 42.9% (~6 d) for Day.AILtoAnth. This trait was a componend of Day.Anth, so the latter also showed a (smaller percentage) reduction due to heat (Table 3.5). Similarly, in durum wheat, heat applied at 1 and 6 cm AIL shortened Day.AILtoAnth by 21.2 % (3 d) and 26.4 % (4 d) respectively. Unexpectedly, Day.AILtoAwnEm was significantly reduced by the heat treatments in hexaploid wheat, even though most of the genotypes on average had their awns emerged prior to the heat treatment.

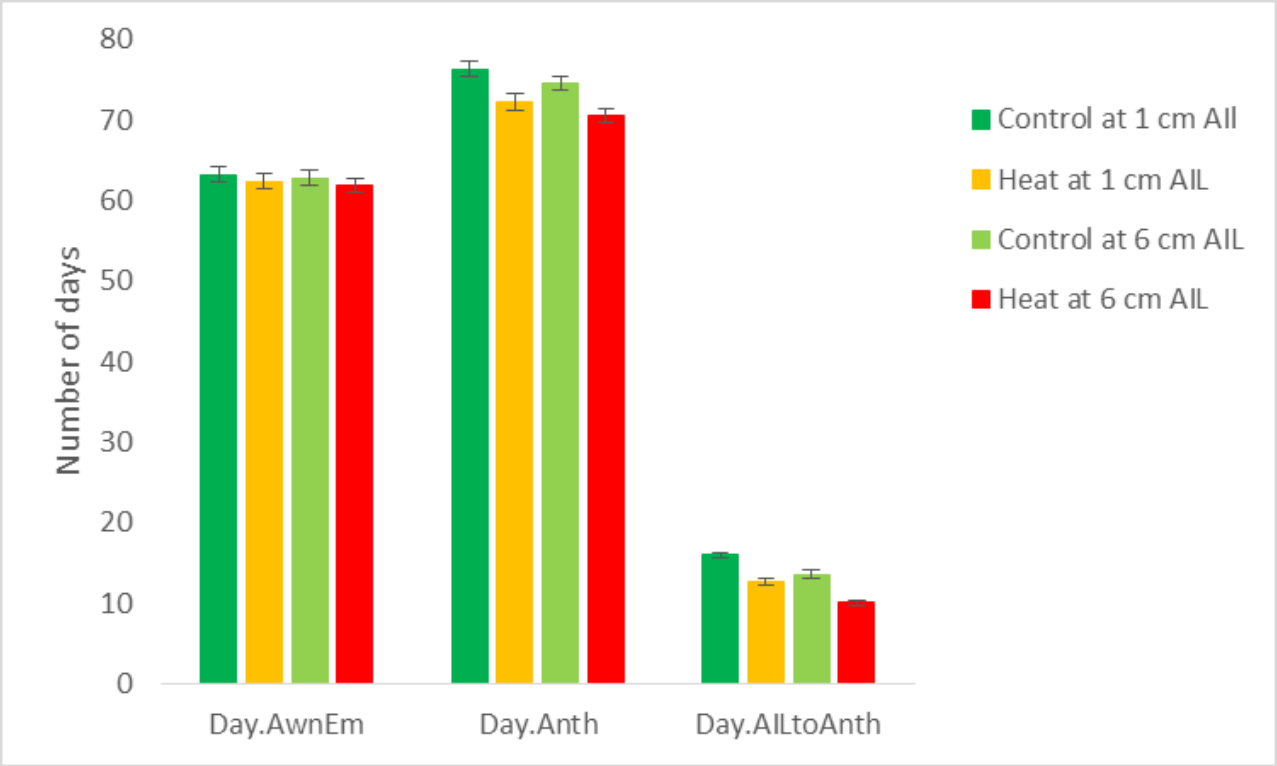

Figure 3. 3 Mean ± S.E. for Day.AwnEm, Day.Anth and Day.AILtoAnth, in control and heat-treated plants of durum genotypes.

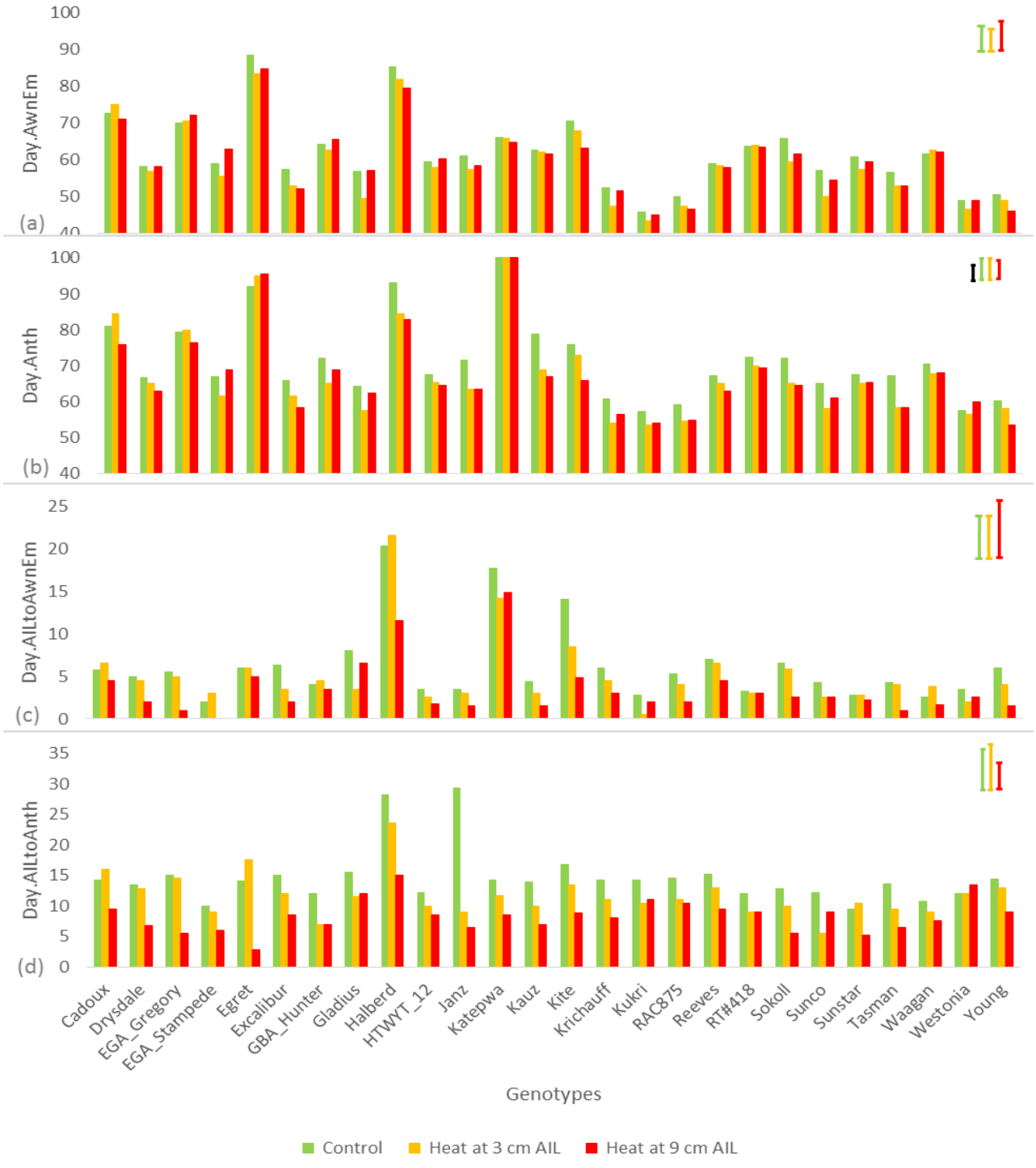

Figure 3. 4 Means of each hexaploid wheat genotype for control and heat-treated plants, for both heat treatment stages, for traits (a) days from sowing to when any awn first became visible above the flag leaf auricle (Day.AwnEm), (b) days from sowing to anthesis (Day.Anth), (c) days from target AIL to awn emergence (Day.AILtoAwnEm) and (d) days from target AIL to anthesis (Day.AILtoAnth). The vertical bars indicate LSD values ($\alpha = 0.05$) for comparisons of means of control and heat within each genotype (black bar), and for mean comparisons across genotypes for control (green bar), heat at 3 cm AIL (yellow bar) or heat at 9 cm AIL (red bar).

3.4.3. Traits related to final organ length, or change in organ length from the commencement of treatment

In both hexaploid wheat and durum, all these traits showed significant genotypic effects (Table 3.5). Significant treatment effects were observed for all the traits in durum. Similarly, in hexaploid wheat, all except AwnL.Mat for the later heat treatment stage and SpkL.Mat and PedL.Mat for both treatment stages showed significant treatment effects. Most of the traits under this category (AwnL.Mat, PedL.Mat, Ht.Mat, AIL.GroPostH, and PlHt.GroPostH) showed non-significant treatment by genotype interaction effects in hexaploid wheat. All except AIL.GroPostH for the 1 cm AIL treatment, and AwnL.Mat, SpkL.Mat, PedL.Mat, and Ht.Mat for the 6 cm AIL treatment, showed significant genotype by treatment interaction effects in durum.

Heat invariably shortened final organ length or growth of organs, consistent with the fact that heat shortened the duration of developmental stages (previous section).

In hexaploid wheat, heat shortened AwnL.Mat by~7 % and~4.7 % for the 3 and 9 cm AIL treatments stages, respectively.

AIL.Mat showed both treatment and genotypic by treatment interaction effects. In hexaploid wheat, heat at 3 and 9 cm AIL stages shortened auricle interval length by 19.8% and 21.8%, respectively. Genotypes differed in their responses of AIL.Mat. The greatest heat responses were observed in Kite and Reeves (38% reduction) for the 3 cm AIL treament and in Kauz (50% reduction) for the 9 cm AIL treatment (Figure 3.5a).

In hexaploid wheat, under control conditions, Cadoux had the greatest plant height (97 cm) and Kite the smallest (59 cm). Heat applied at 3 and 9 cm AIL stages shortened Ht.Mat by 7% and 8%, respectively. The greatest heat responses were observed in Waagan, Kukri and Tasman (15%) for the 3 cm AIL treatment and in Kauz (18%) for the 9 cm AIL treatment.

In hexaploid wheat, under control conditions, auricle interval growth in the period between the target AIL stage to maturity (AIL.GroPostH) averaged 12.4 cm. Plant height growth (PlHt.GroPostH) in the same period averaged 33.8 cm (Table 3.5).

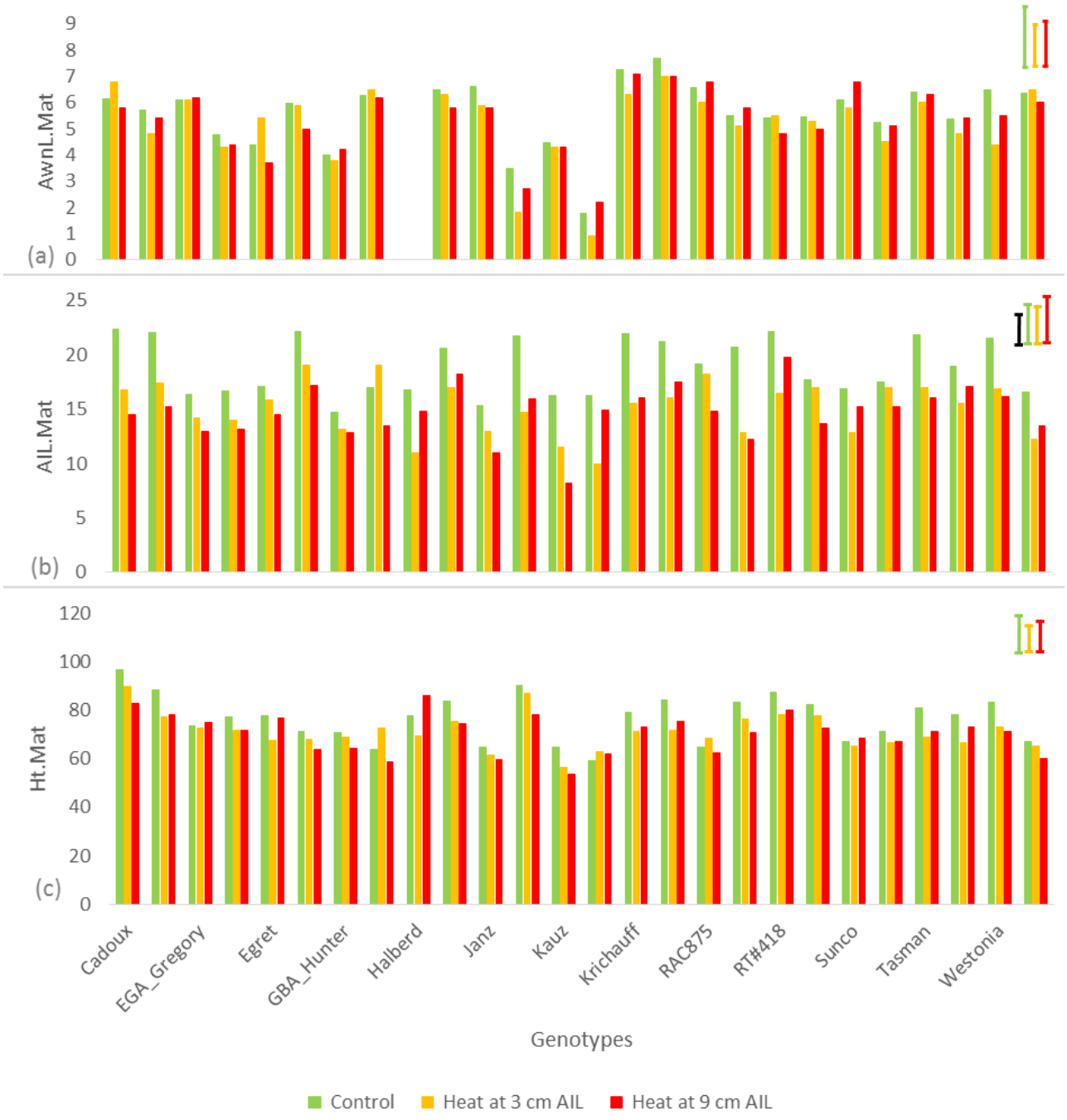

Figure 3. 5a Means for each hexaploid wheat genotype, for control and heat-treated plants, and for both heat treatment stages, for awn length at maturity (a), auricle interval length at maturity (b) and height at maturity (c). Genotype by treatment interactions were significant for auricle interval length at maturity but not for awn length and plant height. Halberd is an awnless variety and therefore has no value for awn length. The vertical bars indicate the LSD values ($\alpha = 0.05$) for within genotype mean comparisons between control and heat-treated plants (black bar), and for mean comparisons between genotypes within control (green bar), heat at 3 cm AIL (yellow bar) or heat at 9 cm AIL (red bar).

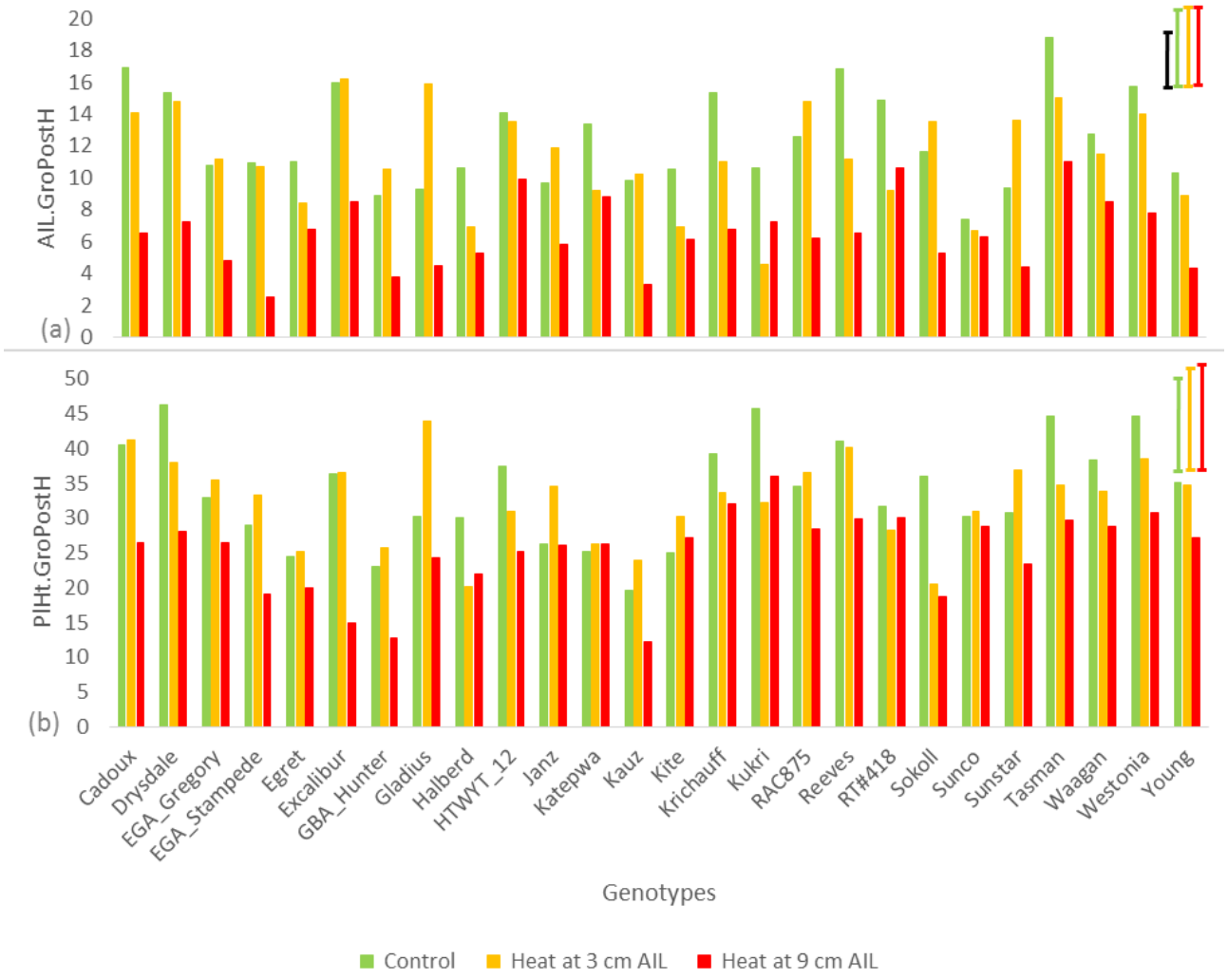

Figure 3. 5b Means of each hexaploid genotype for control and heat-treated plants for post-heat growth of auricle interval (a) and plant height (b). Genotype by treatment interaction was observed only for AIL.GroPostH for the earlier heat stress (3 cm AIL). The vertical bars indicate the LSD values ($\alpha = 0.05$) for within genotype mean comparisons between control and heat-treated plants (black bar), and for mean comparisons between genotypes within control (green bar), heat at 3 cm AIL (yellow bar) or heat at 9 cm AIL (red bar).

In hexaploid wheat, the heat response of AIL.GroPostH averaged 9% and 48% for the 3 and 9 cm AIL treatments, respectively. Heat reduced elongation of the AIL during this period, and on average, plant height. Reductions of AIL.GroPostH by heat was gretest in Kukri (57%) for the 3 cm AIL treatment and in EGA_Stampede (77%) for the 9 cm AIL treatment. On average, heat reduced PlHt.GroPostH by 3.6 % and 25.5 % for the 3 and 9 cm AIL treatments, respectively (Figure 3.5b).

The greatest heat response was in Sokoll (43% reduction) for treatment at 3 cm AIL, and in Excalibur (59% reduction) for treatment at 9 cm AIL.

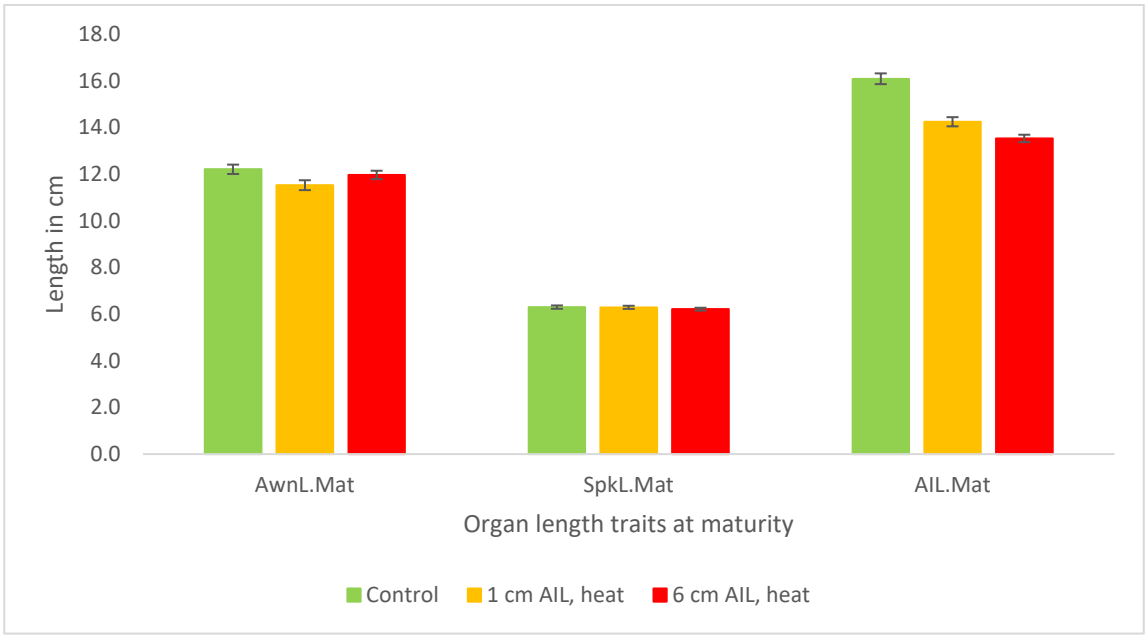

Figure 3. 6 Mean ± S.E. of final organ length at maturity traits in durum genotypes. Length of longest awn at maturity (AwnL.Mat); length of spike from the bottom to top most glume at maturity (SpkL.Mat); auricle interval length at maturity (AIL.Mat); peduncle length at maturity (PedL.Mat); Plant height from the soil surface to the top of the spike (excluding awns) at maturity (Ht.Mat).

In durum, all final organ length traits except SpkL.Mat showed highly significant treatment effects, with the heat treatment on average reducing organ length. AwnL.Mat and PedL.Mat were

more affected by the 1 cm AIL treatment (heat shortened their length by 4 and 11 %, respectively) than the 6 cm AIL treatment (Figure 3.6). However, heat at 6 cm AIL had more impact on AIL.Mat (15 % reduction) than the 1 cm AIL treament. Heat at both stages impacted Ht.Mat, shortening it by an average of 8.7%.

3.4.4. Flag leaf chlorophyll content and chlorophyll change over time in durum wheat

Flag leaf chlorophyll traits were only measured in the durums. These traits all exhibited highly significant genotype and treatment effects. All except RChChl.28-14, showed significant treatment by genotype interaction effects at 6 cm AIL. However, at 1 cm AIL, only RChChl.3-0 and RChChl.28-14 showed the effect (Table 3.6).

Overall patterns of flag leaf chlorophyll content changes over time are summarized in Figure 3.7. Chlorophyll levels in the flag leaf were rapidly increasing at the time of the heat treatment and on average this accumulation was accelerated by the heat treatment. This most likely related to the general observation that developmental processes increase with temperature, up to a certain optimum, due to thermodynamic effects (Parent and Tardieu, 2012). By contrast, when Shirdelmoghanloo et al. (2016) applied heat at a later stage in development to the current study (10 days after anthesis), flag leaf chlorophyll content was levelling out, and the heat treatment accelerated chlorophyll loss.

For the timepoint just after heat treatment, flag leaf chlorophyll content ranged from 29.2 to 48.4 SPAD units in control plants. This was increased by 4.8% and 1% by the heat treatments applied at 1 and 6 cm AIL, respectively (Table 3.5). By 14 d after commencement of treatment, control plants had increased their chlorophyll content slightly, to an average of 27.8 to 51.4 SPAD units. Heat at 1 and 6 cm AIL increased chlorophyll content at this time by 6.7% and 2.5%, respectively. Similarly, chlorophyll in control plants ranged from 27.3 to 52.9 SPAD units by 28 d after the taget AIL was reached, and heat stress applied at 1 and 6 cm AIL increased chlorophyll content at this time by 7.2% and 1.9%, respectively.

Overall increases in chlorophyll content due to heat were greater for 1 cm AIL treatment than for the 6 cm AIL treatment (Table 3.5, Figure 3.7). For the treatment at 6 cm AIL, some genotypes showed a drastic reduction in chlorophyll content due to heat by 28 d, resulting in almost complete

loss of chlorophyll by this time (data not shown), indicating that some genotypes were very sensitive to heat induced chlorophyll loss.

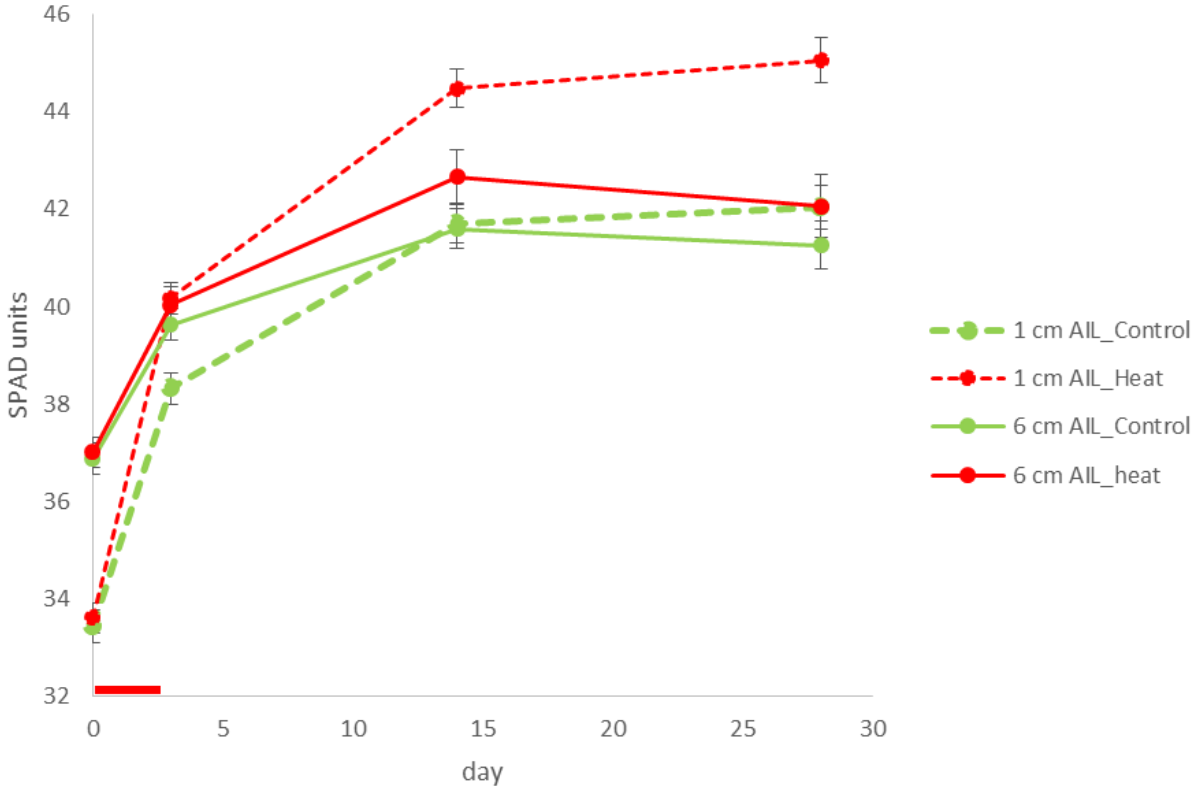

Figure 3. 7 Mean ± S.E. for relative chlorophyll content of the flag leaf in durum genotypes, measured using a SPAD meter. The red bar at the bottom indicates the period of heat treatment. Day zero was defined as the day that the AIL reached the target (Chl0) and the heat treatments commenced.

3.4.5. Number of developed and under-developed spikelets per spike

In both hexaploid wheat and durum, there were significant genotypic effects for the number of developed and under developed spikelets per spike. In hexaploid wheat, heat significantly reduced the number of underdeveloped spikelets per spike (UndvSplt.Spk) and the proportion of spikelets that were underdeveloped (ProUndsplt), more so for the earlier treatment than the later one (22-23% vs. 12-16% reduction, respectively) (Table 3.5). Hence, the treatments were early enough in the hexaploid wheats to affect the length of awns in these lower most spikelets (the basis for defining spikelets as underdeveloped). The direction of this effect was surprising, given that heat treatment decreased awn length on the florets with the longest awns (AwnL.Mat trait), and suggests that heat increased the awn length on the lowermost spikelets of the spike.

No such large effect on the number of underdeveloped spikelets was seen for durum wheat. For spikelet number traits, genotype by treatment interaction effects were only highly significant for ProUndsplt for the earlier heat treatment stage in hexaploid wheat (p=0.001).

Table 3. 7 Means and *p*-values for genotype (Gen), stage (St) and genotype by stage (Gen × St) effects in the heat treated plants, for durum and hexaploid wheat.The green and yellow colours show significant stage and genotype by stage interaction effects ($p < 0.05$), respectively.

	Heat treated durum					Heat treated hexaploid wheat				
	Mean at AIL		Effect			Mean at AIL		Effect		
	1 cm	6 cm	Gen	St	Gen x St	3 cm	9 cm	Gen	St	Gen × St
Day.AIL	60.2	61.5	<.001	0.235	0.843	55.4	57.7	<.001	0.001	0.168
AIL.PreH	1.40	5.40	0.091	<.001	0.11	3.50	7.90	<.001	<.001	0.069
Ht.PreH	35.2	39.8	<.001	0.008	0.012	37.6	44.1	<.001	<.001	0.647
Day.AwnEm	62.5	62.4	<.001	0.91	0.986	59.8	61.0	<.001	0.067	0.497
Day.Anth	72.4	71.4	<.001	0.311	0.014	65.8	64.6	<.001	0.04	0.042
Day.AILtoAwnEm	2.47	1.41	<.001	0.088	0.928	4.40	2.40	0.035	0.051	0.641
Day.AILtoAnth	12.6	10.4	<.001	0.025	0.386	11.3	8.30	0.006	<.001	0.235
Chl.0	33.6	37.0	<.001	0.062	0.401	-	-	-	-	-
Chl.3	40.1	40.0	<.001	0.927	0.097	-	-	-	-	-
Ch1.14	44.4	42.6	<.001	0.186	<.001	-	-	-	-	-
Ch1.28	45.0	42.1	<.001	0.136	<.001	-	-	-	-	-
RChChl.3-0	2.1	0.80	<.001	0.025	0.011	-	-	-	-	-
RChChl.14-3	0.30	0.20	<.001	0.205	0.036	-	-	-	-	-
RChChl.28-14	0.10	0.00	<.001	0.225	0.466	-	-	-	-	-
AwnL.Mat	11.5	11.8	<.001	0.075	0.007	5.20	5.30	<.001	0.496	0.483
SpkL.Mat	6.29	6.23	<.001	0.435	0.044	8.80	8.60	<.001	0.157	0.122
AIL.Mat.	14.1	13.5	<.001	0.23	0.049	15.0	14.7	<.001	0.329	0.071
PedL.Mat	25.1	26.9	<.001	0.017	<.001	27.9	27.6	<.001	0.732	0.836
Ht.Mat	64.4	64.9	<.001	0.447	<.001	69.8	69.5	<.001	0.785	0.448
AIL.GroPostH	11.6	7.90	<.001	0.005	0.154	11.5	6.60	<.001	<.001	0.032
PlHt.GroPostH	33.8	25.0	<.001	0.13	<.001	32.2	25.3	0.021	<.001	0.829
NoDevSplt.Spk	16.1	16.0	<.001	0.523	0.57	17.2	17.8	<.001	0.079	0.141
UndvSplt.Spk	1.54	1.51	<.001	0.813	0.013	3.40	3.70	<.001	0.172	0.824
NoDevSplt.Top	5.69	5.70	<.001	0.878	0.208	6.10	6.30	<.001	0.072	0.073
NoDevSplt.Mid	5.38	5.35	<.001	0.133	0.119	5.80	5.90	<.001	0.393	0.457
NoDevSplt.Bot	5.04	5.02	<.001	0.507	0.179	5.40	5.60	<.001	0.043	0.321
GrNoSplt.1&2.Top	0.57	0.98	<.001	0.032	0.042	0.99	0.94	<.001	0.605	0.732
GrNoSplt.1&2.Mid	0.61	1.08	<.001	0.016	0.002	1.30	1.30	<.001	0.362	0.825
GrNoSplt.1&2.Bot	0.41	0.80	<.001	0.038	0.001	1.40	1.30	<.001	0.142	0.702
GrNoSplt>2.Top	0.12	0.14	<.001	0.579	0.004	0.20	0.10	<.001	0.372	0.006
GrNoSplt>2.Mid	0.24	0.31	<.001	0.431	0.005	0.60	0.60	<.001	0.945	0.596
GrNoSplt>2.Bot	0.20	0.18	<.001	0.713	0.184	0.49	0.48	<.001	0.812	0.387
GrNoSplt.Spk	0.71	1.26	0.006	0.052	0.619	1.65	1.55	<.001	0.303	0.954
GrNo.Spk	11.5	18.7	<.001	0.064	0.002	28.2	27.1	<.001	0.527	0.98

3.4.6. Grain number per spikelet

In hexaploid wheat, treatment and genotype by treatment interactions were mostly non-significant for floret positions >2 in the spikelets (Table 3.5). Treatment by genotype interaction effects were also non-significant for GrNoSplt.1&2.Top or GrNoSplt.1&2.Bot in hexaploid wheat. By contrast, in durum, genotype and treatment effects and their interaction effects were significant for all grain number per spikelet traits.

Overall, heat induced floret sterility was much higher in durum than in hexaploid wheat, even allowing for the different AIL stages used for treatment (Figure 3.9). This seemed consistent with the reputation that durums in Australia are more prone to heat induced floret sterility than hexaploid wheats. However the difference could have also related to the fact that the durums and hexaploids were screened in separate experiments, performed at different times of the year. A more direct comparison is needed to further test the hypothesis that durum is more heat susceptible than hexaploid wheat for fertility effects.

In non-stressed wheat plants, the bottom two floret positions in the spikelets normally show near complete fertility (close to two grains per spikelet (McMaster *et al.*, 1992). This was the expectation in the current experiments, since the plants were largely free of diseases and pests and had good nutrition. By contrast, grain number in the third and above floret positions normally differs across spike segments with the middle third yielding more grains compared with top and bottom part (Evans *et al.*, 1972). The author reported that, grain number in the third and above floret positions of the spikelets also varies with genotype under normal conditions, and varied from 0.2 (cv. Maris Ranger) to 5.9 (cv. Maris Nimrod) grains per spikelet. In the current experiment, the third and above floret positions of the control hexaploid wheat plants bore an average of 0.52 grains per spikelet (0.2, 0.75, and 0.62 seeds per spikelets in top middle and lower third part of the spike, respectively) (Table 3.5; Figure 3.8 and 3.9). Control durum plants averaged 0.7 grains per spikelet at these positions (0.5, 0.9, and 0.7 seeds per spikelets in top, middle and bottom third part of spike, respectively) (Table 3.5; Figure 3.8 and 3.9).

In the hexaploid wheats, four genotypes (Egret, Kite, Katepwa and Halberd) showed high levels of sterility under control conditions (ave. <1.8 grains per spikelet in floret positions 1&2) (Figure 3.8), suggesting they were inherently less fertile, irrespective of the presence of heat stress.

The other genotypes set >1.8 grains per spikelet at floret positions 1&2, and what little sterility these showed under control conditions occurred mainly in the upper third of the spike (Figure 3.8). Likewise in the durum, 29 (26%) of the genotypes averaged <1.8 grains per spikelet at floret positions 1&2. In both hexaploid wheat and durum, the stage of heat treatment had no significant effect on grain set in the third and above floret positions in the spikelets (Table 3.4). Also, fertility under heat was generally the most stable in the middle third of the spike (Tables 3.6 and 3.7).

In hexaploid wheat, there was no significant effect of stage of heat treatment on fertility responses, and stage by genotype interactions were seldom significant (Table 3.7). In hexaploid wheat, there was also good correspondence between fertility responses of genotypes to heat treatment in florets 1&2 vs. florets >2 (Figure 3.8). Therefore, responses of floret fertility to the heat treatments and the genetic determination of its variation appeared to be related to seed set in potentially fertile florets (green and well developed), rather than establishment of the number of potentially fertile florets per spikelet, as the latter would be expected to be confined to the florets at positions >2. Around a third of the genotypes were highly tolerant, showing fertility levels >85% of those observed under control conditions, while the remaining genotypes showed varied levels of sterility, with cv. Westonia being the most sensitive, with 85% loss of fertility under heat.

Contrasting tolerance phenotypes were observed for parents of the following seven existing hexaploid wheat mapping populations: Drysdale × Waagan, Drysdale × Gladius, Excalibur × Kukri, Young × Reeves, Sunco × Tasman, Westonia*2/ Janz, and Westonia × Kauz (Figure 3.8). These populations therefore showed potential for use in identifying QTL for heat-induced floret sterility tolerance.

In contrast to hexaploid wheat, durum showed significant treatments stage effects (Table 3.7) with treatment at 1 cm AIL impacting fertility more than the 6 cm AIL treatment (70 and 52% loss in GrNoSplt.Spk, respectively).

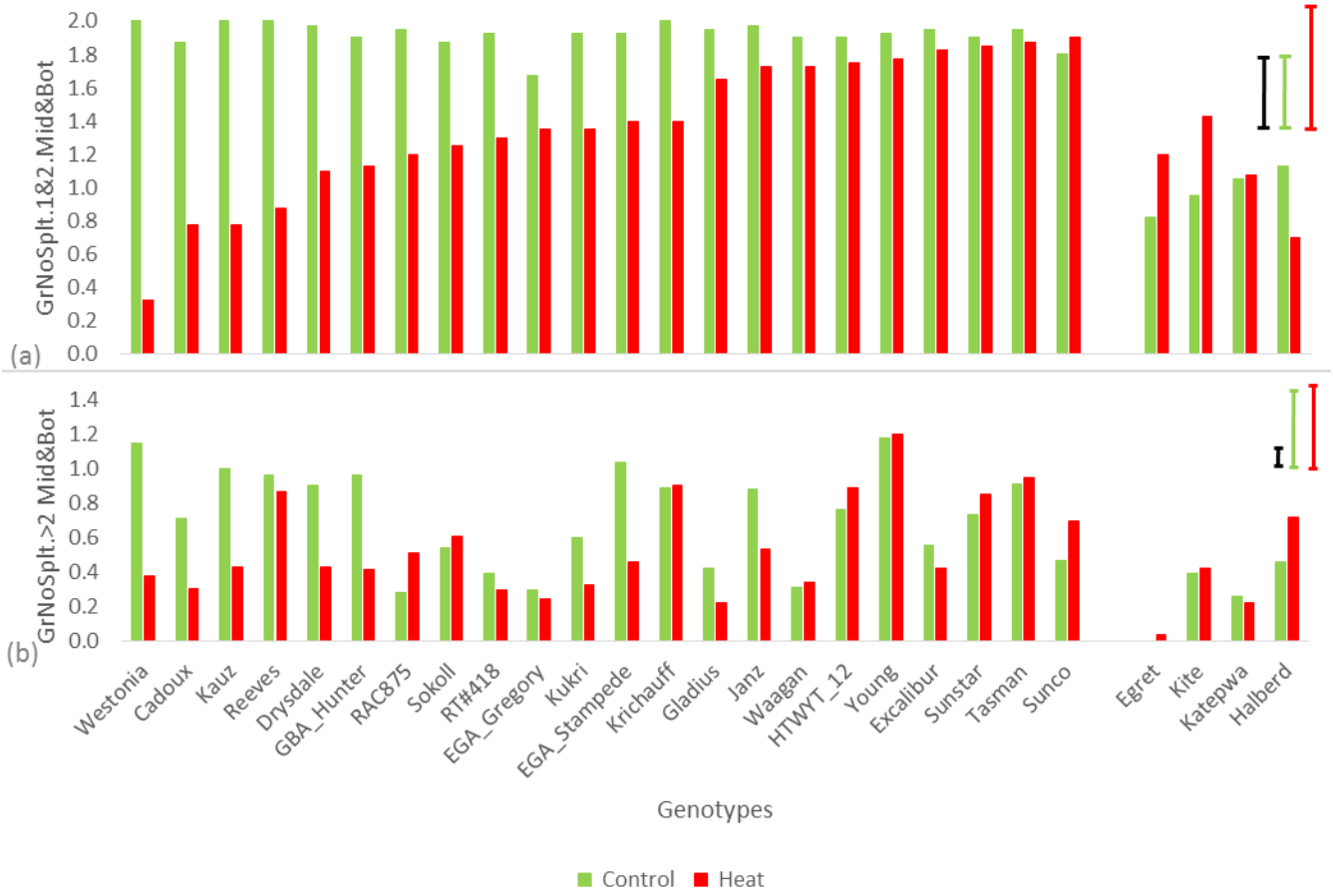

Figure 3. 8 Average grain number in control and heat-treated plants of hexaploid wheat genotypes, for the first and second floret positions in the spikelet (a), or in the third and above floret positions (b). Data are pooled for the 3 and 9 cm AIL treatments, from spikelets from the bottom and middle third of the spike. Genotypes in both figures are ordered according to grains per spikelet under heat in (a), except that the four genotypes that showed low fertility under control conditions are separated to the right. The vertical bars indicate the LSD values ($\alpha = 0.05$) for within genotype mean comparisons between control and heat-treated plants (black bar), and for mean comparisons between genotypes within control (green bar), or heat (red bar).

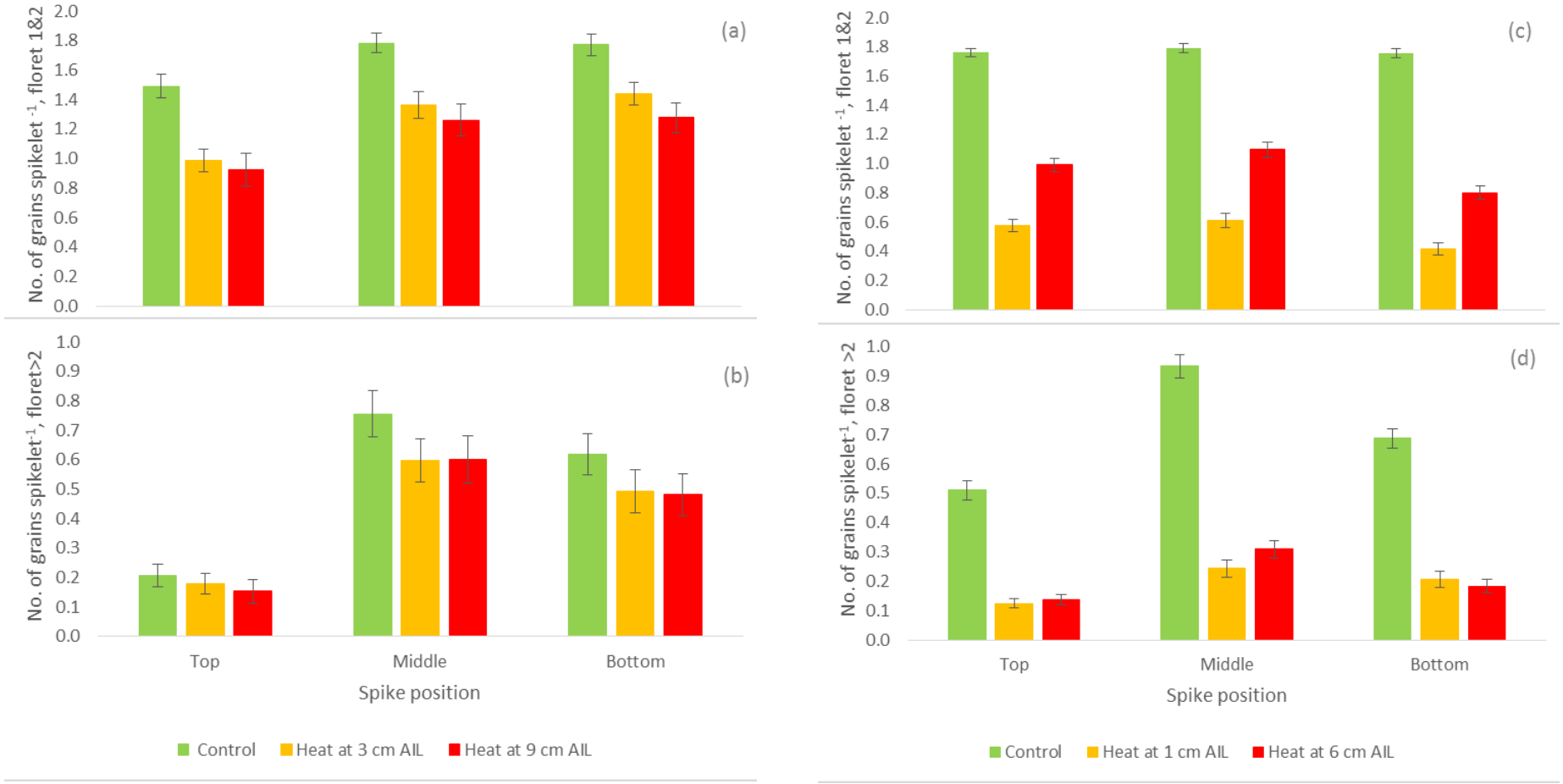

Figure 3. 9 Mean ± S.E. for grains per spikelet, for first and second (a and c) and third and above (b and d) floret positions in the spikelets. Results are shown for hexaploid wheats (a and b) and durum (c and d), respectively. Data from the top, middle and bottom thirds of the spike are presented separately.

Figure 3.10 shows floret fertility under heat plotted against floret fertility in control, while Figure 3.11 shows fertility of heat treated plants treated at the two different stages, plotted against each other, excluding genotypes with poor inherent fertility (<1.7 grains per spikelet under control conditions). In contrast to hexaploid wheat, durum showed poor correlation between the heat responses to the two treatments (Figure 3.11).

The genotypes could be grouped into three main categories: inherently low fertility (high sterility under control conditions), tolerant and intolerant. The genotypes categorized as inherently low fertility are highlighted in Figure 3.10. The genotypes Coulter, Arcangelo, Azeghar-2, Lagonil-2, Bicre, Triticum_Durum, Tamaroi were included in this category.

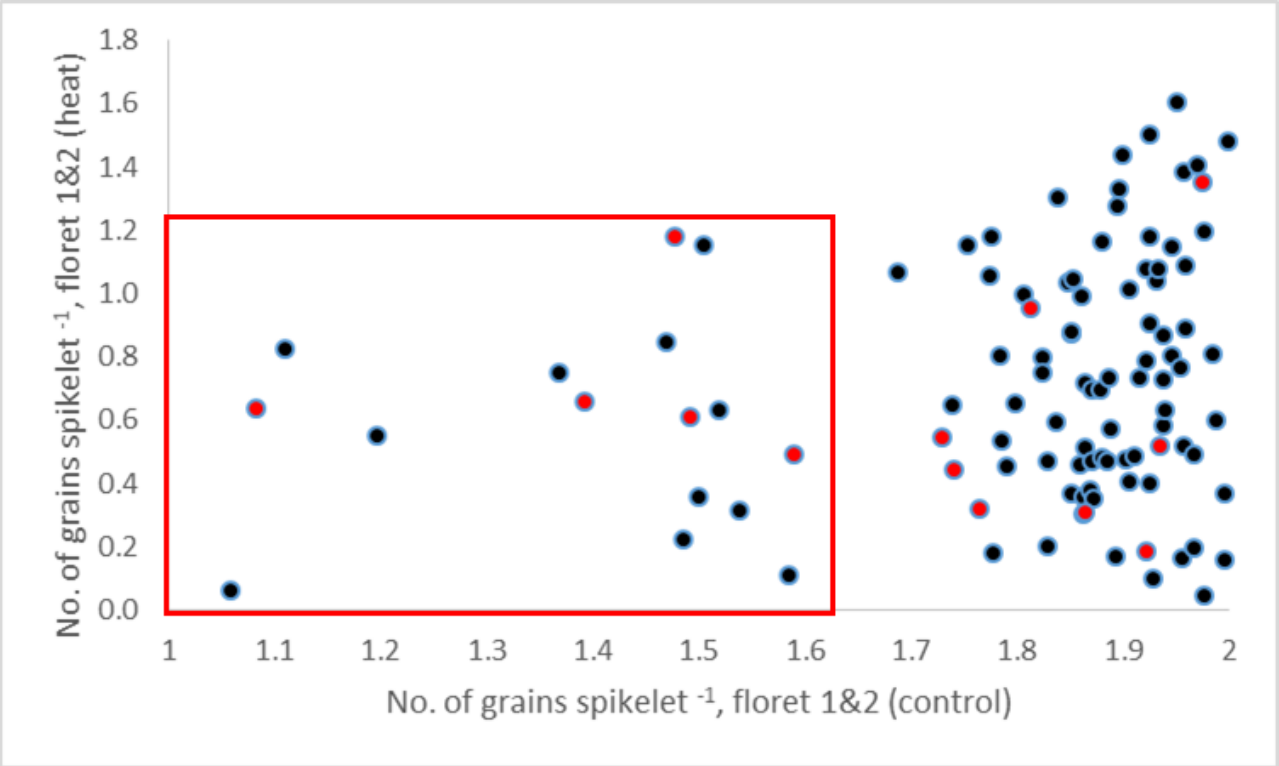

Figure 3. 10 Floret fertility under heat plotted against floret fertility under control for durum. Each spot represents a genotype. Red spots are Australian durums. Inherently low fertility genotypes are highlighted by the red rectangle. Shown are pooled data from spikelets from the bottom, middle and top of the spike, and from 6 cm AIL and 1 cm AIL treatment.

Genotypes in the second category (tolerant) were those that showed high fertility after heat treatments applied at both AIL stages (Figure 3.11; Circled in yellow). Terbol 97-3 from ICARDA

was the most tolerant durum for both heat treatment stages. CIMMYT-67, Plata 16 from CIMMYT, Bigost-1 from ICARDA, Meridiano from Italy, and Altar 84 were other genotypes in this class.

Durum genotypes characterized as intolerant were those that showed low fertility after heat treatment at both stages (Figure 3.11; red rectangle). Among the tested Australian durums, WID 22221, WID 801, WID 802, Kamilaroi, Jandaroi and Kalka were in this class.

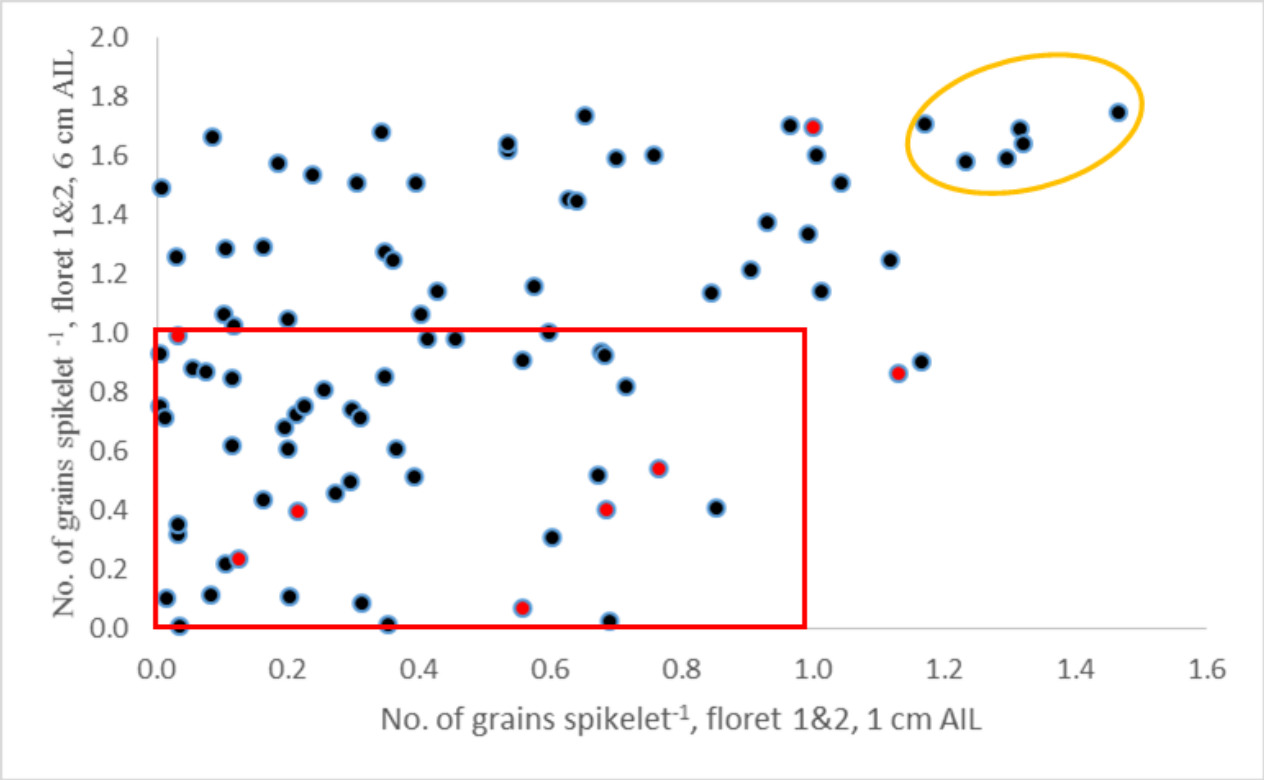

Figure 3. 11 Fertility of durum genotypes after heat treatments: 6 cm AIL vs. 1 cm AIL treatment. Genotypes in the category 'inherently low fertility' are not shown. Shown are data from spikelets from the bottom, middle and top of the spike combined. Each dot represents a genotype. Red dots are Australian durum varieties

The tolerant genotypes identified here could be used as tolerance donors in crosses to locally adapted Australian varieties, and the favourable (tolerance) QTL alleles selected for in subsequent generations using molecular markers. Durum genotypes found to contrast for floret fertility tolerance could be crossed together to develop new mapping populations for discovery of new tolerance QTL.

3.4.7. Heritability

Broad sense heritability values for traits in hexaploid wheat and durum are presented in Table 3.8. Broad sense heritability estimates how strongly genetic factors influence variation in the trait, relative to environmental or random chance factors, and hence provides a good measure of how much direct selection for a trait in a breeding program would result in genetic improvement of the trait. Day.AIL, Ht.PreH, Day.AwnEm and Day.Anth showed consistently high heritability in both hexaploid wheat and durum so these traits should respond best to phenotypic selection. Heritablility was variable (very low to quite high) for Day.AILtoAwnEm and Day.AILtoAnth, low to moderate for chlorophyll traits and moderate to high for organ length, organ growth or spikelet number traits.

Heritability of floret fertility traits was moderate to high, and was not consistently higher or lower in heat treated plants relative to control plants. This suggested that there is good potential for selection of floret fertility under heat stress as a breeding approach to improve heat tolerance in wheat. However, it is also possible that, with different genetic material or conditions to those used here, this situation may not hold as well

Table 3. 8 Heritability (H^2) of traits for each treatment stage, for durum and hexaploid wheat. Colour highlights high (more green) and low (more red) values.

Traits	Durum wheat				Hexaploid wheat			
	1 cm AIL		6 cm AIL		3 cm AIL		9 cm AIL	
	Heat	Control	Heat	Control	Heat	Control	Heat	Control
Traits measured pre-heat								
Day.AIL	0.78	0.77	0.79	0.8	0.91	0.88	0.93	0.94
Ht.PreH	0.69	0.71	0.69	0.61	0.66	0.61	0.88	0.86
Duration of developmental processes								
Day.AwnEm	0.75	0.76	0.77	0.8	0.9	0.88	0.86	0.94
Day.Anth	0.77	0.68	0.8	0.74	0.88	0.83	0.86	0.88
Day.AILtoAwnEm	0.08	0.72	0.66	0.21	0.3	0.7	0.09	0.77
Day.AILtoAnth	0.16	0.17	0.8	0.1	0.01	0.65	0.5	0.15
Flag leaf chlorophyll content and change*								
Chl0	0.45	0.52	0.48	0.44	-	-	-	-
Chl3	0.49	0.41	0.56	0.39	-	-	-	-
Ch114	0.64	0.56	0.53	0.63	-	-	-	-
Ch128	0.67	0.59	0.53	0.65	-	-	-	-
RChChl3_0	0.41	0.13	0.13	0.15	-	-	-	-
RChChl14_3	0.22	0.61	0.15	0.64	-	-	-	-
RChChl28_14	0.22	0.32	0.26	0.24	-	-	-	-
Final organ length, or gain in organ length from the commencement of treatment								
AwnL.Mat	0.76	0.76	0.74	0.63	0.86	0.8	0.78	0.65
SpkL.Mat	0.6	0.85	0.6	0.64	0.81	0.81	0.81	0.84
AIL.Mat	0.46	0.53	0.42	0.65	0.76	0.79	0.62	0.74
PedL.Mat	0.6	0.5	0.81	0.59	0.48	0.66	0.59	0.64
Ht.Mat	0.72	0.69	0.83	0.63	0.68	0.58	0.65	0.71
AIL.GroPostH	0.3	0.39	0.33	0.5	0.62	0.86	0.48	0.51
PlHt.GroPostH	0.23	0.54	0.41	0.64	0.14	0.62	0.4	0.53

Table 3.8 Continued…

Traits	Durum wheat				Hexaploid wheat			
	1 cm AIL		6 cm AIL		3 cm AIL		9 cm AIL	
	Heat	Control	Heat	Control	Heat	Control	Heat	Control
Spikelet number and development of basal spikelets								
NoDevSplt.Spk	0.42	0.41	0.34	0.49	0.7	0.81	0.78	0.36
UndvSplt.Spk	0.68	0.68	0.77	0.62	0.83	0.75	0.59	0.5
NoDevSplt.Top	0.35	0.82	0.39	0.45	0.41	0.78	0.79	0.34
NoDevSplt.Mid	0.83	0.41	0.47	0.44	0.64	0.71	0.75	0.22
NoDevSplt.Bot	0.32	0.39	0.81	0.81	0.65	0.72	0.65	0.37
Grain number per spikelet								
GrNoSplt.1&2.Top	0.47	0.84	0.49	0.36	0.41	0.64	0.65	0.59
GrNoSplt.1&2.Mid	0.52	0.65	0.49	0.59	0.52	0.6	0.95	0.91
GrNoSplt.1&2.Bot	0.46	0.72	0.51	0.56	0.35	0.65	0.77	0.73
GrNoSplt.>2.Top	0.49	0.19	0.46	0.52	0.7	0.55	0.61	0.49
GrNoSplt.>2.Mid	0.53	0.62	0.51	0.45	0.39	0.61	0.51	0.63
GrNoSplt.>2.Bot	0.52	0.56	0.57	0.45	0.57	0.6	0.79	0.55
GrNoSplt.Spk	0.61	0.68	0.6	0.54	0.61	0.67	0.72	0.8
GrNo.Spk	0.58	0.64	0.58	0.45	0.54	0.7	0.6	0.69

3.4.8. Correlation analysis of HSIs

Correlations between HSIs for the various traits, and between trait HSIs and their potentials (trait value under control conditions), for hexaploid wheat (Table 3.9 and 3.11) and durum (Tables 3.10 and 3.12), were analysed.

In hexaploid wheat, floret fertility tolerance was positively correlated with stability of organ length (and growth) (Table 3.9). These relationships were also observed in durum (Table 3.10). In other words, genotypes that were more tolerant for heat-induced floret sterility also tended to maintain their growth better under heat (due to an ability to maintain duration or rate of growth). This suggested that common mechanisms and genes may be involved in tolerance for floret fertility and growth.

In durum, floret fertility tolerance was moderately correlated with stability of anthesis date – a relationship not observed in hexaploid wheat (Tables 3.9 and 3.10).

In durum where chlorophyll traits were measured, floret fertility tolerance showed a few correlations with chlorophyll heat responses that were relatively weak and variable in direction (Table 3.10). Hence, floret fertility tolerance was not closely correlated with chlorophyll stability, in contrast to grain filling heat tolerance in hexaploid wheat which was reported to correlate strongly and positively with chlorophyll stability (Shirdelmoghanloo *et al.*, 2016a; Shirdelmoghanloo *et al.*, 2016b; Shirdelmoghanloo *et al.*, 2016c).

Chlorophyll stability showed some weak to moderate correlations with stability of organ length traits in durum (Table 3.10), although the direction of these correlations was variable.

3.4.9. Correlations between HSI and trait potentials

In hexaploid wheat, floret fertility tolerance was negatively associated with the potentials of the same fertility traits (fertility under control; as indicated by the positive correlation between heat susceptibility indices and the potentials) and number of developed spikelets per spike, but positively correlated with the number of underdeveloped spikelets per spike (Table 3.11). Floret fertility tolerance was also negatively correlated with potentials of

plant height at maturity and of plant height growth in the period after the target AIL was reached, and positively correlated with potentials of some duration of development traits.

In durum, floret fertility tolerance traits showed some significant correlations with the same trait potentials as in hexaploid wheat, but the directions of the effects were inconsistent for the fertility tolerance in different spike and floret positions (Table 3.12). The changing directions of the correlations to organ length and growth trait potentials (in hexaploid wheat and durum) could be escape effects. i.e., at a given AIL, florets at various positions on the spike may have been at a more or less sensitive stage, due to their asynchronous development. The patterns of relationships between floret fertility tolerance and fertility *per se* cannot be explained in the same way. However, it should be noted that correlations between HSI and potential of the same trait may simply be an 'artefact' of the trait value in control being a term used in calculating HSI. The potential for artefactual correlations to arise between non-independent variables has been noted previously (Brett 2004).

Table 3. 9 Correlations between heat susceptibility indices (HSIs) in hexaploid wheat genotypes. Only the 14 traits that showed significant treatment effects are presented.

	Day.AwnEm	Day.Anth	Day.AILtoAwnEm	Day.AILtoAnth	AIL.Mat.	Ht.Mat	AIL.GroPostH	PlHt.GroPostH	UndvSplt.Spk	GrNoSplt.1&2.Top	GrNoSplt.1&2.Mid	GrNoSplt.1&2.Bot	GrNoSplt>2.Bot	GrNoSplt.Spk
Day.AwnEm	-													
Day.Anth	0.52	-												
Day.AILtoAwnEm	0.52	0.46	-											
Day.AILtoAnth	0.30	0.65	0.19	-										
AIL.Mat.	-0.20	0.22	0.17	0.03	-									
Ht.Mat	-0.39	-0.09	-0.17	-0.19	0.46	-								
AIL.GroPostH	-0.24	-0.09	0.02	-0.22	0.69	0.36	-							
PlHt.GroPostH	0.00	0.06	0.00	-0.23	0.08	0.53	0.33	-						
UndvSplt.Spk	0.58	0.04	0.49	-0.15	-0.22	-0.30	-0.14	0.08	-					
GrNoSplt.1&2.Top	-0.46	-0.08	-0.08	-0.38	0.33	0.60	0.23	0.51	-0.30	-				
GrNoSplt.1&2.Mid	-0.43	-0.06	-0.13	-0.34	0.46	0.48	0.31	0.38	-0.37	0.88	-			
GrNoSplt.1&2.Bot	-0.30	0.11	0.00	-0.26	0.28	0.40	0.06	0.42	-0.23	0.91	0.89	-		
GrNoSplt>2.Bot	-0.44	-0.32	-0.27	-0.17	-0.02	0.42	0.11	0.16	-0.17	0.49	0.38	0.35	-	
GrNoSplt.Spk	-0.44	0.00	-0.02	-0.29	0.40	0.52	0.17	0.34	-0.35	0.92	0.94	0.94	0.47	-

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

Table 3. 10 Correlations between heat susceptibility indices (HSIs) in durum genotypes. Only the 21 traits that showed significant treatment effects are presented.

	AIL.PreH	Day.AwnEm	Day.Anth	Day.AILtoAnth	Chl.3	Ch1.14	Ch1.28	RChChl.3-0	RChChl.14-3	AwnL.Mat	SpkL.Mat	AIL.Mat.	PedL.Mat	Ht.Mat	AIL.GroPostH	GrNoSplt.1&2.Top	GrNoSplt.1&2.Mid	GrNoSplt.1&2.Bot	GrNoSplt>2.Top	GrNoSplt>2.Mid	GrNoSplt>2.Bot	GrNoSplt.Spk	GrNo.Spk
AIL.PreH	-																						
Day.AwnEm	0.15	-																					
Day.Anth	0.04	0.23	-																				
Day.AILtoAnth	0.08	-0.01	0.27	-																			
Chl.3	-0.13	0.05	0.10	-0.02	-																		
Ch1.14	0.01	-0.02	-0.02	-0.03	0.38	-																	
Ch1.28	-0.02	0.02	0.00	0.06	0.24	0.82	-																
RChChl.3-0	0.06	0.02	-0.02	0.05	0.54	0.22	0.19	-															
RChChl.14-3	-0.01	0.03	0.05	-0.01	-0.01	0.25	0.30	0.07	-														
AwnL.Mat	0.02	-0.18	-0.17	0.11	-0.16	-0.21	-0.13	0.07	0.15	-													
SpkL.Mat	-0.01	-0.01	-0.03	0.17	-0.18	-0.15	-0.10	0.20	0.21	0.34	-												
AIL.Mat.	0.08	0.06	-0.06	-0.10	-0.28	-0.24	-0.10	-0.14	-0.03	0.13	0.14	-											
PedL.Mat	-0.05	-0.09	-0.02	0.13	-0.10	-0.15	-0.14	0.05	0.02	0.18	0.18	0.24	-										
Ht.Mat	-0.02	-0.05	0.05	0.04	-0.16	-0.17	-0.06	-0.06	-0.02	-0.01	0.19	0.44	0.64	-									
AIL.GroPostH	-0.13	0.03	-0.23	-0.09	-0.20	-0.18	0.01	-0.06	0.00	0.22	0.12	0.83	0.18	0.38	-								
GrNoSplt.1&2.Top	0.03	-0.09	0.06	0.00	0.19	-0.02	0.01	0.22	0.00	-0.09	0.12	0.00	0.07	0.32	-0.12	-							
GrNoSplt.1&2.Mid	-0.15	0.11	-0.12	0.07	-0.03	-0.09	0.04	0.10	-0.05	0.03	-0.06	0.33	0.09	0.23	0.44	0.04	-						
GrNoSplt.1&2.Bot	-0.04	0.11	-0.32	0.03	-0.05	0.09	0.06	0.17	-0.06	-0.04	-0.12	0.11	0.16	0.11	0.25	-0.15	0.44	-					
GrNoSplt>2.Top	0.19	0.02	0.29	0.28	0.04	0.06	0.01	0.02	0.04	0.02	0.00	-0.10	0.00	0.10	-0.10	0.06	-0.05	-0.14	-				
GrNoSplt>2.Mid	0.09	-0.03	0.19	0.01	-0.01	0.06	-0.03	-0.02	0.03	0.04	0.09	0.10	0.11	-0.04	-0.06	0.05	-0.13	-0.28	0.21	-			
GrNoSplt>2.Bot	0.06	-0.04	0.05	0.03	-0.18	-0.10	-0.07	-0.02	0.04	0.02	0.20	0.18	0.24	0.23	0.08	0.08	0.14	-0.04	-0.02	-0.17	-		
GrNoSplt.Spk	0.01	-0.08	0.02	-0.01	0.08	0.01	0.04	0.19	-0.04	0.05	0.09	0.09	0.12	0.34	0.14	0.66	0.39	0.11	0.06	-0.07	0.07	-	
GrNo.Spk	-0.01	-0.05	0.02	0.05	0.14	-0.03	0.03	0.24	-0.02	-0.07	0.15	0.12	0.18	0.40	0.04	0.92	0.34	0.10	0.08	0.04	0.16	0.75	-

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

Table 3. 11 Correlations between trait potentials (value in control plants; in the vertical list) and heat susceptibility indices (HSIs) of traits (listed above), in hexaploid wheat genotypes

	Day.AwnEm	Day.Anth	Day.AILtoAwnEm	Day.AILtoAnth	AIL.Mat.	Ht.Mat	AIL.GroPostH	PlHt.GroPostH	UndvSplt.Spk	GrNoSplt.1&2.Top	GrNoSplt.1&2.Mid	GrNoSplt.1&2.Bot	GrNoSplt>2.Bot	GrNoSplt.Spk
Day.AIL	-0.31	-0.39	-0.53	0.07	0.04	0.00	0.10	-0.18	-0.24	-0.35	-0.19	-0.49	0.06	-0.36
AIL.PreH	0.01	-0.30	-0.06	-0.29	-0.44	-0.19	-0.11	0.00	0.30	-0.16	-0.16	-0.25	-0.05	-0.22
Ht.PreH	-0.51	-0.48	-0.64	-0.18	0.05	0.26	0.32	0.17	-0.35	0.05	0.14	-0.16	0.30	-0.04
Day.AwnEm	-0.11	-0.22	-0.46	0.14	-0.05	-0.25	0.13	-0.06	-0.04	-0.36	-0.26	-0.40	0.14	-0.41
Day.Anth	-0.22	-0.18	-0.40	0.15	0.18	-0.09	0.18	-0.17	-0.21	-0.34	-0.14	-0.43	-0.01	-0.33
Day.AILtoAwnEm	0.23	0.14	0.01	0.05	0.22	-0.42	0.25	-0.12	0.09	-0.41	-0.12	-0.33	-0.34	-0.33
Day.AILtoAnth	0.28	0.39	0.15	0.54	0.19	-0.31	0.04	-0.19	-0.04	-0.19	-0.03	-0.03	0.01	-0.10
AwnL.Mat	-0.01	-0.08	0.29	-0.08	-0.09	0.36	-0.24	0.10	0.23	0.34	0.14	0.32	0.12	0.29
SpkL.Mat	-0.22	0.07	0.10	-0.20	0.03	0.07	0.24	0.36	-0.17	0.30	0.21	0.24	0.19	0.29
AIL.Mat.	-0.14	-0.22	0.00	-0.45	0.33	0.55	0.35	0.42	0.13	0.36	0.30	0.23	0.12	0.29
PedL.Mat	0.05	-0.09	0.28	-0.28	0.11	0.19	0.17	0.28	0.24	0.29	0.29	0.33	-0.07	0.30
Ht.Mat	-0.49	-0.47	-0.41	-0.42	0.29	0.63	0.45	0.53	-0.18	0.42	0.44	0.24	0.33	0.33
AIL.GroPostH	-0.17	-0.14	-0.06	-0.33	0.43	0.54	0.41	0.46	-0.01	0.35	0.32	0.29	0.14	0.31
PlHt.GroPostH	-0.04	-0.14	0.16	-0.42	0.14	0.43	0.25	0.60	0.22	0.43	0.33	0.41	0.04	0.36
NoDevSplt.Spk	-0.35	0.10	-0.26	-0.04	-0.01	0.50	0.11	0.51	-0.40	0.59	0.40	0.47	0.58	0.52
UndvSplt.Spk	0.08	-0.31	-0.15	0.12	-0.11	-0.31	0.01	-0.25	0.25	-0.66	-0.55	-0.66	-0.14	-0.62
NoDevSplt.Top	-0.32	0.13	-0.21	-0.04	0.02	0.46	0.12	0.48	-0.42	0.60	0.44	0.50	0.54	0.55
NoDevSplt.Mid	-0.33	0.09	-0.33	-0.01	-0.03	0.50	0.05	0.48	-0.43	0.58	0.39	0.45	0.56	0.50
NoDevSplt.Bot	-0.36	0.08	-0.24	-0.04	-0.01	0.52	0.12	0.54	-0.33	0.56	0.35	0.44	0.58	0.49
GrNoSplt.1&2.Top	-0.04	0.23	0.33	0.06	0.09	0.30	-0.19	0.23	-0.04	0.55	0.34	0.61	0.12	0.50
GrNoSplt.1&2.Mid	-0.16	0.23	0.13	-0.02	0.05	0.43	-0.24	0.24	-0.15	0.66	0.51	0.74	0.20	0.67
GrNoSplt.1&2.Bot	-0.10	0.25	0.18	-0.05	0.02	0.38	-0.22	0.28	-0.10	0.66	0.46	0.73	0.19	0.64
GrNoSplt>2.Top	-0.13	0.11	0.30	0.04	0.40	0.28	0.08	0.03	-0.13	0.21	0.22	0.27	0.04	0.29
GrNoSplt>2.Mid	-0.17	0.18	0.20	-0.05	0.45	0.47	0.28	0.21	-0.37	0.50	0.45	0.49	0.21	0.55
GrNoSplt>2.Bot	-0.04	0.26	0.16	-0.04	0.35	0.52	0.10	0.23	-0.35	0.55	0.50	0.57	0.22	0.60
GrNoSplt.Spk	-0.11	0.28	0.25	0.00	0.24	0.47	-0.08	0.27	-0.21	0.65	0.50	0.71	0.17	0.67

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

Table 3. 12 Correlations between trait potentials (value in control plants; in the vertical list) and heat susceptibility indices (HSIs) of traits (listed above), in durum genotypes

	Day.AwnEm	Day.Anth	Day.AILtoAnth	Chl.3	Ch1.14	Ch1.28	RChChl.3-0	RChChl.14-3	AwnL.Mat	SpkL.Mat	AIL.Mat.	PedL.Mat	Ht.Mat	AIL.GroPostH	GrNoSplt.1&2.Top	GrNoSplt.1&2.Mid	GrNoSplt.1&2.Bot	GrNoSplt>2.Top	GrNoSplt>2.Mid	GrNoSplt>2.Bot	GrNoSplt.Spk	GrNo.Spk
Day.AIL	0.13	-0.29	-0.12	0.05	-0.07	-0.07	0.17	0.05	0.35	0.11	0.04	0.00	-0.17	0.13	-0.30	0.11	0.06	-0.15	-0.15	0.01	-0.18	-0.26
AIL.PreH	0.09	-0.05	-0.07	-0.23	-0.05	-0.08	-0.20	-0.01	0.06	-0.10	0.12	-0.04	-0.22	0.18	-0.93	0.11	0.19	-0.07	-0.11	0.13	-0.56	-0.80
Ht.PreH	0.09	0.07	-0.01	-0.06	-0.24	-0.11	-0.08	-0.01	0.13	0.08	0.42	-0.07	0.03	0.33	0.04	0.23	-0.21	-0.14	-0.03	0.38	0.00	0.07
Day.AwnEm	0.19	-0.32	-0.12	0.02	-0.13	-0.10	0.13	0.05	0.35	0.07	0.10	-0.03	-0.16	0.22	-0.31	0.21	0.16	-0.19	-0.21	0.01	-0.14	-0.25
Day.Anth	0.16	-0.19	-0.07	0.02	-0.11	-0.09	0.13	0.06	0.39	0.08	0.10	-0.02	-0.17	0.21	-0.40	0.22	0.10	-0.12	-0.15	0.01	-0.18	-0.33
Day.AILtoAwnEm	0.17	-0.35	-0.38	-0.11	-0.21	-0.18	-0.13	0.01	0.15	-0.13	0.25	-0.18	-0.25	0.35	-0.26	0.29	0.06	-0.48	-0.14	0.02	-0.07	-0.24
Day.AILtoAnth	0.11	0.00	-0.33	-0.11	-0.13	-0.08	-0.12	0.03	0.19	-0.17	0.24	-0.17	-0.16	0.37	-0.49	0.35	0.07	-0.28	-0.09	-0.05	-0.11	-0.41
Chl.0	0.02	0.04	0.08	-0.31	-0.29	-0.21	-0.07	0.05	0.21	0.19	0.10	0.09	0.13	0.04	0.03	0.08	0.00	0.10	0.03	-0.19	0.08	0.05
Chl.3	0.03	0.03	0.11	-0.37	-0.27	-0.20	-0.28	0.05	0.19	0.18	0.01	0.01	0.07	-0.03	0.05	0.00	-0.05	0.10	0.07	-0.25	0.06	0.04
Ch1.14	0.04	0.00	-0.03	-0.16	-0.31	-0.24	-0.21	0.16	0.22	0.15	-0.01	0.01	0.07	-0.02	0.08	-0.12	-0.10	0.08	0.06	-0.26	-0.02	0.01
Ch1.28	-0.02	-0.01	-0.06	-0.09	-0.22	-0.34	-0.18	0.15	0.13	0.13	-0.10	0.03	0.03	-0.13	0.08	-0.23	-0.10	0.10	0.12	-0.28	-0.04	-0.01
RChChl.3-0	-0.02	0.02	0.04	-0.08	0.08	0.02	-0.27	-0.01	-0.16	0.07	-0.19	-0.16	-0.06	-0.15	0.02	-0.27	-0.10	0.08	0.06	-0.08	-0.04	-0.02
RChChl.14-3	0.05	-0.03	-0.15	0.16	-0.19	-0.14	-0.04	0.16	-0.02	0.05	-0.01	0.04	0.10	0.05	0.05	-0.15	-0.07	-0.03	0.01	-0.11	-0.09	-0.03
RChChl.28-14	-0.11	0.02	-0.07	0.14	0.17	-0.22	0.07	0.02	-0.09	-0.01	-0.18	0.02	-0.09	-0.23	0.03	-0.20	-0.02	0.05	0.12	-0.11	-0.04	-0.02
AwnL.Mat	-0.10	-0.12	0.01	-0.08	-0.11	-0.09	0.11	-0.09	0.20	0.06	0.05	0.04	0.00	0.02	0.06	-0.02	0.03	-0.17	-0.22	0.09	0.00	0.02
SpkL.Mat	-0.05	-0.01	-0.02	-0.06	-0.26	-0.20	0.07	-0.01	0.09	0.39	0.10	0.24	0.27	0.14	0.06	-0.02	-0.03	-0.16	-0.21	0.42	0.05	0.08
AIL.Mat.	-0.02	0.11	-0.06	-0.05	-0.24	-0.15	-0.07	-0.07	0.03	0.19	0.48	0.11	0.21	0.28	0.18	-0.04	-0.22	-0.25	0.20	0.33	-0.02	0.16
PedL.Mat	0.03	0.08	0.01	-0.12	-0.25	-0.22	-0.07	-0.05	0.13	0.20	0.44	0.38	0.26	0.28	0.19	0.15	-0.06	-0.31	0.03	0.35	0.11	0.23
Ht.Mat	0.04	0.09	0.00	-0.07	-0.25	-0.13	-0.09	-0.05	0.10	0.15	0.49	0.14	0.24	0.35	0.21	0.19	-0.13	-0.28	0.03	0.38	0.11	0.25
AIL.GroPostH	0.01	0.17	0.15	-0.06	-0.22	-0.11	-0.04	-0.06	0.02	0.15	0.42	0.23	0.28	0.26	0.13	-0.06	-0.03	-0.12	0.18	0.21	-0.06	0.14
PlHt.GroPostH	0.09	0.00	-0.04	0.07	-0.01	-0.12	0.21	0.06	0.23	0.26	0.06	0.25	0.02	-0.11	-0.07	-0.11	-0.05	0.00	0.13	0.08	-0.19	-0.07
NoDevSplt.Spk	-0.06	0.12	0.00	0.03	-0.10	-0.01	-0.02	0.08	-0.09	0.06	0.05	-0.05	0.13	0.04	0.33	-0.11	-0.20	-0.04	-0.18	0.22	0.18	0.25
UndvSplt.Spk	0.05	-0.19	-0.19	-0.03	-0.07	-0.03	0.14	-0.12	0.10	-0.02	0.29	0.03	-0.07	0.33	-0.32	0.45	0.19	-0.19	0.13	0.19	-0.14	-0.15
NoDevSplt.Top	-0.08	0.18	0.03	0.05	-0.08	0.03	-0.04	0.05	-0.12	0.05	0.10	0.03	0.21	0.04	0.38	-0.17	-0.27	-0.03	-0.10	0.25	0.18	0.29
NoDevSplt.Mid	-0.09	0.04	0.02	-0.02	-0.12	-0.06	-0.01	0.08	-0.06	0.09	0.04	0.02	0.12	0.07	0.27	-0.03	-0.12	-0.09	-0.17	0.24	0.22	0.23
NoDevSplt.Bot	-0.04	0.08	-0.04	0.06	-0.05	0.01	-0.02	0.10	-0.08	0.04	0.02	-0.09	0.08	0.03	0.24	-0.12	-0.14	0.04	-0.19	0.20	0.11	0.16
GrNoSplt.1&2.Top	-0.11	0.09	0.07	0.17	0.10	-0.07	0.20	0.08	-0.06	0.11	-0.20	0.12	0.11	-0.39	0.58	-0.24	0.09	-0.05	0.22	-0.09	0.19	0.49
GrNoSplt.1&2.Mid	-0.09	0.11	0.05	0.06	0.00	-0.16	0.08	-0.06	-0.16	0.00	-0.02	0.16	0.11	-0.27	0.54	-0.17	0.08	-0.02	0.12	0.11	0.16	0.48
GrNoSplt.1&2.Bot	-0.05	0.18	0.04	0.08	0.14	-0.01	0.01	0.10	-0.24	-0.03	-0.05	0.24	0.25	-0.27	0.45	-0.18	0.09	-0.03	0.09	0.10	0.15	0.38
GrNoSplt>2.Top	0.02	0.02	0.13	0.00	-0.06	-0.09	-0.01	0.20	0.27	0.22	-0.33	0.12	-0.03	-0.28	0.08	-0.36	-0.04	0.08	-0.09	-0.16	-0.07	-0.02
GrNoSplt>2.Mid	-0.04	0.07	0.09	0.06	0.05	-0.06	-0.06	0.15	0.12	0.09	-0.28	0.07	-0.01	-0.32	0.15	-0.43	-0.04	0.12	-0.05	-0.23	-0.03	0.03
GrNoSplt>2.Bot	-0.05	0.07	0.07	0.05	0.07	-0.06	-0.09	0.18	0.08	0.03	-0.33	0.02	-0.03	-0.36	0.13	-0.41	-0.05	0.18	-0.07	-0.20	-0.07	-0.01
GrNoSplt.Spk	-0.09	0.09	0.09	0.07	0.07	-0.09	0.03	0.15	-0.02	0.09	-0.26	0.20	0.11	-0.39	0.39	-0.40	0.05	0.07	0.03	-0.11	0.07	0.28
GrNo.Spk	-0.09	0.14	0.08	0.06	0.03	-0.10	-0.02	0.17	-0.03	0.12	-0.21	0.13	0.09	-0.34	0.38	-0.43	-0.02	0.06	-0.01	-0.05	0.05	0.26

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

3.5. Summary and conclusions

Heat significantly reduced floret fertility in both durums and hexaploid wheats, and there was significant genetic variation for these responses, indicating a potential for genetic analysis of heat tolerance leading to the discovery of tolerance QTL.

Heat induced floret sterility was greater in the durums than in hexaploid wheat, consistent with their reputations in Australia. However, they were tested at separate time of the year, so a more direct comparison of durums and hexaploid wheats is needed to confirm this trend.

At least in hexaploid wheat, heat responses of floret fertility and its genetic variability was no different in the basal most florets in the spikelets as compared to the more apical florets; hence these phenomena related to grain set of well developed florets and not whole-floret abortion at the more apical floret positions.

The screen of 100 exotic durum accessions identified a number of consistently tolerant accessions. These have the potential to be used as tolerance donors in breeding programs to improve the tolerance of Australian durum varieties.

A number of available hexaploid biparental mapping popuations were found to have parents that contrasted for tolerace to heat induced floret sterility, suggesting these families would be suitable for genetic anlysis to discover tolerance QTL.

Tolerance QTL could could be used for marker assisted selection of heat tolerance in breeding programs. Medium to high heritabiliy of floret fertility under heat suggested that direct phenotypic selection for fertility under heat stress conditions could also be a viable breeding strategy for genetic improvement of heat tolerance in durum or hexaploid wheat varieties.

The heat treatment accelerated flag leaf chlorophyll accumulation but this response showed no consistent correlation with tolerance to heat induced floret sterility. This contrasted with the strong relationships previously reported between staygreen and grain filling heat tolerance, suggesting that grain filling and floret fertility tolerance may have a different mechanistic/genetic basis.

The heat treatment shortened the duration of developmental processes and reduced final organ lengths. These responses showed no consistent relationships with tolerance to heat induced floret sterility, suggesting that the two types of responses were controlled by different mechanisms/genes.

Chapter 4: Mapping QTLs for heat induced floret sterility in a Drysdale × Waagan DH wheat mapping population

4.1. Abstract

The time of reproductive development of wheat crops coincides with the period of greatest heat stress in Australia. To identify quantitative trait loci (QTL) controlling heat induced floret sterility tolerance in wheat and determine whether heat tolerance expressed during booting vs. grain filling stages genetically overlap, an F_1-derived population of 144 double haploid (DH) lines developed from crosses between Drysdale and Waagan was used for QTL mapping of tolerance traits. The experiment employed two replications (blocks) for each of the two developmental stages (3 and 9 cm auricle interval) during pollen development targeted for the heat treatment. All traits measured after the heat treatment showed significant responses to the heat treatment. QTL effects for heat tolerance were detected for 14 genomic regions, on 11 chromosomes. Floret sterility tolerance effects were observed at a major locus on 2B and five minor ones, on 1B, 3B, 4B, 4D, and 7A, with individual QTL explaining between 5.4 and 48.7% of the phenotypic variance. The QTL on 2B co-located with a locus controlling resistance to the yellow rust disease, with heat tolerance being coupled with rust resistance. Since the heat tolerances of the two yield components grain size and grain number were controlled independently, breeders should consider applying selection for both of these traits when breeding for hot environments. The floret fertility tolerance QTL on 2B is recommended for further fine mapping and validation studies.

4.2. Introduction

The projected world population coupled with the need for food are expected to increase by more than 50% by the year of 2050 (Munck *et al.*, 2009). However, extreme weather events limit crop production (Coumou and Rahmstorf, 2012; Lesk *et al.*, 2016). The wheat (*Triticum astivum* L.) growing areas of the world experience environmental stresses including drought and heat stress that adversely affect yield (Semenov and Shewry, 2011). This affects productivity by slowing the rate of genetic gain, especially in low yield potential environments (Graybosch and Peterson, 2012). Due to global warming, mean temperatures are predicted to rise by 0.3 ^{o}C per decade, reaching approximately 1 and 3 ^{o}C above the current temperature by the years 2025 and 2100, respectively (Jones *et al.* (1999), which will aggravate the problem (Bita and Gerats, 2013).

Pradhan *et al.* (2012) suggested high temperature had a greater detrimental effect on wheat yield than drought. In Australia, the reproductive stage of wheat coincides with the period of greatest heat stress (Dolferus *et al.*, 2011). Heat stress at post anthesis leads to reductions in grain size (Dolferus *et al.*, 2011; Shirdelmoghanloo *et al.*, 2016c; Tashiro and Wardlaw, 1990). Pre-anthesis heat stress can reduce grain set, and in extreme cases can cause complete sterility (Saini and Aspinall, 1982a). Using the cultivar 'Gabo', Saini and Aspinall (1982a) identified a window of tiller development that was most sensitive to heat-induced floret sterility; this was the time from meiosis to microspore release from the pollen mother cell (Saini and Aspinall, 1982a).

There has been little documented progress in breeding of wheat that is tolerant under field conditions due to a lack of robust selection and assessment methods; heat stress in the field has unpredictable timing, intensity and often co-occurs with drought stress (Semenov and Shewry, 2011). These difficulties could be overcome by finding chromosomal regions and molecular markers associated with heat tolerance, so that breeders could use marker-assisted selection for heat tolerance breeding.

Despite the detrimental effects of heat stress on yield and quality in wheat (Hamed, 2009), to our knowledge no there have been no reports of loci for tolerance to heat induced floret sterility in wheat. However, QTL related to heat-induced sterility in rice (Jagadish *et al.*, 2010; VIVITHA *et al.*, 2016; Ye *et al.*, 2012; Zhao *et al.*, 2016) and failed seed set in the fruits (siliques) of *Arabidopsis thaliana* (Bac-Molenaar *et al.*, 2015) have been described.

Molecular markers for tolerance to heat induced floret sterility should help breeders produce heat tolerant wheat varieties. Accordingly, the aim of the current study was to identify QTL for tolerance to heat induced floret sterility in hexaploid wheat. Shirdelmoghanloo *et al.* (2016c) had used a mapping population of 144 Waagan × Drysdale DH lines to map tolerance to heat applied at early grain filling, resulting in the identification of tolerance QTL on chromosomes 3B and 6B which effected final grain size. This is the same population as used in the current study. Hence, the results of this and the current study should reveal whether heat tolerance at pollen development and grain filling stages genetically overlap.

Shirdelmoghanloo *et al.* (2016c) constructed a high quality linkage map of the Waagan × Drysdale DH lines using the wheat 9k SNP marker array (Cavanagh *et al.*, 2013). The parent varieties

Waagan and Drysdale have also been screened for heat induced floret sterility across a range of developmental stages (Nick Collins and Iman Lohraseb, unpublished data). The sensitive stage was found to be similar to the one described by Saini and Aspinall (1982) using cv. Gabo, which was at meiosis. Auricle interval length (AIL) was used as an indicator of the pollen developmental stage and was defined as the distance between the auricles of the flag leaf and that of the previous leaf. In Chapter 3, heat induced floret sterility was greatest when heat was applied at 3 cm AIL in Waagan and 9 cm AIL in Drysdale. Furthermore, the varieties contrasted for tolerance, with Waagan being classified as tolerant for heat induced floret fertility, and Drysdale intolerant. Accordingly, in this study, the DH lines were subjected to heat stress (37/27 ^{o}C day/night) for three consecutive days when the primary tillers were at 3 or 9 cm AIL and evaluated for floret sterility at maturity, in order to map tolerance QTL.

4.3. Materials and methods

4.3.1. Plant genetic materials

The F_1-derived population of 144 doubled-haploid (DH) lines had been developed from crosses between Drysdale (Hartog*3/Quarrion) as the female, and Waagan (Janz/24IBWSN-244; 24IBWSN-244 is a CIMMYT line) as the male. The construction of the population and its gentic map has been described by Shirdelmoghanloo *et al.* (2016c). The genetic map is composed of 548 genetically non-redundant molecular marker loci, identified using the wheat Illumina 9,000 SNP array (Cavanagh *et al.*, 2013). Markers on the map also include diagnostic markers for the two major phenology genes segregating in this population (*Rht-B1*, *Rht-D1*), as well as a marker for the *Ppd-B1* gene, for which there is no flowering time segregation in this population.

4.3.2. Greenhouse conditions and heat treatment

The experiment was conducted from March to August in 2014 using the Australian Plant Accelerator plant growth facilities, University of Adelaide, Waite Campus, Adelaide. Plants were initially grown in a naturally lit greenhouse compartment, where the average temperature and relative humidity was recorded as 20/17 ºC and 68/76 % day/night respectively (Appendix table 4.1).

The experiment was arranged in a split plot design with four blocks. Genotypes (DH lines and parents) were randomly allocated to main plots and treatments (control and heat) to subplots which comprise two contiguous plots in rows. The heat treatment was applied at different growth stages of the plants, i.e. plants in block one and three were moved to the heat chamber at 3 cm AIL and plants in block two and four at 9 cm AIL.

Black plastic pots (8 × 8 cm at the top and 18 cm depth) were filled with a coir peat based soil containing slow release fertilizer (Maphosa *et al.*, 2014; Shirdelmoghanloo *et al.*, 2016c) and arranged in drained plastic tubs to stop them falling over. Two seeds were sown per pot and after 7 days seedlings were thinned to one per pot. Plants were kept well-watered until maturity. Plants were free of diseases or insect pests during the entire experimental period and no chemical sprays were used.

Half of the plants were heat treated, with each individual plant being heat treated when its primary tiller (main stem) reached a specific target developmental stage. Heat treatment was performed at 3 cm AIL in two replications and 9 cm AIL in the other two replications. Primary and secondary tillers were labelled with different coloured plastic tags so that they could be distinguished for later scoring. Heat treatments were the same as those used in Chapter 3 and as used by Shirdelmoghanloo *et al.* (2016c). The 37°C/27 °C day/night heat treatment of 3 d duration was performed in a growth chamber (Conviron BDW120), with 3 h ramping periods between day/night temperatures. Pots were placed in trays containing about 2 cm of water to minimize drought stress.

4.3.3. Data collection

The various measured traits, relating to duration of developmental processes, final organ length, spikelet number, or development of lower spikelets and floret fertility, and when they were measured, are described in Table 4.1.

4.3.4. Statistical analysis and QTL mapping

Each trait was analysed separately using linear mixed models and residual maximum likelihood for variance parameter estimation. The models accounted for genetic and non-genetic variation and were fitted with the R package ASReml-R (Gilmour *et al.*, 2009). Best linear unbiased predictors (BLUPs) of the genotypes were extracted from the fitted model.

Table 4. 1 Traits measured

Traits abbreviation	Description	Scored on
Measured pre-heat		
Day.AIL	Days from sowing to when primary tiller reached 3 or 9 cm AIL	Primary tiller
AIL.PreH	Exact measured AIL on the day AIL on the primary tiller was found to be closest to the target length	Primary and secondary tiller
Ht.PreH	Tiller height from soil surface to base of the auricle of the flag leaf on the day AIL in the primary tiller reached the target length	Primary and secondary tiller
Duration of developmental processes		
Day.AwnEm	Days from sowing to when any awn first became visible above the flag leaf auricle.	Primary tiller
Day.Anth	Days from sowing to anthesis	Primary tiller
Day.AILtoAwnEm	Days from target AIL day to awn emergence	Primary tiller
Day.AILtoAnth	Days from target AIL day to anthesis	Primary tiller
Final organ length, or change in length in the period including heat treatment and up to maturity		
AwnL.Mat	Length of longest awn at maturity	Primary and secondary tiller
SpkL.Mat	Length of spike from the bottom to top most glume at maturity	Primary and secondary tiller
AIL.Mat	Auricle interval length at maturity	Primary and secondary tiller
PedL.Mat	Peduncle length at maturity	Primary and secondary tiller
Ht.Mat	Plant height from the soil surface to the top of the spike (excluding awns) at maturity	Primary and secondary tiller
AIL.GroPostH	Change in AIL from the day AIL reached the target length, up to maturity, calculated as AIL.Mat - AIL.PreH	Primary and secondary tiller
PlHt.GroPostH	Change in plant height from the day AIL reached the target length, up to maturity, calculated as Ht.Mat - Ht.PreH	Primary and secondary tiller

Table 4.1 Continued

Traits abbreviation	Description	Scored on
Spikelet number and development of basal spikelets		
ProUndsplt	Proportion of spikelets that were underdeveloped. Underdeveloped spikelets were defined as spikelets having awn length less than 50% that of the spikelets from the middle of the spike.	Primary and secondary tiller
UndvSplt.Spk	Number of under developed spikelets per spike.	Primary and secondary tiller
NoDevSplt.Top	Number of developed spikelets in top of the spike.	Primary and secondary tiller
NoDevSplt.Mid	Number of developed spikelets in middle of the spike.	Primary and secondary tiller
NoDevSplt.Bot	Number of developed spikelets in bottom of the spike.	Primary and secondary tiller
NoDevSplt.Spk	Number of well-developed spikelets per spike (NoDevSplt.Top + NoDevSplt.Mid + NoDevSplt.Bot)	Primary and secondary tiller
NoSplt.Spk	Total number of spikelets (NoDevSplt.Spk + UndvSplt.Spk) per spike	Primary and secondary tiller
Floret fertility (excluding bottom under-developed spikelets)		
GrNoSplt.1&2.Top,	Grain number per spikelet in bottom two floret positions, in the top thirds of spike	Primary and secondary tiller
GrNoSplt.1&2.Mid	Grain number per spikelet in bottom two floret positions, in the middle thirds of spike	Primary and secondary tiller
GrNoSplt.1&2..Bot	Grain number per spikelet in bottom two floret positions, in the bottom thirds of spike	Primary and secondary tiller
GrNoSplt.>2.Top,	Grain number per spikelet in floret positions 3 and above in the spikelets in the top thirds of spike,	Primary and secondary tiller
GrNoSplt.>2.Mid	Grain number per spikelet in floret positions 3 and above in the spikelets in the middle thirds of spike,	
GrNoSplt.>2.Bot	Grain number per spikelet in floret positions 3 and above in the spikelets in the bottom thirds of spike,	
GrNoSplt.Spk	Average grain number per spikelet across the whole spike	Primary and secondary tiller
GrNo.Spk	Total grain number per spike	Primary and secondary tiller

Tolerance index

A heat tolerance index was defined as the residuals from a random regression of the heat vs the control BLUPs according to (Mahjourimajd *et al.*, 2016) and (McDonald *et al.*, 2015): Tolerance = [BLUPs of heat – (β * BLUPs of control)]. The coefficient β was calculated as follows:

$$\beta = \rho hc * \sqrt{\sigma^2 \text{heat} / \sigma^2 \text{control}}$$

where ρhc denotes the genetic correlation between heat and control treatment and σ^2 heat and σ^2control is the genetic variance of the heat and control treatment, respectively.

In other words, the tolerance index for a genotype represented the deviation of the observed value under heat from the value that was expected considering both its value in control and the regression across all the genotypes. The heat index is expressed in the original unit except where transformation was needed to produce a more normal frequency distribution. One reason why it was preferred over the more commonly used heat susceptibility index (HSI; heat susceptibility index) (Fischer and Maurer, 1978) was because the heat tolerance index is much less dependent on the value under control than HSI (data not shown).

QTL analysis was performed for traits under control and heat conditions (BLUPs) and for tolerance index, using GenStat version 16th (Payne *et al.*, 2009; Payne, 2009). QTL analysis was initially conducted by 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 and maximum step size to 10 cM with a genome wide significance level of $\alpha = 0.05$. QTLs were grouped into numbered QTL regions on the basis of being linked within ~30cM.

4.4. Results

Mean responses and means of the studied traits under control conditions are described in Tables 4.2 and 4.3, for the primary and secondary tillers, respectively. For simplicity, descriptions of responses were initially focussed on the primary tiller. For the studied traits, significant differences between genotypes were observed under either the control or heat condition, indicating the existence of variability among the tested genotypes. Except for Day.AIL, AIL.PreH and Ht.PreH (the three traits measured before heat treatment), all the studied traits showed significant treatment effects (Appendix

Tables 4.2a and b), indicating responsiveness to the heat treatment. Generally, heat shortened the duration of developmental processes (Figure 4.1), reduced final organ length (AwnL.Mat, SpkL.Mat, AIL.Mat, PedL.Mat, and Ht.Mat) and decreased floret fertility. Heat did not affect NoSplt.Spk for any treatment-stage /genotype combination (since the number was already fixed at the developmental stage investigated) but did affect the percentage of spikelets on a spike that were classified as underdeveloped (Table 4.2 and 4.3).

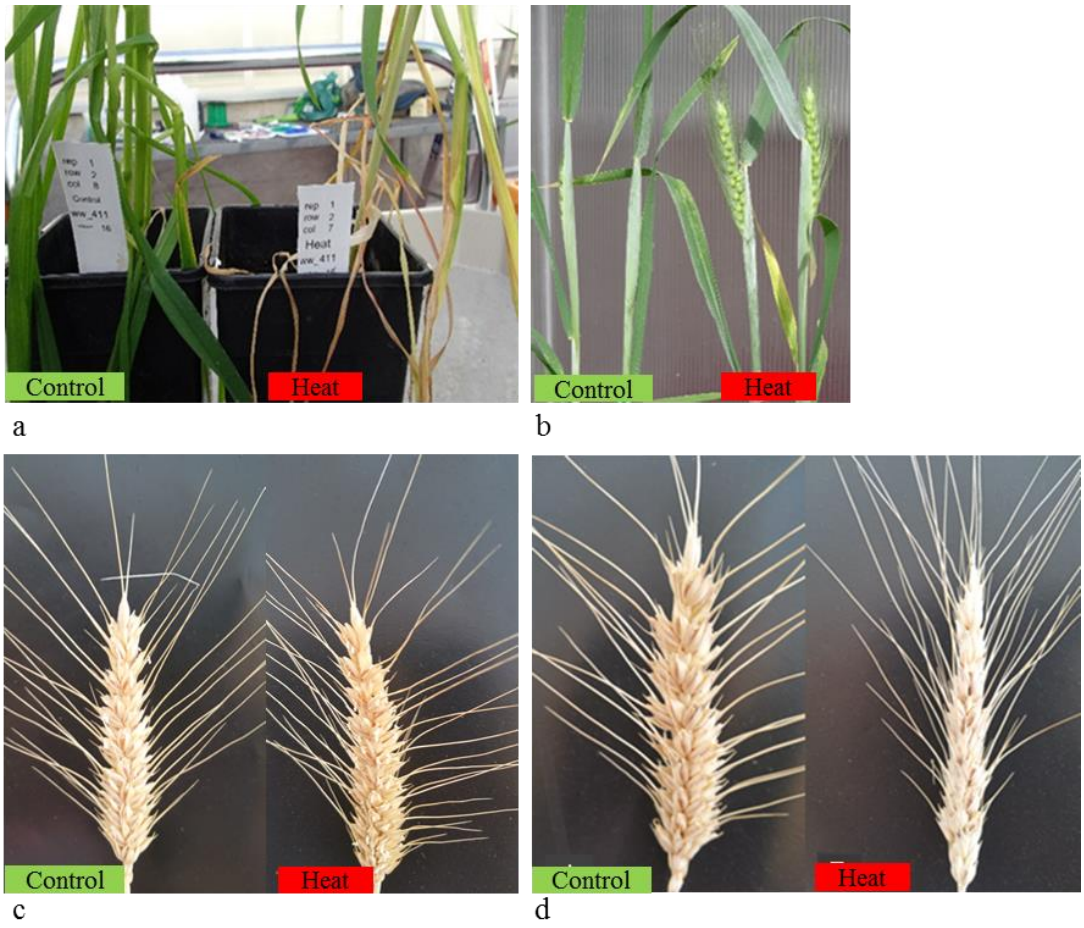

Figure 4. 1 Some typical responses to the 3 d heat treatment applied at booting; a. accelerated chlorophyll loss (discolouration) evident just after the heat treatment, b. 3 d faster spike emergence from the boot; normal floret fertility visible at maturity in the tolerant parent cv. Waagan (c), but a reduction in fertility in the intolerant parent cv. Drysdale (d).

4.4.1. Duration of developmental processes

DH lines took on average 60 d after sowing for awns to emerge (Appendix table 4.2a). All the DH lines had their awn emerged by the 3 cm AIL stage, 17 % of the DH lines (24 plants) had their awns emerged by the 9 cm AIL stage. Thus, the traits Day.AwnEm and Day.AILtoAwnEm could be affected by the heat treatment. Indeed, the treatment at 3 cm AIL did reduce Day.AILtoAwnEm by an average of 2 d (Table 4.2).

Under control conditions, the DH lines took an average of 70 d after sowing to reach anthesis, while they took ~57 d to reach the heat treatment stages (3 or 9 cm AIL). Therefore, anthesis occurred after the heat treatment in all DH lines. Day.Anth was significantly shortened by heat applied at 3 or 9 cm AIL (by ~3 d in each case). In the DHs, heat treatment shortened the period from AIL to Day.Anth (Day.AILtoAnth) by an average of 22 and 34%, for the treatments at 3 and 9 cm AIL, respectively, and shortened the period from AIL to Day.AwnEm (Day.AILtoAwnEm) by an average of 35 and 33%, for the treatments at 3 and 9 cm AIL, respectively.

4.4.2. Final organ length and organ growth during and after heat treatment

Under control conditions, DH plants increased on average 40 cm in plant height and 15 cm in auricle interval, from the day of reaching the target AIL to maturity. Heat reduced plant height by 11 to 32% (13 - 35 cm for the 3 cm AIL treatment and 7 - 27 cm for the 9 cm AIL treatment). Similarly, heat decreased final organ length of AwnL.Mat, AIL.Mat, PedL.Mat and Ht.Mat. Spikes showed a different behaviour, with heat significantly increasing spike lengths at maturity (by 0.39 cm) when heat was applied at 3 cm AIL. Unexpectedly, the trend was that heat reduced AIL, peduncle and plant height more when it was applied later (9 cm AIL) as compared with the earlier stage (3 cm AIL) (Appendix table 4.2a).

4.4.3. Spikelet number and development of basal spikelets

As already mentioned, total number of spikelets per spike was unaffected by heat, which was expected, given that spikelet number is determined in wheat during the stage of terminal spikelet differentiation (31 Zadoks scale), which is well before the stage where the plants were exposed to heat stress (45 Zadoks scale) (Bennett *et al.*, 1973b; Kirby, 1974; Rawson, 1970). However, in the DH

lines, heat stress did decrease the percentage of spikelets that were underdeveloped, by 28.5% and 7.7%, for the treatments at 3 and 9 cm AIL, respectively.

Table 4. 2 Trait responses on primary tillers. Responses are average percent differences in heat vs. control, for the two parents and across all the doubled haploids (DH).

	3 cm AIL			9 cm AIL			Mean for DH in
Trait	Drysdale	Waagan	DH	Drysdale	Waagan	DH	control
Traits measured pre-heat							
Day.AIL	-0.95	0.00	-0.24	5.14	-0.41	-0.11	57.2 d
AIL.PreH	-40.5	-8.57	-11.5	-8.00	7.50	-1.97	6.34 cm
Ht.PreH	2.28	-12.6	-0.86	8.13	5.03	0.61	40.6 cm
Duration of developmental processes							
Day.AwnEm	-3.81	-0.40	-3.40**	1.75	0.95	-1.28**	61.3 d
Day.Anth	-4.06	-3.56	-4.34**	-3.82	-3.89	-4.99**	68.9 d
Day.AILtoAwnEm	-28.0	-6.25	-34.6**	-46.7	5.00	-32.9**	4.08 d
Day.AILtoAnth	-15.0	-21.7*	-22.0**	-43.7**	-25.0	-33.7**	11.8 d
Final organ length, or gain in organ length from the commencement of treatment							
AwnL.Mat	-19.3*	-10.4	-6.01**	0.00	0.00	-2.43*	5.19 cm
SpkL.Mat	-2.71	-7.35	4.16**	-0.82	-5.56	-0.64**	8.41 cm
AIL.Mat	-17.2	-18.4*	-13.7**	-33.5**	-9.27	-18.5**	18.9 cm
PedL.Mat	-17.8	-11.5	-7.72**	-16.9**	-1.71	-11.3**	30.2 cm
Ht.Mat	-10.9	-14.9**	-6.83**	-13.4**	-6.25	-9.12**	77.6 cm
AIL.GroPostH	-10.9	-21.4*	-14.0**	-49.4**	-21.6	-31.8**	12.5 cm
PlHt.GroPostH	-21.4*	-17.0	-11.7**	-36.0**	-19.6	-21.3**	36.7 cm
Spikelet number and development of spikelet at basal position in spike							
NoSplt.Spk	1.12	-7.37	0.428	1.14	-3.92	0.056	21.4
ProUndsplt	-32.2	19.7	-28.5**	17.0	2.89	-7.69**	0.27
Grain number per spikelet located at different positions within the spike							
GrNoSplt. 1&2.Top	-47.8	-41.6*	-56.7**	-44.3	-9.84	-46.2**	1.60
GrNoSplt.1&2.Mid	-48.2*	-6.52	-44.6**	-42.9*	-8.57	-31.9**	1.82
GrNoSplt.1&2.Bot	-23.9	-20.9	-37.9**	-59.1*	-5.26	-38.5**	1.77
GrNoSplt.>2.Top	-66.2	200	39.6**	-100	-50.0	-47.5**	0.17
GrNoSplt.>2.Mid	-41.9	-33.9	-2.94	-65.5	41.5	-39.1**	0.75
GrNoSplt.>2.Bot	-18.8	42.9	7.48**	-70.6*	-18.1	-32.0**	0.50
GrNoSplt.Spk	-40.4*	-19.9	-36.2**	-56.7**	-5.64	-38.5**	2.19

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

4.4.4. Floret fertility (grain number per spikelet)

Under optimal growing conditions (e.g., good disease control, lighting and nutrition), wheat spikelets produce close to an average of two grains in the two lowermost floret positions in the spikelet, whereas the number of grains set in the more apical florets on the spikelet is variable, depending on the genotype. The average grain number per spikelet in the first and second floret position in the control DH lines was 1.6, 1.8 and 1.8 in the top, middle and bottom thirds of the spike (Appendix table 4.2a; the three parts of the spike are described in Chapter 3; Figure 3.1) indicating good levels of fertility in this experiment.

Significant reductions in floret fertility were observed in the DHs, for 9 out of the 12 floret type × treatment time combinations. The reduction in fertility ranged from 32 to 57% (Table 4.2). The exception was spikelet fertility in the more apical florets of the spikelets (GrNoSplt.>2) for the 3 cm AIL treatment, where heat significantly increased fertility in the top and bottom part of the spike, and had no significant effect in the middle part. However, the overall effect on fertility of the entire spike was negative because overall the >2 floret position contributed fewer grains than the floret 1&2 positions. For all fertility traits, Waagan was affected less than Drysdale, consistent with the previous classification of Waagan as being more tolerant than Drysdale.

4.4.5. Responses to heat stress in secondary tillers

When the primary tillers were at 3 and 9 cm AIL, the secondary tillers showed an average AIL of 1.6 and 6.5 cm, respectively, which overlapped with the developmental window for heat induced floret sterility susceptibility. Similar to the primary tillers, the secondary tillers showed a reduction in grain number due to heat (GrNoSplt.Spk) (Table 4.3). Generally, fertility responded less to the earlier treatment (1.6 cm AIL) than the later one (6.5 cm AIL), implying 1.6 cm AIL preceeded the peak in susceptibility. Interestingly, as is the case for the primary tiller, heat significantly increased grain number in the third-and-above floret position for some of the genotype × treatment-stage combinations.

As secondary tillers were at an earlier stage of development than the primary tillers when heat-treated, the secondary tillers were expected to show greater responses to growth parameters. This was the case (greater reduction under heat) for traits AIL.Mat, PedL.Mat, AIL.GroPostH and PlHt.GroPostH (Table 4.2 and 4.3). As in the primary tillers, heat significantly increased final spike

length at maturity, although only with the 1.6 cm AIL treatment (by 0.58 cm; compared with 0.39 cm in the primary tillers).

Table 4. 3 Trait responses on secondary tillers. Responses are average percent differences in heat vs. control, for the two parents and across all the doubled haploids (DH).

	1.6 cm AIL			6.5 cm AIL			Mean for DH
Trait	**Drysdale**	**Waagan**	**DH**	**Drysdale**	**Waagan**	**DH**	**in control**
Traits measured pre-heat							
AIL.PreH	-21.4	-65.2	-3.5	64.7	-21.4	-1.4	4.15 cm
Ht.PreH	-16.5	3.73	-0.6	10.2	10.4	-0.76	35.0 cm
Final organ length, or gain in organ length from the commencement of treatment (cm)							
AwnL.Mat	-4.76	25*	-7.4**	-100***	7.69	-2.7*	5.12 cm
SpkL.Mat	10.8	6.45	7.3**	0	0	-0.32	7.99 cm
AIL.Mat	-15.1*	-12	-10.4**	-18.8*	-3.45	-17.5**	18.2 cm
PedL.Mat	-13.8*	-4.72	-7.0**	-13.4*	-9.76	-10.8**	29.9 cm
Ht.Mat	-8.49	-6.75	-6.8**	-11.8*	2.41	-10.4**	75.2 cm
AIL.GroPostH	-16.1*	-20.5*	-11.8**	-42.9**	7.91	-26.2**	14.0 cm
PlHt.GroPostH	13.6*	-23.8*	-10.6**	-31.1**	-5.94	-20.6**	39.8 cm
Spikelet number and development of basal spikelets							
NoSplt.Spk	0	3.16	0.6	-1.17	1.05	-0.5	21.7
ProUndsplt	-8.24	-31.9**	-27.6**	-4.92	-22.6	-7.89**	0.31
Grain number per spikelet							
GrNoSplt. 1&2.Top	-30.5*	-14.3*	-38.4**	-70.5**	-9.35	-48.5**	1.50
GrNoSplt.1&2.Mid	-4.65	-20.4*	-36.4**	-52.5***	0	-39.7**	1.80
GrNoSplt.1&2.Bot	-21.1*	-6.08	-26.5**	-48.4**	-2.5	-39.5**	1.70
GrNoSplt.>2.Top	0	0.03	155**	-68.4*	100**	-22.3**	0.10
GrNoSplt.>2.Mid	-80**	3.04	19.3**	-69.7**	87.7**	-29.7**	0.60
GrNoSplt.>2.Bot	-54.5**	0.15	14.6**	-67.9*	40.5**	-20.7**	0.40
GrNoSplt.Spk	-30.3**	-9.71	-24.1**	-59.7***	1.99	-39.3**	2.10

* and ** indicate significant difference between control and heat-treated plants respective to each tested material at $p < 0.05$ and $p < 0.01$, respectively

4.4.6. Quantitative trait loci

Under the experimental conditions applied in this study, QTL were detected in 29 genomic regions (numbered QTL1-QTL29), which were located on all chromosomes except for 1D, 3D and 7D (Appendix table 4.4 and 4.5). No QTL was detected for the trait Day.AwnEm.

Some phenology and fertility traits were also mapped in this population by (Shirdelmoghanloo *et al.*, 2016c) (date of anthesis, grain number and plant height at maturity), generally revealing the same QTL (under control conditions) between the two studies for these traits (not shown). The population segregates for both major semi-dwarf genes *Rht-B1* and *Rht-D1* but does not segregate for any known major locus for vernalisation or photoperiod response (Shirdelmoghanloo *et al.*, 2016c). There were only two flowering time QTL, which were very minor, on chromosomes 2B, and 4B, determining differences in time to anthesis of only 1.3 to 2.8 d (Appendix table 4.5).

QTL effects for heat tolerance indexes (heat responses) were detected for 14 regions, located on 11 chromosomes (Appendix table 4.3). Of these, six QTL (QTL2 on 1B1, QTL7 on 2B1, QTL12 on 3B2, QTL16 on 4B, QTL17 on 4D, and QTL26 on 7A2) were associated with floret sterility tolerance, with individual QTL explaining between 5.4 and 48.7% of the phenotypic variance (Table 4.4). Both parents contributed favourable alleles (for stability of floret fertility) and in some cases heat tolerance QTL co-located with QTL for traits measured under control or/and heat conditions, or heat response QTL for other traits as described in more detail below.

QTL regions detected for floret fertility tolerance in the primary tiller

QTL7 on chromosome 2B

Heat sterility tolerance QTL effects were strongest and most frequently observed across floret and tiller type at the QTL7 region on short arm of chromosome 2B (linkage group 2B1; Table 4.4, 4.5 and 4.6). The region explained from 9.1 to 48.7% of the phenotypic variance and Waagan contributed the allele for heat tolerance. *Per se* floret fertility QTL were detected here only under the heat condition (Waagan allele being positive). The region co-located with QTL for awn length *per se* at maturity (Drysdale contributing the positive allele) under both control and heat conditions.

QTL17 (Rht-D1) on chromosome 4D

The QTL17 region containing the segregating height locus *Rht-D1* also showed heat tolerance QTL for floret fertility. In one case (trait GrNoSplt.1&2Top), the dwarfing allele from Drysdale was associated with tolerance, while in another (trait GrNoSplt.>2Mid) it was associated with intolerance. The Waagan allele also increased GrNoSplt.1&2Bot *per se* on both tiller types under both heat and control conditions, for the treatment at 3 cm AIL.

As expected from the knowledge that *Rht-D1* segregated in this population, the region also influenced final organ length (for AIL.Mat and Ht.Mat) with the dwarfing allele from Drysdale being negative. It also influenced stability of these organ length traits under heat, with the dwarfing allele being associated with stability. It accounted for 42% and 45% of the phenotypic variability for the AIL.Mat and Ht.Mat heat responses, respectively. This region also influenced the proportion of spikelets that were underdeveloped, under all conditions and in both tiller types, with the dwarfing allele from Drysdale increasing this trait, but the region did not affect heat responsiveness of the trait.

QTL26 on chromosome 7A

Tolerance QTL for grain number per spike and grain number per spikelet (over the whole spike) was detected on the short arm of chromosome 7A (linkage group 7A2) with the Waagan allele conferring tolerance. The Waagan allele also increased the number of developed spikelets per spike (as defined in Table 4.1; bottom, middle, top & overall; for both treatment stages) under control and heat conditions, but only on the secondary tillers. However, the region showed no stability QTL effects for this trait.

QTL2 on chromosome 1B

A floret sterility tolerance QTL was detected on the short arm of 1B (QTL2; linkage group 1B1) for heat applied at 3 cm AIL, on both primary (GrNo.Spk) and secondary (GrNoSplt.Spk) tillers, explaining about 8% of the phenotypic variability in both cases. Drysdale contributed the tolerance allele. This region also showed fertility *per se* trait QTLs under heat but not under control conditions.

Floret fertility tolerance QTLs detected only in the secondary tillers

QTL12 on chromosome 3B

A sterility tolerance QTL for grain number per spikelet for the bottom third of the spike was detected on the long arm of chromosome 3B (80.9-105.8 cM; linkage group 3B2). It explained 10.7 to 14.4 % of the phenotypic variability and Waagan contributed the tolerance allele. No significant fertility *per se* QTL were identified here under either control or heat conditions. QTL for height at maturity *per se* in both heat and control plants were located here, with additive effects of 1.43 to 1.63 cm, with Waagan contributing the positive allele.

QTL16 on chromosome 4B

This region gave a tolerance QTL effect for GrNoSplt.1&2Mid on the secondary tillers, with Drysdale providing the allele for stability. It also showed several fertility *per se* QTL effects under both heat and control conditions (on secondary tillers), or control conditions only (primary tillers), for various floret types and treatment timings, with Drysdale contributing the positive allele.

The QTL16 region also showed tolerance effects for peduncle length (PedL.Mat; on secondary tillers) and plant height during & post heat (PlHt.GroPostH; on primary tiller), explaining 15 and 12% of the phenotypic variance, respectively, with Drysdale providing stability. However, no significant QTL were detected here for the *per se* traits for either control or heat conditions. The QTL16 region also affected response of auricle interval length at maturity (AIL.Mat) explaining 2% of the variation, with Waagan conferring the stability allele. QTL16 also showed AIL.Mat *per se* QTL effects, with the Drysdale allele increasing its length under both control and heat conditions. Hence, heat stability for organ length and growth traits was contributed by both parents at this genomic location, depending on the trait.

The QTL16 region also influenced stability under heat for the number of underdeveloped spikelets per spike (UndvSplt.Spk), explaining more than 11% of the variation, with Drysdale contributing the stability allele. This region also showed *per se* QTL for UndvSplt.Spk, with the Waagan allele increasing UndvSplt.Spk.

Table 4. 4 The six chromosome regions containing QTL for tolerance to heat-induced floret sterility, detected in the Drysdale × Waagan mapping population.

Tolerance QTL	Positive allele donor	Linkage group	cM location	Closest marker/s
QTL2	Drysdale	1B	53.2-62.5	wsnp_Ex_c22377_31571527
QTL7	Waagan	2B1	79.5	wsnp_JD_c3732_4781170
QTL12	Waagan	3B2	80.9-105.8	wsnp_Ex_c9594_15882022
QTL16	Drysdale	4B	127.5	wsnp_Ku_c11570_18860306
QTL17 (*Rht-D1*)	Drysdale or Waagan	4D	0-3	Rht-D1/ wsnp_CAP11_c356_280910
QTL26	Waagan	7A2	43-67	wsnp_Ex_c2268_4251636/wsnp_Ex_c12102_19361467

Table 4. 5 Significant QTL effects for tolerance to heat-induced floret sterility, observed at three positions within the spike (top, middle, bottom third), in the Drysdale × Waagan mapping population, for primary tillers. Additive effects for the fertility tolerance QTL (based on tolerance index) are shown, with % variance explained in parentheses.

	3 cm AIL treatment							9 cm AIL treatment						
	Top		Middle		Bottom		Whole spike	Top		Middle		Bottom		Whole spike
	1&2	>2	1&2	>2	1&2	>2		1&2	>2	1&2	>2	1&2	>2	
QTL2	-	-	-	-	-	-	1.15(8)	-	-	-	-	-	-	-
QTL7	0.33(26)	-	0.44(31)	-	0.22(25)	0.01(18)	2.50(32)	0.35(25)	-	0.47(25)	0.08(23)	0.74(43)	0.05(18)	4.96(49)
QTL12	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QTL16	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QTL17 (*Rht-D1*)	-	-	-	-	-	-	-	0.25(12)	-	-	-	-	-	-
QTL26	-	-	-	-	-	-	-	0.18(7)	-	0.24(7)	-	-	-	1.64(6)

-, no significant tolerance QTL detected

Table 4. 6 Occurrence of significant QTL effects for tolerance to heat-induced floret sterility, observed at three positions within the spike (top, middle, bottom third), in the Drysdale × Waagan mapping population, for secondary tillers. Additive effects of the fertility tolerance QTL (based on tolerance index) are shown, with variance explained in parentheses.

	3 cm AIL treatment							9 cm AIL treatment						
	Top		Middle		Bottom		Whole spike	Top		Middle		Bottom		Whole spike
	1&2	>2	1&2	>2	1&2	>2		1&2	>2	1&2	>2	1&2	>2	
QTL2	-	-	-	-	-	-	0.12(8)	-	-	-	-	-	-	-
QTL7	0.07(9)	-	-	-	-	-	0.99(15)	0.38(19)	-	-	0.04(10)	0.29(21)	-	2.69(31)
QTL12	-	-	-	-	0.12(11)	-	-	-	-	-	-	0.24(15)	-	-
QTL16	-	-	0.0(9)	-	-	-	-	-	-	-	-	-	-	-
QTL17 (Rht-D1)	-	-	0.0(15)	0.0(15)	-	-	-	-	-	-	-	-	-	-
QTL26	-	-	-	-	-	-	-	-	-	-	-	-	-	-

-, no significant tolerance QTL detected

Other QTL regions detected for floret fertility per se only under heat stress

Chromosome regions showing QTL effects for *per se* traits under heat but not control conditions, and not showing any significant tolerance QTL effect, were candidates for weak tolerance loci. There were three such regions for floret fertility: QTL22 on chromosome 6A and two regions on chromosome 7B (QTL28 and QTL29). For QTL22 and QTL28, Waagan contributed the positive allele while for QTL29 it was Drysdale. QTL28 and 29 effects were detected for floret 1&2 positions on both tiller types (primary and secondary). QTL22 was co-located with Ht.PreH *per se* QTL effect, while the other two chromosome regions were not associated with any other trait.

Other QTL regions detected for heat tolerance of final organ length and spikelet number

In addition to QTL17 (*Rht-D1*), the QTL15 (*Rht-B1*; dwarfing allele for Waagan) on chromosome 4B influenced heat stability of the organ length traits Ht.Mat and AIL.Mat. The effect for Ht.Mat was detected in secondary tillers with the early-stage heat treatment, while the effect for AIL.Mat was detected in primary tillers with the late-stage treatment. The QTL region explained 41% and 45% of the phenotypic variance for response of Ht.Mat and AIL.Mat, respectively. In both cases, the Waagan allele was positive for stability, but negative for the organ length *per se* traits for various tillers/treatment stage combinations under heat or control conditions. This region also affected UndvSplt.Spk, where the Drysdale allele contributed heat stability and lowered *per se* trait values.

QTL5 on chromosome 2A influenced heat response of Ht.Mat, where Drysdale contributed stability. The same allele also contributed to Ht.Mat, AIL.Mat *per se*, and their growth from the time of treatment to maturity, under heat or control conditions.

At QTL13, on linkage group 4A2, the Drysdale allele conferred stability for AIL.Mat and Ht.Mat but lower *per se* values, under both conditions.

QTL25 on the short arm of chromosome 7A (linkage group 7A2) influenced AIL.Mat stability. Here, the Waagan allele contributed stability and decreased *per se* values under both, heat and control conditions.

QTL20 on chromosome 5D (40.2cM; linkage group 5D2) affected awn length stability on the primary tiller for the early stage heat treatment, explaining 11.32% of the phenotypic variance for the trait, with the Waagan contributing the stability allele.

At QTL27 on chromosome 7B the Drysdale allele contributed heat stability of awn length on secondary tillers, explaining 9% the phenotypic variance, and increased the *per se* trait on both tiller types for both treatment stages, in both control and heat treated plants. The Drysdale allele also delayed the time from heat treatment to anthesis on primary tillers, for the early stage of heat treatment.

QTL9 on chromosome 2D4 (7.58 to 18.8 cM) was the only region that showed QTL for stability of spike length at maturity and explained~9% of the phenotypic variance. This effect was detected on the secondary tillers with the early stage of heat treatment. The Drysdale allele contributed stability and increased spike length *per se* under both, heat and control conditions.

QTL4 on chromosome 2A showed QTL for heat stability of the number of developed and under developed spikelet number per spike. These effects were detected for the early stage of heat treatment on the secondary tiller. The Waagan allele contributed tolerance for developed spikelets, while conversely, the Drysdale allele contributed stability for underdeveloped spikelets. However, no *per se* QTL effect for these traits was detected here.

QTL21 on chromosome 6A also affected stability for underdeveloped spikelet number. This was detected for both treatment stages in the primary tillers, with the Drysdale allele contributing tolerance.

4.5. Discussion

There has been little significant progress in breeding wheat for heat tolerance and no other QTL has yet been described in wheat for tolerance to heat induced floret sterility. With an objective to developing molecular markers that could be used to aid selection in heat tolerance breeding, a Drysdale × Waagan mapping population was used to dissect the genetic basis for tolerance to heat induced floret sterility in wheat.

While the overall effect of heat on floret fertility was negative, it was interesting that positive effects of heat on fertility were sometimes observed in the florets at positions >2 on the spikelets,

suggesting that heat could have a second effect on floret fertility (positive), that was particular to these floret positions. These positive effects were observed in Waagan and the DHs, but not in Drysdale. Perhaps in Drysdale, heat induced floret sterility (owing to this variety's intolerance) always outweighed any positive effect. The mechanism by which heat can promote fertility in the apical florets of the spikelet is currently unknown. At the QTL17 (*Rht-D1*) locus, the dwarfing allele from Drysdale was associated with tolerance in the floret 1&2 positions, but intolerance at the >2 floret positions, suggesting that the two effects (positively and negative) might be affected by related phenomena.

Six genomic regions (1B (QTL2), 2B (QTL7), 3B (QTL12), 4B (QTL16), 4D (QTL17) and 7A (QTL26)) were found to be associated with heat-induced floret sterility tolerance. Both parents contributed to heat tolerance. Waagan, the heat-tolerant parent, contributed the tolerance allele at QTL7, QTL12 and QTL26, while the susceptible parent Drysdale contributed the tolerant allele at QTL2 and QTL16. QTL7 was the strongest of the fertility tolerance QTLs, explaining 49% of the phenotypic variability. QTL7 perhaps largely accounted for why Waagan was the more tolerant of the two varieties.

4.5.1. Floret sterility tolerance QTL suspected of being artefacts

QTL17 (*Rht-D1*)

The Drysdale × Waagan DH population segregated for both *Rht* genes (38 double dwarf, 76 semi dwarf and 30 double tall types), which also affected final AIL. These *Rht* loci did not affect flowering time, suggesting that they did not influence the duration of developmental processes. However, at a given AIL, spikes in double-tall and double-dwarf DH plants may have been at earlier and later stages of development, respectively, than those of semi dwarf DH plants, as the AIL would have taken longer to reach the target length in the double-dwarf plants. This notion was supported by the QTL for *per se* Day.AILtoAnth observed under both control and heat condition at *Rht-B1* and *Rht-D1*, with the dwarfing alleles shortening this interval, indicating that at a given AIL, the double dwarf plants were further advanced in their progress towards maturity. Hence, the 'floret fertility tolerance' effects observed at the *Rht-D1* locus might have been due to 'escape' (not tolerance *per se*), due to alteration in the relationship between the AIL and spike developmental stage. This would need to be confirmed by further study.

Effects on heat responses of fertility were detected at *Rht-D1*, but for an unknown reason, not at *Rht-B1*. At *Rht-D1,* the dwarfing allele contributed 'tolerance' for GrNoSplt.1&2.Top but 'intolerance' for GrNoSplt.1&2.Mid. This switch of allele effects would be consistent with escape. The auricle interval lengths used to target heat treatments in this study were chosen based on susceptible windows of development determined in the parent varieties Waagan and Drysdale, which are semi dwarfs (each only carrying one of the two *Rht* genes). So, for example, in double dwarf plants at the target AIL, florets 1&2 could have been past the susceptible developmental window (hence the dwarf allele confers tolerance), while florets at >2 positions (which develop after florets at positions 1&2) might have been in the susceptible window (hence the dwarf allele confers intolerance).

The *Rht* loci also affected fertility *per se*, whereby the tall alleles at both, *Rht-B1* and *Rht-D1* promoted floret fertility *per se* under the two temperature conditions. The loci did not affect total spikelet number, so the fertility effect may relate to a greater available source supply in the double tall plants (due to more shoot mass) for a given number of spikelets. The tall alleles also reduced the proportion of spikelets that were underdeveloped, which perhaps also related to greater source supply.

A previous study (Law and Worland, 1984) reported that *Rht* dwarfing alleles (*Rht-B1b, Rht-D1b,* and *Rht-B1c*) enhanced floret sterility caused by high temperatures during booting, which perhaps relates to the aforementioned escape and/or source/sink effects.

At both *Rht-B1* and *Rht-D1* the dwarfing allele protected AIL.Mat and Ph.Mat from length reductions due to heat (were positive for the tolerance indexes), probably because double-dwarf plants were closer to their final height at the target AIL stage, allowing less opportunity for the heat treatment to have an impact on further growth.

QTL16

QTL16 on 4B was 51 cM below *Rht-B1* and hence would not have been due to the *Rht-B1* gene. It was the strongest flowering time QTL (traits Day.Anth and Day.AwnEm) although the effect was minor; the Waagan allele delayed anthesis and awn emergence by only 2.8 and 3 days, respectively. The fertility tolerance effect associated with the Drysdale allele was seen only for a specific spike segment (GrNoSplt.1&2.Mid) and only with the earliest stage treatment (3 cm AIL) in secondary tillers. As it was only detected in secondary tillers, it raised the question of whether it arose from a genetic effect that influenced the relative timing of development of the primary vs. secondary

tillers. However, the absence of an AIL.PreH or Ht.PreH effect in the secondary tillers at this locus did not support such a theory.

At this locus, the Drysdale allele decreased the numbers of spikelets per spike and proportion of spikelets that were underdeveloped but increased floret fertility *per se.* Shirdelmoghanloo *et al.* (2016c) also showed that the later flowering time allele (Waagan) increased shoot biomass. The net result of these effects may have been a higher source:sink ratio associated with the Drysdale allele, favouring greater floret fertility stability under heat. Nonetheless, co-location with a flowering time effect still raises suspicion that the QTL16 tolerance effect arose by escape.

QTL12

The fertility tolerance QTL12 on 3B (QTL12) co-located with QTL for height *per se* traits under both treatment conditions and stages. This was one of seven minor height QTL (after *Rht-B1* sand *Rht-D1*) with additive effects of up to 3.3 cm for height at maturity. As argued for *Rht-D1,* a height effect could have affected the AIL vs. spike stage relationship leading an escape effect, although the lack of a significant Day.AILtoAnth QTL effect here provided no evidence in favour of that theory. Because the QTL12 floret fertility effect appeared only in secondary tillers, it also raised the question of whether it resulted from a genetic effect causing differences in relative development rates of the primary vs. secondary tillers. However, the absence of AIL.PreH or Ht.PreH effect in the secondary tillers at this locus did not support that idea. In summary, there was some evidence the QTL12 floret fertility effect arose by escape, due to the height effect.

4.5.2. Tolerance loci for heat induced floret sterility

QTL7

QTL7 on the short arm of chromosome 2B (linkage group 2B1) showed the strongest (explained 49% of the phenotypic variability) and most consistent fertility tolerance QTL effects over the range of floret developmental stages. In main stems of the DH lines carrying the Drysdale allele at the 2B locus, the 9 cm AIL stage treatment reduced fertility by an average of 20 grains out of a potential number of 37 per spike (observed in the control). The DH lines carrying the Waagan allele lost an average of 7 (fewer grains) per spike. In other words, the presence of the favourable allele more than halved the impact of the heat stress on grain number under these conditions. How the QTL could influence yield in the field could not be estimated, as we don't yet know the impact of heat induced floret sterility on wheat productivity, or whether the QTL might be expressed differently in the field relative to the greenhouse. Heat stress in the field often occurs with drought stress, which may affect the expression of the tolerance. It is therefore important that the effect of the QTL be measured using field trials, to determine the potential use of the QTL to breeders. It is unclear how the co-located AwnL.Mat *per se* QTL could be related to floret fertility tolerance mechanisms and it seems likely that these co-located QTL reflect the effects of separate but closely linked genes.

Ppd-B1 is a major phenology locus in wheat which affects flowering time in a photoperiod dependent manner (Beales *et al.*, 2007) and is located on chromosome 2B (Kuchel *et al.*, 2006; Shindo *et al.*, 2003; Shirdelmoghanloo *et al.*, 2016c). *Ppd-B1* was mapped in the Drysdale × Waagan DH population using a SNP within the gene, but the alleles segregating in this population did not appear to functionally differ, as no flowering time locus was detected there (Shirdelmoghanloo *et al.*, 2016c). Day.Anth *per se* QTL under either condition was detected on 2B1 (at 26 cM) but this was about 50 cM from the fertility tolerance QTL. Therefore, the floret fertility tolerance effect of QTL7 was shown to be independent of *Ppd-B1*.

The Drysdale × Waagan DH population was field trialed three times in Southern NSW, where some level of natural rust infection occurred. This was most likely wheat yellow (stripe) rust (*Puccina striiformis* f. sp. *tritici*), based on observation of rusts known to be present in this area during these seasons (Prof Robert Park, personal communication). The infection levels were scored and the data used in QTL analysis (Livinus Emebiri and Hamid Shirdelmoghanloo, unpublished data). The strongest rust resistance QTL recorded in the 2012 Wagga Wagga trial was on 2BS, at 93.9 cM on

linkage group 2B1 (wsnp_Ex_c13351_21042379), and explained 66% of the phenotypic variability, with Waagan contributing the resistance allele. In the 2013 Wagga Wagga trial, a tolerance QTL was detected almost at the same position (84.8 cM; wsnp_RFL_Contig4483_5312236(C)) but Drysdale was the resistance allele contributor (indicating the presence of a different rust type/isolate) and it explained only 8.8% of the phenotypic variability. These rust resistance QTL were located very close to the fertility heat tolerance locus QTL7 detected in the greenhouse/chamber experiment, positioned at 79.5 cM on 2B1. Rust resistance genes known to be located on chromosome 2B and present in Waagan and Drysdale include *Yr27* (for yellow rust, present in Waagan) and *Lr23, (*for leaf (brown) rust, present in Drysdale) (McDonald *et al.*, 2004).

The strong rust resistance effect from the Waagan allele of the the 2B locus observed in the 2012 trial could have been the yellow rust resistance gene *Yr27*. This gene originated from a Canadian farmer's selection named McMurachy from where the gene was transferred to Selkirk, and subsequently to a range of CIMMYT cultivars (McDonald *et al.*, 2004). Waagan was developed from crosses between Janz and the CIMMYT line 24IBWSN-244; the latter could have contributed the *Yr27* resistance gene to Waagan.

QTL2 and QTL26

QTL2 on 1B influenced fertility tolerance in both primary and secondary tillers, for GrNo.Spk and GrNoSplt, respectively (Appendix table 4.4). Furthermore, it produced *per se* fertility QTL effects only under heat and was not associated with QTL for any other traits. Therefore, it appeared that this fertility tolerance locus was not a developmental artefact.

The QTL26 fertility tolerance effect on chromosome 7A (positive allele from Waagan) was observed only in the primary tiller with the 9 cm AIL heat stress. Besides floret fertility tolerance, QTL26 influenced grain number per spikelet *per se* traits and spikelets per spike *per se* traits (under either control or heat conditions; only in the secondary tillers; with the Waagan allele being positive). Co-location of these traits suggests that the traits may be functionally linked (although they were detected on different tiller classes).

The additive effects of QTL2 and QTL26 on floret sterility tolerance was ~12 and 7 -fold smaller than that of QTL7, respectively. Therefore, they show considerably less promise than QTL as a basis for breeding heat tolerant wheat.

The three QTLs that appeared to be genuine tolerance loci were named using the conventional system for wheat: QHFert.aww-1B (for QTL2); QHFert.aww-2B (for QTL7) and QHFert.aww-7A (for QTL26, with H for heat tolerance, Fert floret fertility, and aww Australia wheat Waite).

4.5.3. Relationship of heat tolerance QTL with tolerance at grain filling

The Waagan × Drysdale population used in this study had previously been analysed by Shirdelmoghanloo *et al.* (2016c) under heat stress applied at 10 days after anthesis to study the effect of heat on grain filling (final grain size), while in the current study, treatment was done much earlier, at ~13.5 and 10 days before anthesis (corresponding to the 3 and 9 cm AIL stages, respectively) to target floret fertility effects. Shirdelmoghanloo *et al.* (2016c) identified two heat tolerance loci for grain filling, on linkage groups 3B1 (0-3.15 cM) and 6B3 (9.06-18.11 cM). Those QTL did not co-locate with any of the tolerance loci identified for floret fertility in the current study. This indicates that the genetic control of tolerance at the two different developmental stages is independent.

The grain filling heat tolerance QTL co-located with QTL effects for chlorophyll and shoot weight responses (stability for all three being coupled), suggesting that the mechanism of grain filling heat tolerance was related to that of chlorophyll and shoot weight stability. One fertility tolerance locus QTL16 co-located with a chlorophyll heat stability effect from the previous study (traits FlSe and ChlR27; QTL18 in Shirdelmoghanloo *et al.* (2016c), with the tolerance allele for floret fertility (from Drysdale) providing chlorophyll stability under heat. However, a causal relationship between chlorophyll and floret fertility heat tolerance cannot be confidently attributed to QTL16, due to other confounding developmental QTL effects observed at this position, which include *per se* and response QTL for almost all of the studied traits. The other five floret fertility tolerance loci identified (including the major one on chromosome 2B) did not co-locate with any chlorophyll or shoot weight stability QTL effect from the previous study. Overall, the evidence points to mechanisms of chlorophyll and shoot weight stability being linked to those of grain filling heat tolerance, but not those of floret fertility heat tolerance.

Per se QTL effects for SpkL.Mat identified in the current study were located in the 3B QTL region where the major QTL for grain filling heat tolerance was found (Waagan contributed tolerance allele). Hence, these two effects may be functionally linked via an as yet unknown mechanism, or alternatively may be encoded by unrelated but closely linked genes.

4.5.4. Screening/phenotyping for heat-induced floret sterility traits

Spikelet differentiation and development begins in the middle of the spike and proceeds toward the base and upper part of the spike. Within each spikelet, differentiation begins with the most basal florets and proceeds upward (Bonnett, 1936). The florets at the higher positions on the spikelets tend to suffer more readily from sterility due to limitation of source supply, perhaps because their vasculature is connected less directly to the rachis (Kirby, 1988; McMaster, 1997; Sofield *et al.*, 1977). It has been observed that, when some sterility occurs under control conditions, it tends to occur mostly in the upper portion of the spike (Chapter 3). These factors justify scoring the different floret types and spike segments separately.

Consideration of floret 1&2 positions separately gave fertility tolerance QTL that were not detected by summing the fertility across all floret types (grains per spikelet trait) (Table 4.4, 4.5 and 4.6). Similarly, fertility tolerance QTL12, 16 and 17 were only detected when different parts of the spike were considered separately (bottom, middle and top part, respectively) (Tables 4.4 and 4.5) Thus, scoring different floret positions or spike segments independently may provide some advantage for the detection of additional minor QTL, provided that these are genuine.

Although there has been one major window of susceptibility to heat-induced floret sterility described in wheat, other vulnerable stages have been identified in barley and Arabidopsis (Bac-Molenaar *et al.*, 2015; Sakata *et al.*, 2000). In theory, data from the secondary tillers, which is initiated after the primary tiller, may help to detect fertility tolerance QTL expressed at a slightly earlier developmental stage. Provided it did not represent an escape artefact, the fertility tolerance locus QTL12 on 3B, detected only in the secondary tillers and with the earliest time of heat treatment, could represent such a tolerance mechanism.

Scoring secondary tillers was also expected to yield greater growth responses to heat (and hence stronger/more QTL for them), as these tillers were at an earlier stage of development at the time of heat treatment than the primary tillers. This was partly found to be the case. Of the studied growth response traits, AIL.Mat and PlHt.GroPostH showed significant tolerance QTL effects only for the primary tiller, whereas Ht.Mat, PedL.Mat and SpkL.Mat showed tolerance QTL effects only in the

secondary tillers, and AwnL.Mat response QTL loci were detected in both the primary and secondary tillers.

Plants were subjected to heat stress when the primary tiller was at 3 or 9 cm AIL. There were fertility tolerance QTL identified (QTL2 and QTL16) for the earlier treatment stage that were not detected with later stage treatment. Similarly, the fertility tolerance locus QTL26 was detected only with the later stage heat stress. These QTL might represent tolerance mechanisms expressed at slightly different stages of tiller development.

4.6. Conclusion and implications for plant breeding

A major QTL for floret sterility tolerance was detected on chromosome 2B and five minor QTLs on 1B, 3B, 4B, 4D and 7A, explaining 5.4 to 49% of the phenotypic variance, with the tolerance alleles coming from either parent, depending on the QTL Some floret fertility tolerance QTL co-located with QTLs for absolute organ length traits or heat responses of other traits, suggesting that some of these tolerance effects represented escape artefacts.

QTL where heat tolerance was associated with enhanced performance under control conditions might provide the opportunity to improve both tolerance and *per se* performance simultaneously in breeding. The QTL regions on chromosomes 1B, 2B and 7A have this potential. Wheat yellow rust (*Puccina striiformis* f. sp. *tritici*) is one of the most important wheat diseases worldwide, causing up to 60% or greater losses in yield (Chen, 2005; Luo *et al.*, 2009). The floret fertility tolerance QTL on 2B was linked in coupling with a resistance gene to yellow rust (probably *Yr27*), at least in one season. This might enable breeders to improve both heat tolerance and rust resistance simultaneously. Since the tolerance of the yield components grain size and grain number were found to be controlled independently, breeders should select for both of those tolerances separately, to provide maximum gains in heat tolerance. Since QTL7 with its large fertility tolerance effect was mapped under well-watered conditions in the greenhouse, it is important to test its effects in the field, to assess its potential usefulness to breeders.

Chapter 5: Fine-mapping of QHFert.aww-2B, a major QTL influencing heat-induced floret fertility in bread wheat (*Triticum aestivum* L.)

5.1. Abstract

Further fine mapping of heat tolerance for floret fertility (QHFert.aww-2B) was facilitated by the Diversity Among Wheat geNome (DAWN) platform, doubled haploids (DHs) and heterogeneous inbred families (HIFs) derived from Drysdale × Waagan mapping cross. Out of the 38 designed KASP assays in the region, 17 showed polymorphism between the parental lines and mapped into the region. Based on this analysis, the QTL was narrowed down to a 9 cM interval between markers wsnp_Ex_c5412_9565527 and AHW_DW-054. The known DNA sequence in this interval totalled 31.5 Mb (9 genomic sequence scaffolds) and contained 203 predicted genes. The QTL was mapped 21.2 cM from the centromere, and showed a physical to genetic ratio which was around half the average for the wheat genome. The existence of further recombinants between the flanking markers suggested that there was an opportunity to delimit the locus further. Of 14 tested HIF families, two (WW30647 and WW30893) showed strong marker-trait association and suggested a dominant mode of expression of the tolerance allele from Waagan. Pairs of Near Isogenic Lines (NILs; 32 lines total) were selected from eight HIF families and sent to Wagga Wagga for field multiplication and field evaluation. Further fine mapping of the QTL should allow the cloning of the causative gene and provide reliable markers for the locus that may help breeders select for tolerance to heat induced floret sterility.

5.2. Background

Diversity Among Wheat geNome (DAWN), an in-house University of Adelaide wheat genomics platform (U. Baumann *et al*., unpublished). DAWN combines several wheat genomics data sets, namely the recently released NRGene wheat cv. Chinese Spring genome sequence assembly, the Bioplatforms Australia (BPA) whole genome shotgun sequencing data sets (https://researchdata.ands.org.au/bpa-wheat-cultivars/2614), the International Wheat Genome Sequencing Consortium (IWGSC) RNA-Seq data set (Consortium, 2014) and the Munich Information Centre for Protein Sequences (MIPS HCS) gene model alignment locations integrated with POTAGE (R. Suchecki, N. S. Watson-Haigh, and U. Baumann, unpublished). The RNA-Seq data set represents five different wheat tissues (root, leaf, stem, spike and grain) each sampled at three different developmental stages, providing a broad overview of gene expression during development. It was

generated from hexaploid bread wheat cv. Chinese Spring by Choulet *et al.* (2014). NRGene is a company that recently collaborated with IWGSC to generate a high quality *de novo* whole genome shotgun genome sequence of the wheat cv. Chinese Spring (CS). The BPA genomic sequence assemblies are of 16 wheat genotypes (11 Australian wheat varieties, including Drysdale, and five from abroad). The varieties were selected based on their genetic diversity and relevance to the Australian wheat research community and were subjected to 10 × genomic sequence coverage using Illumina HiSeq sequencing (Edwards *et al.*, 2012). In DAWN, the BPA contigs are aligned onto the NRGene assembly and allow identification of SNP polymorphisms between the BPA genotypes and Chinese Spring. Sequences, such as genetic markers, can be mapped onto the assembly (specific scaffolds) using a BLAST function. These scaffold IDs can then be used to identify further intervening/flanking scaffolds (and hence genes and SNPs) in the region, as mentioned in the Material and Method section.

To positionally clone a QTL, it needs to be mapped as a single point locus. One approach to do this involves the method that Tanksley and Nelson (1996) and Tuinstra *et al.* (1997) referred to as heterogeneous inbred family (HIF) analysis. In this approach, recombinant inbred lines (RILs) that are still heterozygous in the QTL region are identified using molecular markers, the heterozygous plants are allowed to self-pollinate and the resulting progeny families are then used for marker- and phenotype analyses to identify those HIF families that are still segregating for the QTL (i.e., showing the marker–trait association in the region). In each HIF family, the boundaries of the segregating (heterozygous) chromosome segments are localized with molecular markers. Together, this information is used to localize the QTL to a particular marker interval. Variation of the trait in the HIF families is relatively easy to ascribe to the QTL, because each HIF family has a uniform (fixed) genetic background, minimizing the influence of any other QTL.

Sibling lines homozygous for contrasting alleles at the target locus can be marker-selected from HIF families and represent Near isogenic lines (NILs) (Loudet *et al.*, 2002). NILs are defined as pairs of lines that differ for a chromosome segment carrying a locus of interest but are otherwise very similar in their genetic background. Such NILs can be useful for molecular and physiological studies of the QTL, and can be field-trialled to quantify QTL effects in the field. This approach of generating NILs is much easier and quicker (if a RIL population is already available) than the more traditional method of backcrossing (Allard, 1960).

Using HIF analysis, re-phenotyping of recombinants from the DH population, and targeted generation of additional markers using the DAWN tool, the strongest QTL for heat tolerance for floret fertility (QHFert.aww-2B) identified in the Drysdale × Waagan DH population (Chapter 4) has been fine-mapped. NILs for the QTL were selected that will facilitate future physiological and molecular characterization as well as field studies of the locus.

5.3. Material and methods

5.3.1. Scaffold assignment and development of SNP-based KASP markers

To develop new markers in the vicinity of the 2B QTL, SNPs in genes located in the region were identified using DAWN running on the Integrative Genomics Viewer (IGV) platform. Firstly, ~200-300 bp sequences containing SNPs from the 9k Illumina wheat SNP array that were mapped in the vicinity of the 2B locus (Chapter 4) were obtained from the publication of (Cavanagh *et al.*, 2013), and located in NRGene scaffolds using BLAST searches. A near perfect match (>98% sequence identify over the entire length) was taken to indicate identification of the correct position. The bioinformatics research group of Adelaide University (U. Baumann et al) then kindly identified scaffolds located between the marker-containing scaffolds using information based on the IWGSC WGA v0.4 CS assembly (referred to as the "NRGene" assembly). Scaffolds and associated information, including gene models and SNPs between CS and the BPA varieties, were viewed in the IGV window of DAWN. NRGene scaffolds also had cM positions assigned (with 0 cM being at the top of the short arm of the chromosome) based on relationships to PopSeq (Chapman *et al.*, 2015). Using the nucleotide positions of the SNPs in the scaffolds and the Fetch-Seq tool in DAWN, ~200 bp sequence segments containing the SNPs were retrieved from either the corresponding IWGSC bread wheat scaffolds or Chinese Spring NRGene assembly scaffolds. The ~200 bp sequences were then used to design KASP marker assays (Semagn *et al.*, 2014) using Kraken software (Wood and Salzberg, 2014). The KASP primers were named with the prefix 'AHW_DW', referring to the Adelaide University Heat Wheat research group and the Drysdale × Waagan mapping cross.

Each KASP assay was tested on cvs. Drysdale and Waagan to determine whether it identified polymorphism between these parents. The polymorphic markers were then used on the DH and RILs for mapping.

For mapping, marker orders were defined as those that required the least numbers of double recombination events – as determined by visualization of the marker scores in DH and RIL-derived lines in Excel, using conditional formatting to colour Drysdale and Waagan derived alleles red and green respectively, and heterozygous scores yellow. Genetic (cM) distances were calculated from recombination fractions using the Kosambi mapping function (Kosambi, 1943).

5.3.2. Re-phenotyping recombinant DH lines

The experiment was conducted from July 2016 to December 2016 using the Australian Plant Accelerator growth facilities, where the average temperature and relative humidity was recorded as 23/20 ºC and 51/59 % day/night respectively (Appendix table 6.1). Temperature was 23/19 C day /night around booting stage (heat treatment stage).

Selected DH lines that were recombinant in the QTL region were used for repeated phenotyping. Per DH, eight plants were grown (one per pot), with six used for heat treatment when the primary tiller reached the stage of 6 cm auricle interval length (AIL) and the other two were kept as control plants. Tolerance assays were performed as described in Chapter 3 and 4. Only the lower two thirds of the spike were scored for floret fertility, using only the basal two floret positions in the spikelets.

5.3.3. Fine mapping using the HIF approach

The experiment was conducted from last week of January to July in 2016 using the same facilities as described in chapter 4. The average temperature and relative humidity was recorded as 24/20 ºC and 57/65 % day/night respectively (Appendix table 5.1). Temperature was 24.5/21 day/night and 9 days over 30 °C around the booting stage (heat treatment stage) due to high outside temperatures.

Seed packets for 604 $F_{2:5}$ Drysdale × Waagan RIL lines, obtained from Livinus Emebiri at NSW-DPI Wagga Wagga, were used as the starting point for this study. These had previously been selected from a larger population of 2,627 lines (derived from 43 F_1 plants) on the basis of being potentially semi-dwarf types, based on field measurements of height. The 604 lines also did not include the most extreme early or late flowering lines from the population.

5.3.4. DNA extraction

Genomic DNA was extracted from ~50 mm long leaf segments collected from 2 week old seedlings using the method of (Miller *et al.*, 1999), with some modifications (Pallotta *et al.*, 2000). The tissue was collected in 1.1 mL racked microtubes (8 × 12 format; Thermo Fisher Scientific), frozen by immersing the base of the tubes in liquid nitrogen, freeze-dried, ground using ball bearings in a tube shaker, combined with 600 µL of extraction buffer (0.1M Tris-HCl pH 7.5, 0.05M EDTA pH 8.0 and 1.25% SDS) and vortexed. The racked tubes were then centrifuged very briefly, incubated in a 65ºC oven for 30 min, cooled at 4 ºC for ~ 15 min, and 300 µL of 6M ammonium acetate added to each tube. The racked tubes were vortexed and incubated at 4 ºC for about 15 min before centrifuging at 3,273× g for 15 min. The supernatant (600 µL) was combined with 360 µL of isopropanol and DNA precipitated by centrifugation at 3,273× g for 15 min. The liquid was discarded and the pellet washed using 400 µL of 70% ethanol. The dried pellet was resuspended in 40 µg/mL bovine pancreatic RNAse A (Sigma) and the DNA left at 4 ºC overnight to enable RNA digestion and complete resuspension of the DNA. Finally, nucleic acid concentrations were determined by absorbance at 260 nm using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies) and the integrity of the DNA assessed by subjecting 2 µL of the DNA, mixed with 1.5 µL loading dye and 6.5 µL water, to electrophoresis in 0.7% agarose gels containing 5µl in 100 mL of SYBR™ Safe DNA Gel stain (Invitrogen) for DNA visualization over a blue-light transilluminator.

5.4. Results and Discussion

5.4.1. Designing and mapping of additional markers for fine mapping

Genotypes sequenced in the BPA project included Drysdale but not Waagan. Therefore another dataset (Breeders' 35K Axiom® genotyping data set) was used to identify a BPA-sequenced wheat genotype that looked most dissimilar to Drysdale on chromosome arm 2BS, so that it could be used as a surrogate for Waagan in looking for polymorphism with Drysdale in the BPA sequence. Of the BPA-sequenced genotypes, 10 (as well as Waagan) had 35k SNP scores available at http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB/axiom_download.php. SNPs assigned to the whole of 2BS by Allen *et al.* (2017) were then used in a Pearson correlation analysis. Gladius was found to be most dissimilar to Drysdale on 2BS (Figure 5.1), and was therefore used to identify polymorphism with Drysdale.

Of the SNPs displayed in DAWN, only those polymorphic between Drysdale and Gladius were considered for KASP assay development. The selections were further reduced, based on various other criteria: SNPs evenly spread across and within the scaffolds were chosen, SNPs were favored if they were in a sequence stretch (~200 bp window size) with ≥ 50% GC content and were covered by relatively high depth of genomic sequence read coverage, particularly in Drysdale. Furthermore, SNPs that appeared to be in genes based on the gene models and number of matches to the RNA-Seq reads, were favoured.

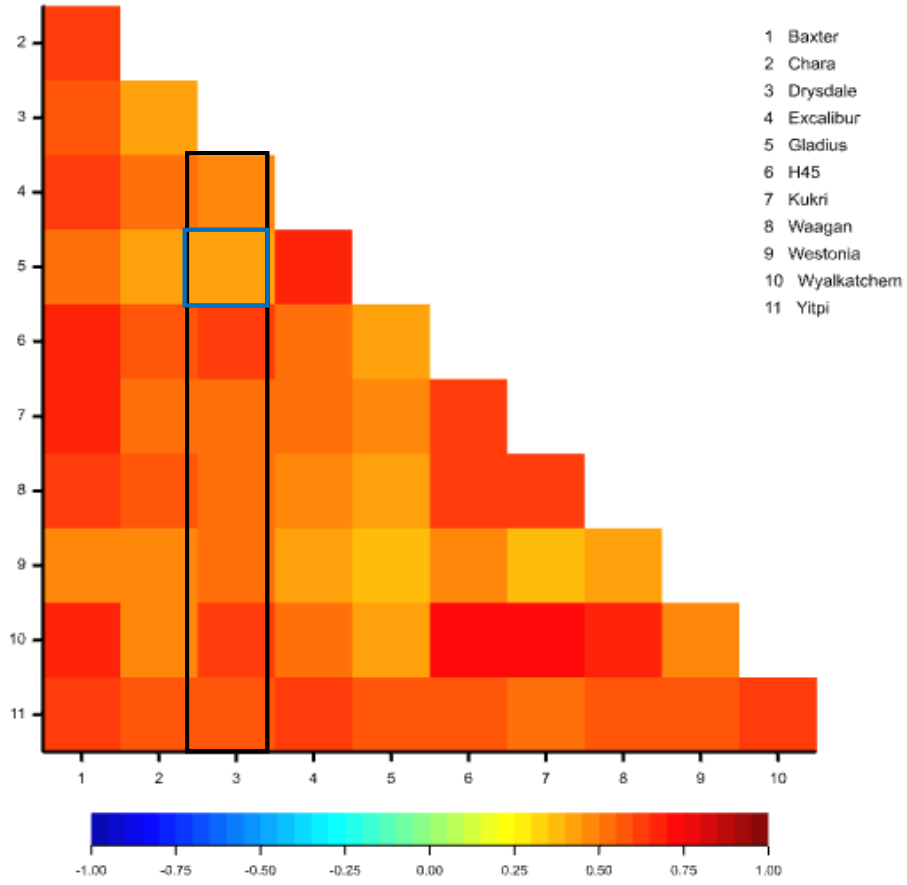

Figure 5. 1 Pearson's correlation matrix among 11 wheat genotypes sequenced by BPA, based on calls at SNPs located on 2BS, obtained using the wheat 35K Axiom® genotyping data set. Red and blue tones represent the strength of positive and negative associations, respectively. Boxes highlight comparisons to cv. Drysdale.

The 21.9 cM interval (later re-calculated as 23.7 cM in this chapter) between SNP markers KASParMAS048 and wsnp_Ex_c944_1810245, which the QTL mapping had indicated covered the

2B QTL (Chapter 4), was chosen for marker development. In total, 28 scaffolds were identified in the region between and containing these markers, totalling 103.7 Mb of sequence (Table 5.1). The scaffolds containing the KASParMAS048 marker were not integrated into the chromsome 2B assembly (was an 'unassigned' scaffold). Consequently, scaffolds located between KASParMAS048 and the next marker proximal to it (wsnp_Ex_c5412_9565527) could not be identified. Besides this interval of unknown physical length, there were 27 gaps of unknown size between the 28 scaffolds. Such gaps in this assembly could be anywhere from a few bases to a Mb long and contain other unassigned scaffolds (N. Watson-Haigh, personal communication).

A total of 38 SNPs located on the identified scaffolds and indicated by DAWN to be polymorphic between Drysdale and Gladius were used to design new KASP assays. Of these KASP assays, 21 did not discriminate between Drysdale and Waagan and/or could not be mapped reliably (Appendix table 5.3). These may reflect failure of the Drysdale/Gladius comparison to predict Drysdale/Waagan SNPs, i.e., because Waagan and Drysdale carried the same haplotypes in some parts of the region, although Waagan and Gladius differed. Identification of Drysdale/Waagan SNPs might have been further limited by the fact that DAWN only displays SNPs that are polymorphic between Chinese Spring and one or more of the BPA-sequenced varieties, i.e., this may cause some Drysdale/Waagan SNPs to be missed in cases where a unique base is present in Waagan, which is not represented in DAWN. Furthermore, primers were based on the cv. Chinese Spring sequence, and may therefore have some polymorphism in Waagan or Drysdale which might prevent the primers from binding or extending the target sequence in the parents and mapping lines.

Table 5. 1 Details of the 28 wheat cv. Chinese Spring NRGene genomic sequence scaffolds spanning the QGrNoSplt.Spk.aww-2B1 region. SNP markers from the 9k Illumina array that were originally mapped in the Drysdale × Waagan population to define the QTL are in the first column. The green-highlighted scaffolds show the interval to which the 2B QTL was further delimited in this chapter.

9k array SNP (and Drysdale × Waagan 2B map location)	Scaffold number	Centi Morgan (cM)[a]	Scaffold length (bp)	HiC_orientation[b]	AGP_start[c]	AGP_end
KASParMAS048 (74.25cM)	212400	NA	5,249	NA	NA	NA
wsnp_Ex_c5412_9565527 (76.51cM)	110402	40.25	1,346,254	-1	63,223,042	64,569,295
	88768	42.73	8,054,127	-1	64,569,396	72,623,522
wsnp_JD_c3732_4781170 (79.52cM)	144010	47.24	5,207,403	-1	72,623,623	77,831,025
	57860	50.54	2,282,171	-1	77,831,126	80,113,296
	39667	50.41	3,443,914	1	80,113,397	83,557,310
	85263	50.61	1,166,808	-1	83,557,411	84,724,218
	108993	50.46	5,378,381	1	84,724,319	90,102,699
	4059	50.39	2,923,624	-1	90,102,800	93,026,423
	16147	50.79	1,708,548	-1	93,026,524	94,735,071
wsnp_RFL_Contig4483_5312236 (84.8cM)	10981	51.55	3,877,756	1	94,735,172	98,612,927
	84097	54.48	1,354,875	1	98,613,028	99,967,902
	29500	54.71	963,240	NA	99,968,003	100,931,242
	7646	54.75	559,903	NA	100,931,343	101,491,245
	70877	54.67	3,427,453	-1	101,491,346	104,918,798
	32988	54.66	1,064,740	NA	104,918,899	105,983,638
	64317	54.55	2,322,765	1	105,983,739	108,306,503
	46227	54.75	1,432,663	1	108,306,604	109,739,266
	88642	54.79	203,436	NA	109,739,367	109,942,802
	118017	54.46	803,584	NA	109,942,903	110,746,486
	92379	55.52	2,874,901	-1	110,746,587	113,621,487
	44821	55.66	18,925,372	-1	113,621,588	132,546,959
	2319	55.67	14,072,397	-1	132,547,060	146,619,456
	89096	55.44	887,386	NA	146,619,557	147,506,942
	46064	55.64	593,322	NA	147,507,043	148,100,364
wsnp_Ex_c13351_21042379 (93.92cM)	30390	55.73	3,317,848	1	148,100,465	151,418,312
	56324	55.74	5,362,512	-1	151,418,413	156,780,924
	140242	55.57	2,307,445	1	156,781,025	159,088,469
wsnp_Ex_c944_1810245 (96.18cM)	106441	56.64	7,786,175	1	159,088,570	166,874,744

[a] Distance from the centromere in the NRGene assembly, based mostly on the POPSEQ position; [b] Orientation of scaffolds determined by the HiC method (chromosome-confirmation capture coupled with high-throughput sequencing; (Mascher and Stein, 2014); [c] AGP (A Golden Path), a format system used to indicate start and end positions of contigs within a sequence. [d] Scaffold not incorporated into the chromosome 2B assembly, so location within it is unknown.

Table 5. 2 Details of successful KASP assays in the QHFert.aww-2B region. The green-highlighted markers were the original flanking markers used to define the 2B QTL interval. Markers highlighted in yellow represent SNPs from the 9k SNP array that were previously mapped in the Drysdale × Waagan DH population (Shirdelmoghanloo et al., 2016c).

Marker/assay	Scaffold number	Base position of SNP within the scaffold	Primer	Primer sequence (5′ to 3′)
KASParMAS048*	212400; Un assigned to a chromosome		Allele X	GAAGGTGACCAAGTTCATGCTGTTTCCAGTGATGTCGTCGCACA
			Allele Y	GAAGGTCGGAGTCAACGGATTTCCAGTGATGTCGTCGCACG
			Common	TCAGCAGCACCATTTGACAGGCAA
AHW_DW-001	88768	1,320,431	Allele X	GAAGGTGACCAAGTTCATGCTGGGAAGACACCACCAGTGTTAC
			Allele Y	GAAGGTCGGAGTCAACGGATTATGGGAAGACACCACCAGTGTTAT
			Common	ACAACAGTATGTATCTGGTAGTGAACCTT
AHW_DW-010	144010	5,018,840	Allele X	GAAGGTGACCAAGTTCATGCTCCATGGATATCCAGATGGTGCG
			Allele Y	GAAGGTCGGAGTCAACGGATTCCATGGATATCCAGATGGTGCA
			Common	GACACACTGAAGAGTGCTGGGAAAA
wsnp_JD_c3732_4781170	144010	2,224,521	Allele X	GAAGGTGACCAAGTTCATGCTCCATATAGGCAAAATCAATCTTGGA
			Allele Y	GAAGGTCGGAGTCAACGGATTCCATATAGGCAAAATCAATCTTGGC
			Common	CTCCTCCGGCATTGGGTCTTTTTAA
AHW_DW-031	57860	1,287,002	Allele X	GAAGGTGACCAAGTTCATGCTGCCTCGTAGACCTGCATCGTAA
			Allele Y	GAAGGTCGGAGTCAACGGATTCCTCGTAGACCTGCATCGTAG
			Common	CATTCTCCTCCTACCTCTCCTGTT
AHW_DW-037	39667	1,286,058	Allele X	GAAGGTGACCAAGTTCATGCTATCATCCGAGAGGCATCGAGG
			Allele Y	GAAGGTCGGAGTCAACGGATTCATCATCCGAGAGGCATCGAGA
			Common	CGATCATTGAGGGCCTCTACATCAA
AHW_DW-032	39667	3,098,557	Allele X	GAAGGTGACCAAGTTCATGCTGTTCAACAAAGACTTGGCCGT
			Allele Y	GAAGGTCGGAGTCAACGGATTCTGTTCAACAAAGACTTGGCCGC
			Common	GGGGCCATCTGGGGCGCAT

* The KASParMAS048 assay was available prior to this study

Table 5. 2: Continued…

Marker/assay	Scaffold number	Base position of SNP within the scaffold	Primer	Primer sequence (5′ to 3′)
AHW_DW-054	16147	1,605,224	Allele X	GAAGGTGACCAAGTTCATGCTATGAAATTTAAAATAACTATGATACATGTACTC
			Allele Y	GAAGGTCGGAGTCAACGGATTATGAAATTTAAAATAACTATGATACATGTACTA
			Common	CTGTTTCGCGAAACAAAATAGCCCATATA
AHW_DW-053	16147	919,868	Allele X	GAAGGTGACCAAGTTCATGCTGATTAGATCTGAGAGGACCGCG
			Allele Y	GAAGGTCGGAGTCAACGGATTAGATTAGATCTGAGAGGACCGCA
			Common	GTTCGGCTTCTCCCTCTGGTCAT
AHW_DW-036	16147	518,525	Allele X	GAAGGTGACCAAGTTCATGCTGAATGTACACAAAGTAAGAATGACAATATCA
			Allele Y	GAAGGTCGGAGTCAACGGATTAATGTACACAAAGTAAGAATGACAATATCG
			Common	TATATGCCGCCGGCAAGGATTAATTATAT
AHW_DW-011	10981	613,666	Allele X	GAAGGTGACCAAGTTCATGCTCCTCAAGGCACTAGTAGCACGA
			Allele Y	GAAGGTCGGAGTCAACGGATTCTCAAGGCACTAGTAGCACGG
			Common	GGATTATTGTTTGAGGGTTCGGGCAT
AHW_DW-024	10981	1,349,508	Allele X	GAAGGTGACCAAGTTCATGCTCGATATTACTTGGAATGGTCATTATCTAT
			Allele Y	GAAGGTCGGAGTCAACGGATTCGATATTACTTGGAATGGTCATTATCTAC
			Common	TGCCACATCGGAAGCCTTTATAATAACTA
AHW_DW-027	10981	3,520,917	Allele X	GAAGGTGACCAAGTTCATGCTGCAACATATTTCAATAATCTTCAACCGCA
			Allele Y	GAAGGTCGGAGTCAACGGATTCAACATATTTCAATAATCTTCAACCGCG
			Common	GAAATATACTGCCGTGGATGGACGAT
AHW_DW-030	84097	197,147	Allele X	GAAGGTGACCAAGTTCATGCTGAGGCAGTGATGGACAGCACTT
			Allele Y	GAAGGTCGGAGTCAACGGATTGAGGCAGTGATGGACAGCACTA
			Common	GACAGATCGAGGCTAGGAGGTATTT

Table 5. 2: Continued…

Marker/assay	Scaffold number	Base position of SNP within the scaffold	Primer	Primer sequence (5′ to 3′)
AHW_DW-013	84097	655,782	Allele X	GAAGGTGACCAAGTTCATGCTGATGTAGCCTCAACAAGGATAATCC
			Allele Y	GAAGGTCGGAGTCAACGGATTGATGTAGCCTCAACAAGGATAATCA
			Common	CGATGCTTTTAGAATCTCTGGTTAACCAA
AHW_DW-014	70877	2,497,250	Allele X	GAAGGTGACCAAGTTCATGCTCCTTGTGAAGAGCCATGTAAGACA
			Allele Y	GAAGGTCGGAGTCAACGGATTCTTGTGAAGAGCCATGTAAGACG
			Common	CAAGGAGAGCTTGCTTGCAAGTGAA
wsnp_Ex_c13351_21042379	30390	2,607,304	Allele X	GAAGGTGACCAAGTTCATGCTATTCAAGGTGTTCAACGAAGCACCA
			Allele Y	GAAGGTCGGAGTCAACGGATTCAAGGTGTTCAACGAAGCACCG
			Common	GCATTTTAAGACTTACAAGTGAGCTTCTAT
AHW_DW-004	56324	3,019,233	Allele X	GAAGGTGACCAAGTTCATGCTGATGTTGCTACAGTTGCGCACG
			Allele Y	GAAGGTCGGAGTCAACGGATTCGATGTTGCTACAGTTGCGCACA
			Common	CATCATCGATGGCTGCCCTCACAT
AHW_DW-003	140242	1,837,906	Allele X	GAAGGTGACCAAGTTCATGCTTGCTCCTCTCCCATCCTTGG
			Allele Y	GAAGGTCGGAGTCAACGGATTGTTGCTCCTCTCCCATCCTTGA
			Common	CGCCAGTAAATGAGTTCCCAATCCTT
AHW_DW-005	106441	2,322,417	Allele X	GAAGGTGACCAAGTTCATGCTGACAATAGATTAAGGGCACATAGTTG
			Allele Y	GAAGGTCGGAGTCAACGGATTATGACAATAGATTAAGGGCACATAGTTA
			Common	CATGTAGCCGGTTTTGGGGTAAGAT
wsnp_Ex_c944_1810245	106441	5,336,480	Allele X	GAAGGTGACCAAGTTCATGCTGAATTCGAGTTTCGTGATTCCGAGAT
			Allele Y	GAAGGTCGGAGTCAACGGATTAATTCGAGTTTCGTGATTCCGAGAC
			Common	TATGGGGATCTGCTGCCTCCATATA

Thus, 17 useful KASP marker assays were generated in the region using SNPs identified in DAWN. Additionally, the five markers from the 9k SNP array that had originally been mapped in the 2B QTL region were used to design KASP assays, but only three of them worked reliably and mapped to the original locus positions; the ones that failed are indicated in Appendix table 5.3. Marker KASParMAS048 (in the *Ppd-B1* gene) from the original marker map represents a KASP assay that was scored in the Drysdale × Waagan DH population by (Shirdelmoghanloo *et al.*, 2016c). Thus, a total of 21 KASP markers, based on SNPs from 13 different scaffolds, were available in the region (Table 5.2). These were scored in all 144 Drysdale × Waagan DH lines using the orignal DNAs from study of Shirdelmoghanloo *et al.* (2016c). DNA of 17 recombinant DH lines for the interval between KASParMAS048 and wsnp_Ex_c944_1810245 was also re-extracted from three newly grown plants per line (one DNA extraction per plant), and the plants were individually scored for all the KASP markers. The new and old marker data agreed for all but two of the DH lines (WW28466 and WW28372; Figure 5.3). The original marker data of those lines were disregarded for the map construction, as they were based on only one DNA sample, in contrast to the new scores, which were based on multiple DNA samples per line.

The marker scores were used to produce a revised genetic map of molecular markers in the 2B QTL region (Appendix Table 5.2; Figure 5.4). The cM positions of the markers mapped in the Drysdale × Waagan DH population largely agreed with the order of cM positions of the matching scaffolds in the NRGene assembly (Figure 5.3), although the absolute positions somewhat differed, e.g., markers wsnp_Ex_c5412_9565527 and wsnp_Ex_c944_1810245 were at 76.5 and 85.5 cM on the Drysdale × Waagan map, but at 40.25 and 50.79 cM on the NRGene map, respectively.

5.4.2. Phenotypic analysis of DH lines to place the QHFert.aww-2B locus on the new marker map

As QHFert.aww-2B was a very strong QTL, an attempt was made to locate it as a single point locus using the DH lines. As a first step, Drysdale × Waagan DH lines that were non-recombinant for markers across the interval (i.e., for which the 2B QTL genotype could be certain) were used to define the frequency distribution of the phenotype for lines of the two genotypic classes (Waagan or Drysdale allele for the tolerance locus). A total of 66 Drysdale types and 46 Waagan types were identified as non-recombinant across the interval between

markers KASParMAS048 and wsnp_Ex_c944_1810245. Then, the frequency distributions of the fertility scores from Chapter 4 were plotted separately for the two groups of lines (Figure 5.2). The frequency distributions were partially distinct, allowing definition of floret fertility ranges indicative of a Drysdale or Waagan genotype at the 2B locus (respectively, <1.0 and >1.5 grains per spikelet in floret 1&2 positions, in heat-treated plants). By this definition, the overlap range (1.0-1.5) could not be used to predict the 2B locus genotype. In this way, the 20 recombinants for the 2B interval were each categorized for their likely genotype (allele) at the QHFert.aww-2B locus (Figure 5.3).

The phenotype data obtained in Chapter 4 was based on only four heat treated plants (and four control plants) per Drysdale × Waagan DH line. To improve the accuracy of phenotype information for fine mapping, 17 of the DH recombinants for the 2B interval (the same ones that were genotyped using re-extracted DNA) were re-phenotyped for heat induced floret sterility, using six heat treated plants and two control plants per line. The same fertility ranges as before were used to infer which lines carried the Drysdale or Waagan alleles at the 2B QTL using the new phenotype data. The two phenotype datasets predicted the opposite 2B QTL allele for lines WW28404 and WW28466 (Figure 5.3; Appendix table 5.2), but qWW28466 as previously mentioned also differed for its marker profile between the two phenotyping experiment and was probably mislabelled. For all other recombinant lines, the 2B QTL allele calls were consistent between the two phenotyping rounds, for lines where a call could be made in both cases (Figure 5.3; Appendix table 5.2).

The 2B QTL allele calls were then used to infer which side of each recombination point the 2B QTL locus was located on (Figure 5.3). There were some inconsistencies in the location of the 2B QTL suggested by this method by different DH lines. This was probably mainly due to the fact that additional minor QTL affecting the phenotype score in the Drysdale × Waagan DH population, identified in Chapter 4, were unaccounted for in this approach.

Nonetheless, the method allowed identification of the 9.1 cM interval between the markers wsnp_Ex_c5412_9565527 and AHW_DW_054 as being the most likely interval for the 2B QTL locus (Figure 5.3 and 5.4). wsnp_Ex_c5412_9565527 represented a SNP from the 9k SNP array that was not successfully converted to a KASP assay, hence its position was based solely on the original 9k SNP array calls. Four and one recombinant suggested the 2B QTL was to the proximal and distal side of wsnp_Ex_c5412_9565527, respectively. Four and

zero recombinants suggested the 2B QTL was distal and proximal of AHW_DW_054, respectively (Figure 5.3 and Appendix table 5.2). There was one recombinant line (WW28510) separating the six markers in this interval into two groups, but unfortunately the phenotype score for that line was in the overlapping range, preventing the 2B locus genotype from being called (Figure 5.3 and Figure 5.4).

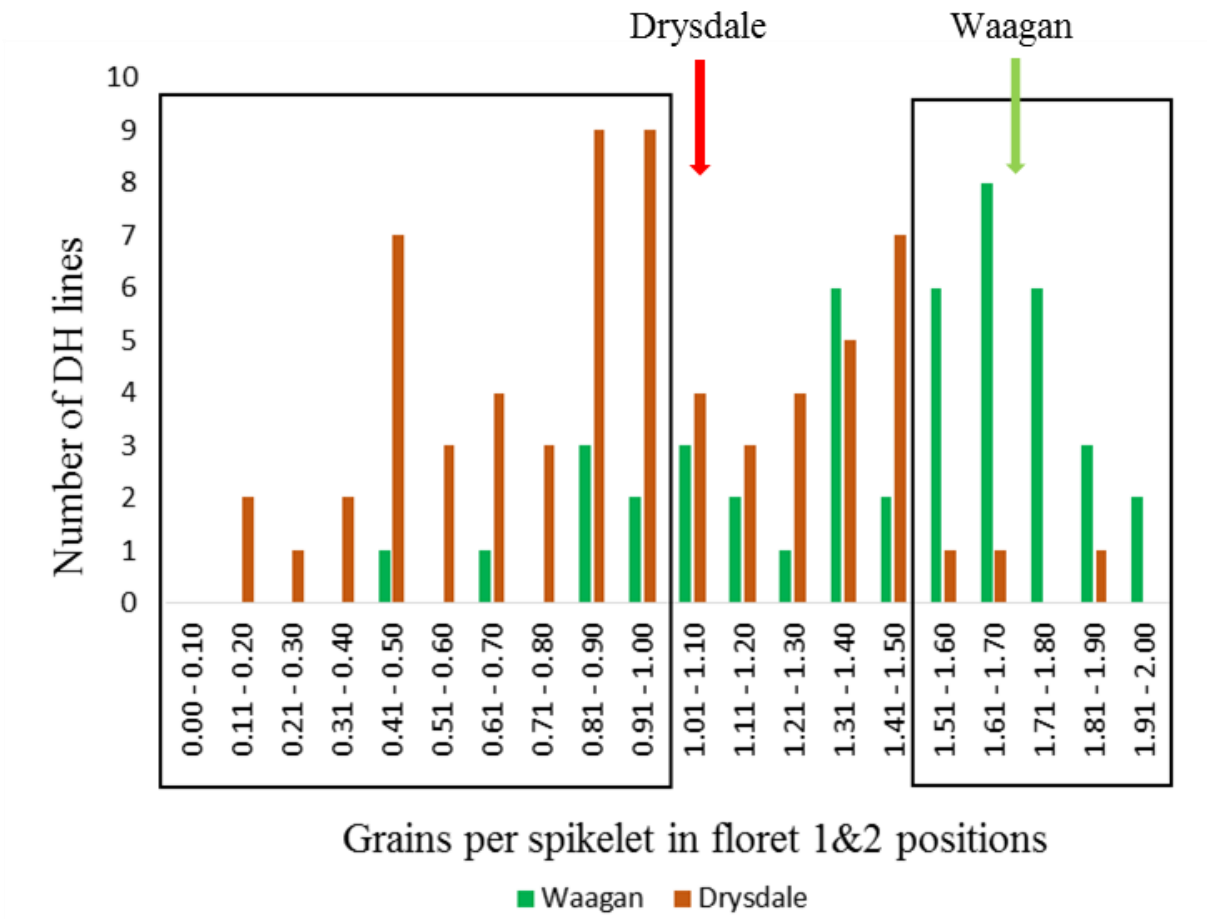

Figure 5. 2 Frequency distributions for floret fertility of heat-treated plants (average of treatments at 3 and 9 cm AIL stage, from the middle and bottom third of the spike; phenotype data from Chapter 4) in Drysdale × Waagan DH lines that were non-recombinant for markers across the QHFert.aww-2B interval (carrying either Drysdale or Waagan marker alleles). The mean scores for cv. Drysdale and Waagan plants are indicated by arrows.

The 9.1 cM refined QTL interval contained nine known scaffolds ranging in size from 1.2 Mb to 8 Mb and totalling ~31.5 Mb sequence. The overall physical to genetic distance ratio in the interval was therefore 3.5 Mb/cM. High-quality genetic maps of the wheat genome have

total lengths of around 2,500 cM (Semagn *et al.*, 2006; Shirdelmoghanloo *et al.*, 2016c; Somers *et al.*, 2004), and the total physical size of the wheat genome is around 17,000 Mb (Feuillet and Eversole, 2007), making the average physical to genetic ratio for wheat around 6.8 Mb/cM. Hence, the estimated physical to genetic ratio of the 2B QTL interval was about half that of the genome-wide average for wheat, suggesting that the locus was in a region of relatively high recombination, giving a favourable outlook for positional cloning of the underlying gene.

Figure 5. 3 Mapping the QHFert.aww-2B on 2B by using the DH lines, and treating the QTL as a single point locus. The 2B QTL allele call was defined by the frequency distributions in Fig. 5.2 (≥1.5 and < 1.0 grains/spikelet in floret positions 1&2, for Waagan and Drysdale allele calls, respectively). The yellow arrows indicates the position of the tolerance locus relative to that of the recombination point, based on the 2B QTL allele call for that line in the last column.

		KASPaMAS048	wsnp_Ex_c5412_9565527	AHW_DW_001	AHW_DW_010	wsnp_JD_c3732_4781170	0071AHW_DW_031	AHW_DW_037	AHW_DW_032	AHW_DW_054	AHW_DW_053	0076AHW_DW_036	AHW_DW_011	AHW_DW_024	AHW_DW_027	AHW_DW_030	AHW_DW_013	AHW_DW_014	wsnp_Ex_c13351_2104237	AHW_DW_004	AHW_DW_003	AHW_DW_005	wsnp_Ex_c944_1810245	Fertility M&B 1&2 Heat	Fertility M&B 1&2 Heat (second re	2B QTL allele call
Drysdale x Waagan DH 2B cM		74.3	75.7	79.9	79.9	79.9	80.6	80.6	80.6	84.0	84.0	84.7	85.4	85.4	85.4	85.4	85.4	85.4	94.5	94.5	94.5	96.5	96.5			
NRGene 2B cM		38.7	40.3	42.7	47.2	47.2	50.4	50.5	50.5	50.8	50.8	50.8	51.6	51.6	51.6	54.5	54.5	54.7	55.7	55.7	55.6	56.6	56.6			
DH	Description																									
WW28538		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	2.1		Waagan
WW28531_WW28533		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	2.1		Waagan
WW28404	repeat genotype	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	0.7		Drysdale
WW28404	repeat pheno/geno-typed	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD		1.60	Waagan
WW28536	repeat genotype	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	1.3		
WW28536	repeat pheno/geno-typed	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD		0.68	Drysdale
WW28389	repeat genotype	WW	WW	WW	WW	WW	WW	WW		WW	WW	WW		WW	WW	WW	WW	WW	DD		DD	DD	DD	1.4		
WW28389	repeat pheno/geno-typed	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD		0.87	Drysdale
WW28468		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	1.5		
WW28481	repeat genotype	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	1.5		
WW28481	repeat pheno/geno-typed	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD		1.53	Waagan
WW28501	repeat genotype	WW	WW	WW	WW	WW	WW	WW		WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	1.7		Waagan
WW28501	repeat pheno/geno-typed	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD		1.12	
WW28379	repeat genotype	WW	WW	WW	WW	WW	WW	WW		WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	1.7		Waagan
WW28379	repeat pheno/geno-typed	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD		1.57	Waagan
WW28450		WW		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	0.9		Drysdale
WW28477		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW		WW	WW	DD	DD	1.7		Waagan
WW28370		DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	0.5		Drysdale
WW28369		DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	1.1		
WW28493		DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.5		
WW28527	repeat genotype	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.6		Waagan
WW28527	repeat pheno/geno-typed	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW		1.78	Waagan
WW28520	repeat genotype	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.8		Waagan
WW28520	repeat pheno/geno-typed	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW		1.58	Waagan
WW28539		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.7		Waagan
WW28423	repeat genotype	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.4		
WW28423	repeat pheno/geno-typed	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		1.69	Waagan
WW28510		DD	WW	WW	WW	WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.1		
WW28360		WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.8		Drysdale
WW28380	repeat genotype	WW	WW	DD	DD	DD	DD	DD			DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.9		Drysdale
WW28380	repeat pheno/geno-typed	WW	WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		0.69	Drysdale
WW28547	repeat genotype	WW	WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.9		Drysdale
WW28547	repeat pheno/geno-typed	WW	WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		0.23	Drysdale
WW28466	repeat genotype	WW	DD	DD	WW			DD	DD	DD	WW		WW	WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.7		Drysdale
WW28466	repeat pheno/geno-typed	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	WW	WW	WW	WW	WW		0.82	Drysdale
WW28371		DD	WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.9		Drysdale
WW28372	repeat genotype	DD	WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.6		
WW28372	repeat pheno/geno-typed	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD		1.72	Waagan
WW28471_WW28473	repeat genotype	DD	DD	DD	DD	DD	DD	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.2		
WW28471	repeat pheno/geno-typed	DD	DD	DD	DD	DD	DD	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW		1.30	
WW28480	repeat genotype	DD	DD	DD	DD	DD	DD	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.5		
WW28480	repeat pheno/geno-typed	DD	DD	DD	DD	DD	DD	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW		1.17	
WW28490		DD	DD	DD	DD	DD	DD	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	0.8		Drysdale
WW28382	repeat genotype	DD	DD	DD	DD	DD	DD	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.3		
WW28382	repeat pheno/geno-typed	DD	DD	DD	DD	DD	DD	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW		0.28	Drysdale
WW28518		DD	DD	DD	DD	DD	DD	DD		WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	0.6		Drysdale
WW28391_WW28394	repeat genotype	DD	DD	DD	DD	DD	DD	DD		DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	0.7		Drysdale
WW28391	repeat pheno/geno-typed	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW		0.54	Drysdale
WW28526_WW28528		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	WW	WW	WW	WW	WW	0.9		Drysdale
WW28405		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.2		Drysdale

n.a., non-allocated chromosome

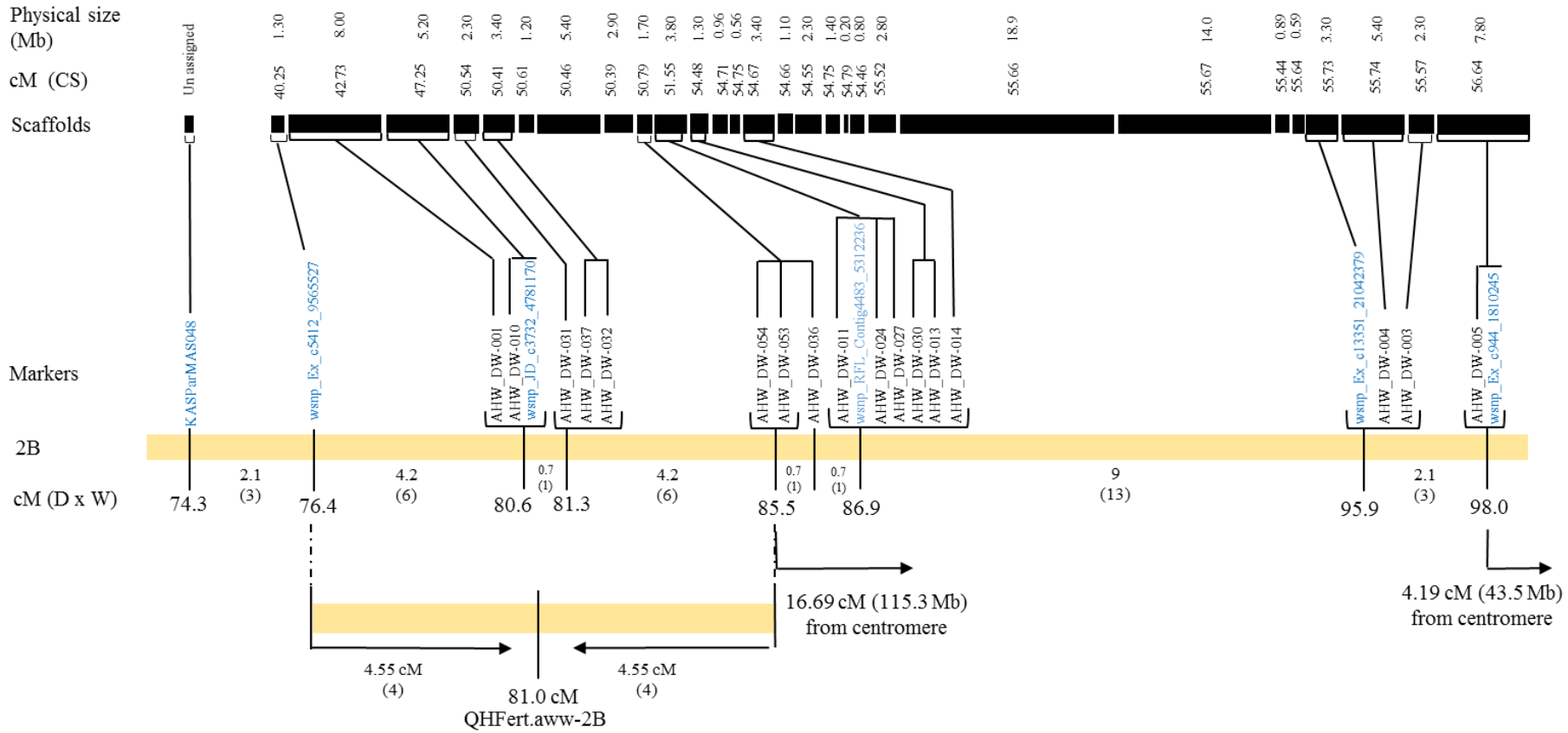

Figure 5. 4 Genetic and physical maps of the QHFert.aww-2B region. The genetic map was made using the population of 144 Drysdale × Waagan DH lines. The cM distances for each marker interval (with number of recombinants observed in the DH population in brackets) and cM distance from the 2BS telomere, are both shown. Blue markers show SNPs mapped using the 9k SNP array in Chapter 4. The map at the bottom summarizes the position of the 2B QTL defined as a single point locus. The markers are shown in the orders they occur within the scaffolds.

The markers from the 9k SNP array that were closest to the centromere of chromosome 2B on the Drysdale × Waagan map of Shirdelmoghanloo *et al.* (2016c) were wsnp_RFL_Contig3802_4108582 and wsnp_RFL_Contig454_5360785, which identified scaffolds in the NRGene assembly that were assigned to the short and the long arm of the chromosome, respectively, and which were only 1.5 cM apart. wsnp_RFL_Contig3802_4108582 was at position 102.2 cM on the Drysdale × Waagan map, and the 2B tolerance locus was mapped at 81.0 cM. Hence, the 2B QTL was positioned 21.2 cM from the centromere (Figure 5.4).

Different sections of wheat chromosomes vary widely in their recombination rates. Notably, regions around the centromere, representing around 27% of each chromosome, don't recombine at all (Saintenac *et al.*, 2008). The fact that the 2B QTL interval was located 21.2 cM from the centromere of chromosome 2B was therefore consistent with the notion that it was a region of higher-than-average recombination frequency.

The *Ppd-B1* locus on the short arm of chromosome 2B strongly influences flowering time in a day length dependent manner (González *et al.*, 2005b). Potentially, a gene such as *Ppd-B1* with a large effect on plant development could be the source of the QHFert.aww-2B QTL effect, by influencing general plant physiology and/or the method of staging plants for heat treatment based on AIL. However the Drysdale × Waagan population didn't segregate for any flowering time QTL at this position (Shirdelmoghanloo *et al.* (2016c); Chapter 4), indicating that the cv. Drysdale and Waagan *Ppd-B1* alleles were functionally indistinguishable with respect to flowering time control. Furthermore, the KASParMAS048 marker, based on a SNP located in the *Ppd-B1* gene, was separated from the 2B floret fertility QTL by seven recombinants (Figure 5.3). Hence, the QHFert.aww-2B floret fertility heat tolerance gene is not *Ppd-B1*.

5.4.3. Fine mapping of the QHFert.aww-2B using segregating HIF families

For each of the 604 semi dwarf $F_{2:5}$ Drysdale × Waagan RIL lines, five seeds from the original packet were sown, and leaf tissue from the five seedlings were bulked for DNA extraction. The DNA samples were screened using three KASP markers in the QHFert.aww-2B region and some additional KASP markers for the minor floret fertility heat tolerance loci on chromosomes 3B (QHFert.aww-3B) and 7A (QHFert.aww-7A2) (Chapter 4). Fourteen

RILs were identified as being heterogeneous (carrying both alleles) for markers in the 2B QTL region but largely fixed (carrying one allele) for markers at the other two loci, and these were used in further analysis (Table 5.3). The 14 selected lines were then used for mapping by sowing 20 more seeds per line from the original seed packets. Per line, 14 plants were used for heat treatment at 6 cm AIL and 6 plants were used as control. Four KASP markers from the 2B QTL region were used for genotyping each plant. Plants of the Waagan and Drysdale parent varieties were also included as controls for phenotyping and genotyping.

The data and its interpretation are shown in Figure 5.5. Families with strong marker-trait association (WW30647 and WW30893) (Figure 5.5: families 1 and 2) suggested a dominant mode of expression of the tolerance allele from Waagan, with the marker-heterozygotes showing similar (high) levels of tolerance as the plants that were homozygous for the Waagan marker alleles. Four families showed strong to weak marker-trait associations, with two of the latter (WW30889 and WW30676) representing potential recombinant families between the 2B QTL and one or both of the flanking markers (Figure 5.5: families 3, 4, 5 and 6). Two families (Figure 5.5: 7 and 8) showed no clear marker-trait association despite the fact that all three markers were segregating in the same phase within the families. The remaining six of the families were not segregating for the marker wsnp_JD_c3732_4781170 (which co-segregated with the 2B QTL locus in the DH population) (Figure 5.5: families 9, 10, 11, 12, 13 and 14) and showed no clear marker-trait association with either of the flanking markers, perhaps because the 2B locus was not segregating in these families (two of these showed no or little segregation for either flanking marker).

Although these families represented RIL lines, some may have still been segregating for one or more minor loci that influence the trait (e.g., loci on chromosomes 1B, 4B and 4D, 3B and 7A; Chapter 4), thereby hampering detection of marker-trait associations on 2B. The two families that showed the clearest marker-trait associations (WW30647 and WW30893) were not derived from a parent that was recombinant for the markers, and therefore these did not assist with fine mapping. However, because these two families are likely to be fixed (homozygous) for other loci that influence the trait, they were considered to be of most use for future fine mapping efforts. To this end, seed of heterozygous plants from these families were kept. These could be used to marker-identify progeny plants that are recombinant in the region, which could then be genotyped for the 2B locus by phenotyping in their progeny.

Table 5. 3 Fourteen Drysdale × Waagan RIL families that showed heterogeneity for markers in the QHFert.aww-2B region on 2B. Calls are shown for markers in the region of the 2B QTL and minor tolerance loci on chromosomes 3B and 7A. Positions of markers are defined by their linkage groups and cM locations in the study of Shirdelmoghanloo et al. (2016c).

	KASParMAS048	wsnp_Ex_c5412_9565527	wsnp_JD_c3732_4781170	wsnp_Ex_c13351_21042379	wsnp_Ex_c944_1810245	wsnp_Ex_rep_c101457_86818160	wsnp_Ra_c10710_17570054	wsnp_Ku_c3371_6259457	wsnp_JD_rep_c50820_34666611	wsnp_Ku_c139_279238	wsnp_Ex_c2268_4251636	wsnp_Ex_c40247_47349166	wsnp_Ex_c41050_47971100
9k SNP marker	2B1	2B1	2B1	2B1	2B1	3B2	3B2	3B2	3B2	7A2	7A2	7A2	7A2
cM	74.25	76.51	79.52	93.92	96.18	96.32	124.95	146.57	151.85	14.92	43.15	46.03	48.19
WW30647	DW	DW	DW	DW	DW	WW	DW	DD	WW	WW	WW	WW	WW
WW30784	DW	WW	DW	DW	DW	WW	DD	DD	DD	DD	DD	DD	DD
WW30789	DW	DW	DW	WW	WW	DD	DD	DD	DW	DD	DD	DD	DD
WW30692	Bad	DW	DW	DD	WW	WW	WW	DD	DD	DD	DD	DD	DD
WW30860	DW	DW	WW	DD	DD	WW	WW	DD	WW	DD	DD	DD	DD
WW30709	WW	DW	WW	WW	WW	DD	WW	WW	WW	WW	DD	DD	DD
WW30818	WW	DW	WW	WW	WW	WW	DD	DD	WW	DD	DD	DD	DD
WW30652	DD	DD	DW	DW	DW	DD	WW	WW	WW	DD	DD	DD	DD
WW30676	WW	WW	DW	WW	DD	WW	WW	WW	WW	DW	DD	DD	DD
WW30889	WW	WW	DW	DD	DD	DD	WW	DD	DD	DD	WW	WW	WW
WW30909	DD	DD	DD	DW	DW	WW	DD	DD	DD	DW	WW	WW	WW
WW30893	DD	DD	DD	DW	DW	DD	DD	DD	DD	WW	WW	WW	WW
WW30657	DD	DD	WW	DD	DW	DD	DD	WW	WW	WW	WW	WW	WW
WW30743	WW	WW	WW	DD	DW	DD	DD	DD	DD	WW	WW	WW	WW

DD, WW and DW stands for Drysdale, Waagan and heterozygous type respective.

Family	Progeny	KASParMAS048	wsnp_JD_c3732_4781170	wsnp_Ex_c944_1810245	Treatment	Fertility
Parents	Drysdale	DD	DD	DD	Control	1.7
	Drysdale	DD	DD	DD	Heat	0.0
	Waagan	WW	WW	WW	Control	2.0
	Waagan	WW	WW	WW	Heat	1.8
WW30647	Parent	DW	DW	DW		
1	WW30647_9	WW	WW	WW	Control	1.9
	WW30647_16	WW	WW	WW	Control	1.9
	WW30647_5	DW	DW	DW	Control	1.4
	WW30647_13	DW	DW	DD	Control	2.0
	WW30647_19	DD	DD	DW	Control	1.7
	WW30647_2	DD	DD	DD	Control	1.8
	WW30647_14	WW	WW	WW	Heat	1.1
	WW30647_17	WW	WW	WW	Heat	1.3
	WW30647_12	DW	WW	DW	Heat	0.7
	WW30647_10	DW	DW	DW	Heat	0.4
	WW30647_8	DW		DW	Heat	0.7
	WW30647_20	WW	DD	DD	Heat	0.2
	WW30647_1	DD	DD	DD	Heat	0.0
	WW30647_3	DD	DD	DD	Heat	0.1
	WW30647_4	DD	DD	DD	Heat	0.1
	WW30647_15	DD	DD	DD	Heat	0.2
	WW30647_18	DD	DD	DD	Heat	0.0
	WW30647_7	DD		DD	Heat	0.0
WW30893	Parent	DD	DD	DW		
2	WW30893_12	WW	WW	WW	Control	1.3
	WW30893_7	WW	DW	DD	Control	2.0
	WW30893_9	DD	DD	WW	Control	1.1
	WW30893_3	DD	DD	DW	Control	1.9
	WW30893_14	DD	DD	DD	Control	1.8
	WW30893_17	DD	DD	DD	Control	1.6
	WW30893_16	WW	WW	DD	Heat	1.4
	WW30893_8	WW			Heat	1.9
	WW30893_6	DW	DW	DW	Heat	0.9
	WW30893_19	DD	DW	WW	Heat	1.1
	WW30893_18	DD	DW	DW	Heat	0.5
	WW30893_1	DD	DD	DD	Heat	0.2
	WW30893_2	DD	DD	DD	Heat	0.0
	WW30893_4	DD	DD	DD	Heat	0.2
	WW30893_5	DD	DD	DD	Heat	0.0
	WW30893_10	DD	DD	DD	Heat	0.1
	WW30893_11	DD	DD	DD	Heat	0.2
	WW30893_13	DD	DD	DD	Heat	0.1
	WW30893_15	DD	DD	DD	Heat	0.4
	WW30893_20	DD	DD	DD	Heat	0.0

Family	Progeny	KASParMAS048	wsnp_JD_c3732_4781170	wsnp_Ex_c944_1810245	Treatment	Fertility
Parents	Drysdale	DD	DD	DD	Control	1.7
	Drysdale	DD	DD	DD	Heat	0.0
	Waagan	WW	WW	WW	Control	2.0
	Waagan	WW	WW	WW	Heat	1.8
WW30889	Parent	WW	DW	DD		
3	WW30889_18	DW	WW	WW	Control	1.8
	WW30889_8	DD	WW	WW	Control	1.6
	WW30889_16	DD	DW	WW	Control	0.9
	WW30889_3	DD	DD	WW	Control	1.8
	WW30889_5	DD	DD	WW	Control	1.5
	WW30889_12	DD	DD	WW	Control	2.0
	WW30889_4	WW	WW	WW	Heat	0.7
	WW30889_6	WW	WW	WW	Heat	0.7
	WW30889_10	WW	WW	WW	Heat	0.4
	WW30889_13	WW	WW	WW	Heat	0.7
	WW30889_15	WW	WW	WW	Heat	1.7
	WW30889_9	WW	DW	WW	Heat	0.3
	WW30889_14	DW	DW	WW	Heat	0.0
	WW30889_17	DW	DW	WW	Heat	0.3
	WW30889_19	DW	DW	WW	Heat	0.6
	WW30889_1	DD	DD	WW	Heat	0.0
	WW30889_2	DD	DD	WW	Heat	0.0
	WW30889_7	DD	DD	WW	Heat	0.5
	WW30889_11	DD	DD	WW	Heat	0.0
WW30676	Parent	WW	DW	DD		
4	WW30676_3	WW	WW	DD	Control	1.5
	WW30676_6	WW	DW	DD	Control	1.6
	WW30676_11	WW	DW	DD	Control	1.8
	WW30676_16	WW	DW	DD	Control	2.0
	WW30676_20	WW	DW	DD	Control	2.0
	WW30676_9	WW	DD	DD	Control	1.8
	WW30676_2	WW	WW	DD	Heat	0.8
	WW30676_4	WW	DW	DD	Heat	1.0
	WW30676_5	WW	DW	DD	Heat	0.7
	WW30676_7	WW	DW	DD	Heat	0.7
	WW30676_12	WW	DW	DD	Heat	0.2
	WW30676_14	WW	DW	DD	Heat	0.9
	WW30676_18	WW	DW	DD	Heat	1.0
	WW30676_19	WW	DW	DD	Heat	0.5
	WW30676_10	WW	DD	DD	Heat	0.4
	WW30676_13	WW	DD	DD	Heat	0.6
	WW30676_15	WW	DD	DD	Heat	0.3
	WW30676_17	WW	DD	DD	Heat	0.5
	WW30676_1	WW	?	DD	Heat	0.0

Family	Progeny	KASParMAS048	wsnp_JD_c3732_4781170	wsnp_Ex_c944_1810245	Treatment	Fertility
Parents	Drysdale	DD	DD	DD	Control	1.7
	Drysdale	DD	DD	DD	Heat	0.0
	Waagan	WW	WW	WW	Control	2.0
	Waagan	WW	WW	WW	Heat	1.8
WW30652	Parent	DD	DW	DW		
5	WW30652_15	DD	DW	DW	Control	1.8
	WW30652_6	DD	DW	DD	Control	2.0
	WW30652_19	DD	DD	DW	Control	2.0
	WW30652_3	DD	DD	DD	Control	1.7
	WW30652_9	DD	DD	DD	Control	2.0
	WW30652_13	DD	DD	DD	Control	1.6
	WW30652_10	DD	WW	WW	Heat	1.1
	WW30652_2	DD	WW	DD	Heat	1.4
	WW30652_20		WW	DW	Heat	1.1
	WW30652_18	DD	DW	WW	Heat	0.4
	WW30652_7	DD	DW	DW	Heat	1.2
	WW30652_14	DD	DW	DW	Heat	1.3
	WW30652_11	DD	DW	DD	Heat	1.1
	WW30652_1	DD	DD	DW	Heat	1.0
	WW30652_8	DD	DD	DW	Heat	0.6
	WW30652_17	DD	DD	DD	Heat	0.8
WW30692	Parent	Bad	DW	WW		
6	WW30692_11	WW	WW	WW	Control	1.8
	WW30692_16	WW	WW	WW	Control	1.6
	WW30692_1	DD	DW	DW	Control	1.8
	WW30692_4	DD	DD	DW	Control	1.1
	WW30692_2	WW	WW	WW	Heat	0.0
	WW30692_5	WW	WW	WW	Heat	0.9
	WW30692_7	WW	WW	WW	Heat	1.1
	WW30692_9	WW	WW	WW	Heat	1.1
	WW30692_14	WW	WW	WW	Heat	1.5
	WW30692_10	WW	WW	DW	Heat	1.8
	WW30692_12	WW	WW	DW	Heat	1.5
	WW30692_19	WW	DW	WW	Heat	1.2
	WW30692_15	WW	DW	DW	Heat	0.8
	WW30692_20	DW	DW	DW	Heat	0.8
	WW30692_3	DW	DW	DD	Heat	1.0
	WW30692_18	DD	DD	WW	Heat	1.7
	WW30692_17	DD	DD	DD	Heat	1.0

Family	Progeny	KASParMAS048	wsnp_JD_c3732_4781170	wsnp_Ex_c944_1810245	Treatment	Fertility
Parents	Drysdale	DD	DD	DD	Control	1.7
	Drysdale	DD	DD	DD	Heat	0.0
	Waagan	WW	WW	WW	Control	2.0
	Waagan	WW	WW	WW	Heat	1.8
WW30784	Parent	DW	DW	DW		
7	WW30784_12	WW	WW	WW	Control	1.9
	WW30784_18	WW	WW	WW	Control	1.7
	WW30784_6	DW	DW	DW	Control	1.8
	WW30784_2	DD	DD	DD	Control	1.7
	WW30784_15	DD	DD	DD	Control	1.9
	WW30784_9	DD	DD		Control	1.8
	WW30784_1	WW	WW	WW	Heat	1.5
	WW30784_4	WW	WW	WW	Heat	1.6
	WW30784_5	WW	WW	WW	Heat	0.8
	WW30784_13	WW	WW	WW	Heat	1.3
	WW30784_16	WW	WW	WW	Heat	1.2
	WW30784_20	WW	WW	WW	Heat	1.4
	WW30784_17	WW	WW	DW	Heat	1.8
	WW30784_19	DW	DW	DW	Heat	1.5
	WW30784_3	DD	DD	WW	Heat	0.4
	WW30784_7	DD	DD	DD	Heat	1.5
	WW30784_11	DD	DD	DD	Heat	1.2
	WW30784_8		DD	DD	Heat	1.4
WW30789	Parent	DW	DW	WW		
8	WW30789_3	WW	DD	DD	Control	1.9
	WW30789_6	WW	DD	DD	Control	2.0
	WW30789_9	WW	DD	DD	Control	1.9
	WW30789_12	WW	DD	DD	Control	2.0
	WW30789_18	WW	DD	DD	Control	1.9
	WW30789_2	WW	WW	DD	Heat	2.0
	WW30789_4	WW	WW	DD	Heat	0.7
	WW30789_10	WW	WW	DD	Heat	1.5
	WW30789_7	WW	DW	DD	Heat	1.0
	WW30789_11	WW	DW	DD	Heat	0.4
	WW30789_16	WW	DW	DD	Heat	1.0
	WW30789_5	WW	DD	DD	Heat	0.5
	WW30789_13	WW	DD	DD	Heat	0.8
	WW30789_14	WW	DD	DD	Heat	1.9
	WW30789_17	WW	DD	DD	Heat	0.7
	WW30789_19	WW	DD	DD	Heat	1.2
	WW30789_20	WW	DD	DD	Heat	0.8

Family	Progeny	KASParMAS048	wsnp_JD_c3732_4781170	wsnp_Ex_c944_1810245	Treatment	Fertility
Parents	Drysdale	DD	DD	DD	Control	1.7
	Drysdale	DD	DD	DD	Heat	0.0
	Waagan	WW	WW	WW	Control	2.0
	Waagan	WW	WW	WW	Heat	1.8
WW30909	Parent	DD	DD	DW		
9	WW30909_2	DD	DD	WW	Control	1.6
	WW30909_5	DD	DD	DD	Control	1.8
	WW30909_7	DD	DD	DD	Control	1.8
	WW30909_14	DD	DD	DD	Control	2.0
	WW30909_19	DD	DD	DD	Control	1.7
	WW30909_20	DD	DD	WW	Heat	0.1
	WW30909_1	DD	DD	WW	Heat	0.2
	WW30909_3	DD	DD	WW	Heat	0.4
	WW30909_9	DD	DD	WW	Heat	0.3
	WW30909_12	DD	DD	WW	Heat	0.0
	WW30909_13	DD	DD	WW	Heat	0.4
	WW30909_15	DD	DD	WW	Heat	0.3
	WW30909_17	DD	DD	WW	Heat	0.8
	WW30909_18	DD	DD	WW	Heat	0.0
	WW30909_6	DD	DD	DW	Heat	0.2
	WW30909_4	DD	DD	DD	Heat	1.0
	WW30909_8	DD	DD	DD	Heat	0.5
	WW30909_11	DD	DD	DD	Heat	0.3
	WW30909_16	DD	DD	DD	Heat	0.6
WW30657	Parent	DD	WW	DW		
10	WW30657_1	DD	WW	WW	Control	2.0
	WW30657_4	DD	WW	DD	Control	1.8
	WW30657_7	DD	WW	DD	Control	1.9
	WW30657_14	DD	WW	DD	Control	1.7
	WW30657_17	DD	WW	DD	Control	2.0
	WW30657_11		WW	WW	Control	2.0
	WW30657_6	DD	WW	WW	Heat	0.8
	WW30657_13	DD	WW	WW	Heat	1.1
	WW30657_18	DD	WW	WW	Heat	1.1
	WW30657_12	DD	WW	DW	Heat	1.2
	WW30657_15	DD	WW	DW	Heat	0.3
	WW30657_2	DD	WW	DD	Heat	1.1
	WW30657_3	DD	WW	DD	Heat	0.9
	WW30657_5	DD	WW	DD	Heat	1.4
	WW30657_8	DD	WW	DD	Heat	1.4
	WW30657_9	DD	WW	DD	Heat	0.8
	WW30657_10	DD	WW	DD	Heat	1.2
	WW30657_16	DD	WW	DD	Heat	0.8
	WW30657_20	DD	WW	DD	Heat	1.3

Family	Progeny	KASParMAS048	wsnp_JD_c3732_4781170	wsnp_Ex_c944_1810245	Treatment	Fertility
Parents	Drysdale	DD	DD	DD	Control	1.7
	Drysdale	DD	DD	DD	Heat	0.0
	Waagan	WW	WW	WW	Control	2.0
	Waagan	WW	WW	WW	Heat	1.8
WW30860	Parent	DW	WW	DD		
11	WW30860_3	WW	WW	DD	Control	1.8
	WW30860_6	WW	WW	DD	Control	1.9
	WW30860_9	WW	WW	DD	Control	1.9
	WW30860_11	WW	WW	DD	Control	1.9
	WW30860_18	WW	WW	DD	Control	1.6
	WW30860_14	DW	WW	WW	Control	2.0
	WW30860_2	WW	WW	DD	Heat	1.6
	WW30860_4	WW	WW	DD	Heat	1.8
	WW30860_5	WW	WW	DD	Heat	1.7
	WW30860_7	WW	WW	DD	Heat	2.0
	WW30860_8	WW	WW	DD	Heat	1.1
	WW30860_10	WW	WW	DD	Heat	1.6
	WW30860_13	WW	WW	DD	Heat	2.0
	WW30860_15	WW	WW	DD	Heat	1.1
	WW30860_16	WW	WW	DD	Heat	1.2
	WW30860_19	WW	WW	DD	Heat	0.7
	WW30860_20	WW	WW	DD	Heat	1.3
WW30709	Parent	WW	WW	WW		
12	WW30709_2	WW	WW	WW	Control	2.0
	WW30709_11	WW	WW	WW	Control	1.7
	WW30709_17	WW	WW	WW	Control	1.6
	WW30709_20	WW	WW	WW	Control	2.0
	WW30709_7	DD	WW	WW	Control	1.9
	WW30709_6	WW	WW	WW	Heat	0.8
	WW30709_9	WW	WW	WW	Heat	0.5
	WW30709_14	WW	WW	WW	Heat	1.6
	WW30709_16	WW	WW	WW	Heat	0.5
	WW30709_18	WW	WW	WW	Heat	0.3
	WW30709_5	WW	WW	DD	Heat	0.5
	WW30709_1	DW	WW	WW	Heat	0.8
	WW30709_3	DW	WW	WW	Heat	1.3
	WW30709_8	DW	WW	WW	Heat	0.2
	WW30709_10	DW	WW	WW	Heat	0.7
	WW30709_4	DD	WW	WW	Heat	0.7
	WW30709_12	DD	WW	WW	Heat	0.6
	WW30709_13	DD	WW	WW	Heat	0.9
	WW30709_19	DD	WW	WW	Heat	0.3

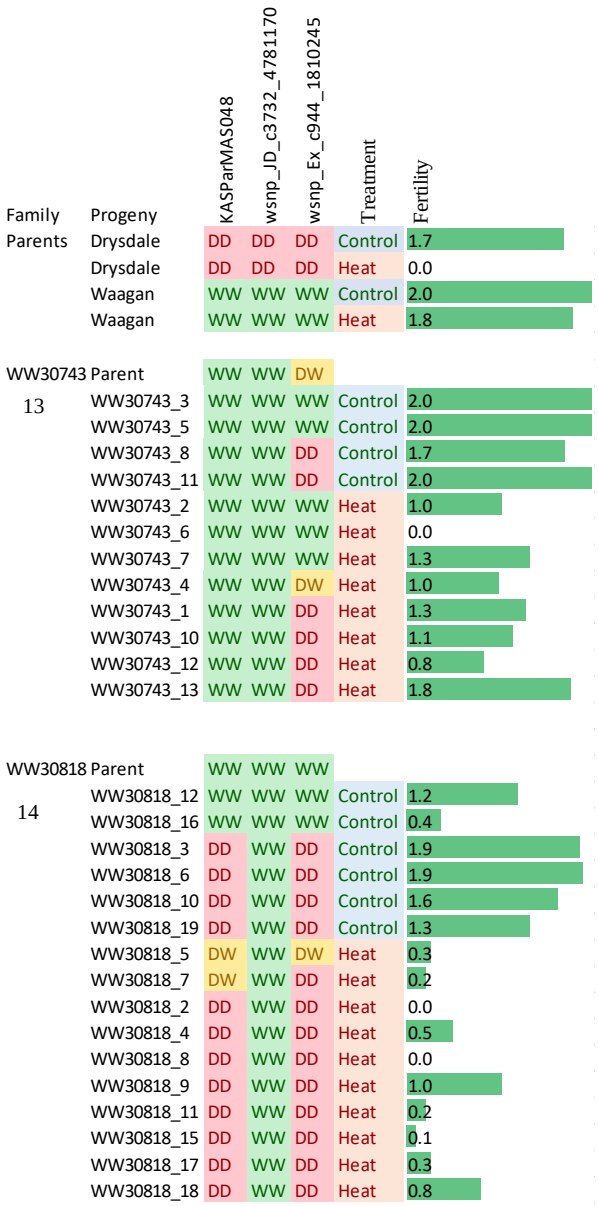

Figure 5. 5 Association of floret fertility under both control and heat (treatment at 6 cm AIL; data bars) with marker scores on individual plants in 14 RIL families. Fertility is the average number of grain per spikelet in the two lower floret positions, in the middle and bottom thirds of spike. Families showed either strong marker-trait association (1 and 2), weak marker-trait association (3 and 4), no association but possible recombinant with the middle marker (5, 6, 7 and 8), or no association and no segregation of the middle marker (9, 10, 11, 12, 13 and 14). Parental lines with their genotypic and phenotypic association under both heat and control condition are indicated at top of each category

5.4.4. Development of near isogenic lines (NILs)

A separate planting was used to marker-select NILs for the QHFert.aww-2B region from progeny of Drysdale × Waagan RIL families that were still segregating in the QTL region. RIL families for this purpose were chosen on the basis of five KASP markers in the 2B QTL region and several KASP markers at each of two additional minor tolerance loci on chromosomes 3B and 7A. In total, 14 $F_{2:5}$ RIL families that were heterogeneous in the 2B QTL region, but largely homozygous at the other two loci, were chosen (Table 5.2). Twenty seeds were sown from each family and their individual DNA assayed for three of the chromosome 2B markers. In total, 32 plants were selected for propagation as NILs – two of each homozygous 2B allele type from each of 8 families (Table 5.4). Seeds from these plants were harvested and samples sent to Livinus Emebiri, NSW-DPI Wagga Wagga, for field multiplication and field evaluation.

Table 5. 4 NILs for the QHFert.aww-2B region selected from Drysdale × Waagan RIL families. KASP marker scores are shown: WW and DD indicate homozygous Waagan and Drysdale allele, respectively. The locations of the markers are defined by the cM positions of the original 9k SNP array markers mapped on linkage group 2B1 by Shirdelmoghanloo et al. (2016c)

Family	KASP marker	KASParMAS048	wsnp_JD_c3732_4781170	wsnp_Ex_c944_1810245	Family	KASP marker	KASParMAS048	wsnp_JD_c3732_4781170	wsnp_Ex_c944_1810245
	cM	74.25	79.52	96.18		cM	74.25	79.52	96.18
WW30647	WW30647_25	WW	WW	WW	WW30889	WW30889_17	WW	WW	DD
	WW30647_80	WW	WW	WW		WW30889_18	WW	WW	DD
	WW30647_37	DD	DD	DD		WW30889_15	WW	DD	DD
	WW30647_71	DD	DD	DD		WW30889_16	WW	DD	DD
ww30784	ww30784_40	WW	WW	WW	WW30709	WW30709_16	WW	WW	WW
	ww30784_73	WW	WW	WW		WW30709_18	WW	WW	WW
	ww30784_42	DD	DD	DD		WW30709_17	DD	WW	WW
	ww30784_86	DD	DD	DD		WW30709_19	DD	WW	WW
WW30652	WW30652_1	DD	WW	DD	WW30789	WW30789_12	WW	WW	WW
	WW30652_4	DD	WW	DD		WW30789_18	WW	WW	WW
	WW30652_11	DD	DD	DD		WW30789_20	DD	DD	WW
	WW30652_8	DD	DD	DD		WW30789_7	DD	DD	WW
WW30676	WW30676_14	WW	WW	DD	WW30909	WW30909_10	DD	DD	WW
	WW30676_7	WW	WW	DD		WW30909_12	DD	DD	WW
	WW30676_2	WW	DD	DD		WW30909_18	DD	DD	DD
	WW30676_4	WW	DD	DD		WW30909_9	DD	DD	DD

5.4.5. Genes in the QHFert.aww-2B interval

The 9.1 cM QTL interval defined by the flanking markers wsnp_Ex_c5412_9565527 and AHW_DW_054 contained nine known scaffolds, varying in size from 1.2 to 8.1 Mb. These nine scaffolds contained 203 predicted genes (Appendix table 5.4). Scaffold 88768 carried the most genes (50) and scaffold 85263 contained the fewest (two; Figure 5.6).

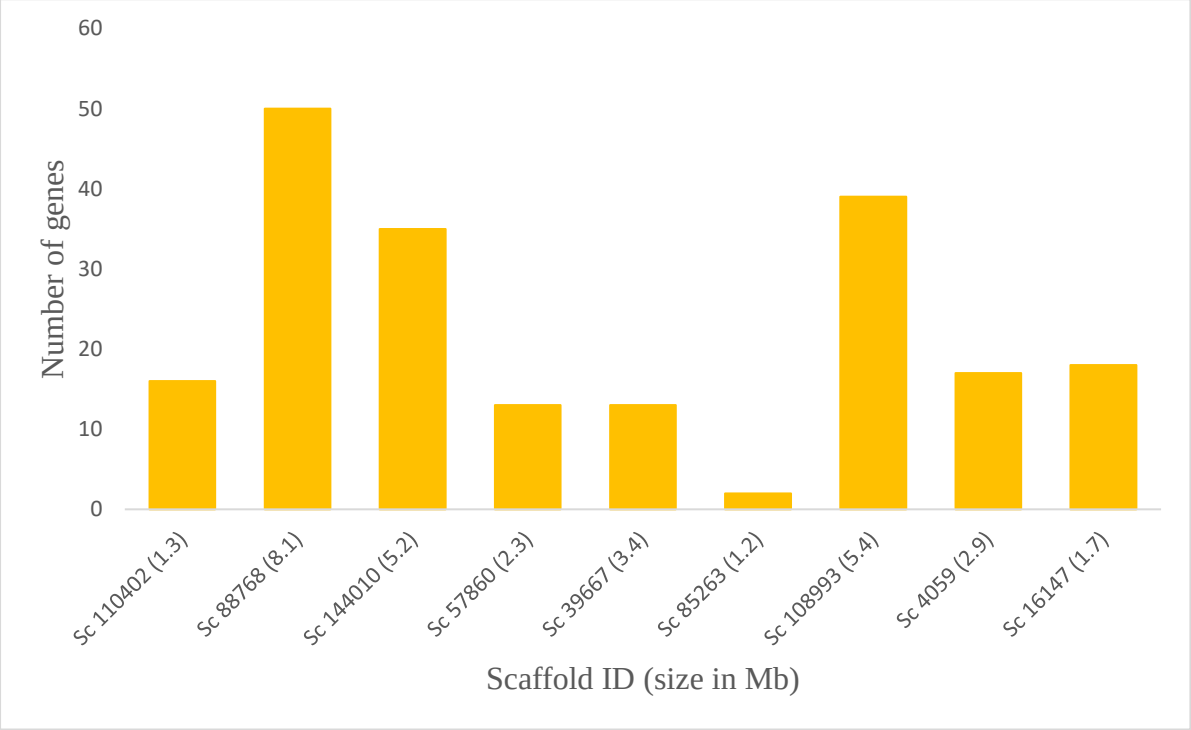

Figure 5. 6 Number of predicted genes per scaffold in the QHFert.aww-2B interal. Numbers in parenthesis shows the Mb size of each scaffold.

The position of the QTL at 81.0 cM on the Drysdale × Waagan map placed it at around the two groups of markers at 80.6 and 81.3 cM, although the precise order of the QTL locus with respect to these markers could not be determined. Therefore, the 2B tolerance gene seems most likely to be located on one of the five scaffolds containing these markers (scaffolds 110402, 88768, 144010, 57860 and 39667). These scaffolds contain 127 genes (Appendix table 5.4).

5.5. Conclusion and future perspectives

Positional cloning of a heat tolerance QTL is a potential avenue for developing diagnostic markers which could then be used by breeders to select for tolerance to heat induced

floret sterility. The major QTL for tolerance to heat-induced floret sterility on chromosome arm 2BS was narrowed to an 9.1 cM interval between markers wsnp_Ex_c5412_9565527 and AHW_DW-054 using phenotyping of recombinant DH lines and addition of 17 new markers to the region. The interval contained known genomic sequence scaffolds totalling 31.5 Mb, and these scaffolds contained 203 predicted genes. The ten DH recombinants between flanking markers have potential to assist in further fine mapping. NILs for the 2B tolerance QTL were generated by exploiting residual heterogeneity in the RIL lines. These will be useful in further molecular and physiological characterization of the 2B locus effects and for field-validating the tolerance.

Chapter 6: Identifying developmental stages most sensitive to heat-induced floret sterility in bread wheat (*T. aestivum* L.) cultivars Drysdale and Waagan

6.1. Abstract

This experiment was designed to identify the most heat susceptible stage of pollen development with respect to floret fertility in the wheat cvs Drysdale and Waagan. Drysdale as a heat susceptible cultivar was most sensitive at developmental stages coinciding with auricle interval length (AIL) 7 cm to before 13 cm AIL. This corresponded to early meiosis to late uni-nucleate microspore stage. The cultivar Waagan is heat tolerant and the effect of heat on pollen development was therefore subtle and difficult to assign to a specific developmental stage. Defining pollen developmental at different AIL revealed that in Waagan, the AIL was 1.5 cm shorter than that of Drysdale when meiosis began. This reiterates the importance of precise staging of developmental processes accounting for genotypic variability within wheat genotypes. Compared to Drysdale, Waagan took longer (~5 d) from sowing to reach at particular AIL. Spike length at the particular stages was also defined in both genotypes. For the future assaying of floret fertility and its developmental stage on the same floret (both before and after heat treatment), would be recommended as a way of more accurately determining sensitive stage.

6.2. Introduction

The stages of reproductive development in wheat from early booting to early grain filling are particularly sensitive to abiotic stresses (heat, cold and drought), and stresses during this time can result in loss of grain yield and low grain quality (Oliver *et al.*, 2005). The timing of heat stress therefore determines whether grain number or size is affected; heat before anthesis leads to spikelet sterility and thereby grain loss, whereas heat after anthesis negatively effects grain weight (Tashiro and Wardlaw, 1990).

With respect to the effect of heat on floret sterility, both male and female reproductive organs are sensitive to high temperature and their response vary with genotype. However, ovules are generally less heat sensitive than the anthers (Wahid *et al.*, 2007a; Willits and Peet, 1998). In wheat, male and female meiosis are well synchronized within individual florets (Bennett *et al.*, 1973a). As the male organs (anthers) are much easier to examine for meiotic

stages than the female organs (ovules), the former is often used as an indicator of meiotic stage for both, male and female organs of a floret.

High temperature accelerates (and shortens) most plant developmental processes, including meiosis. For example, Bennett *et al.* (1972) found that in Chinese Spring, meiosis takes only 18 h at 25 ºC but 24 h at 20 ºC, and in the diploid grass *Dasypyrum villosum,* meiosis was reported to last for 29 h at 20 ºC, 21 h at 28 ºC and only 17 h at 35 ºC (Stefani and Colonna, 1996).

Another factor to consider when characterizing stages of wheat microsporogenesis (male gametogenesis) is the asynchrony of development of florets at different positions on the spike. Floret differentiation and development begins in the spikelets located in the middle of the spike, then continues upwards and downwards, while within the spikelets, floret development proceeds from the basal floret positions upwards. Under non-stress conditions, anther development in the top and bottom third of the spike lags 2 to 3 days behind that of the middle third for florets in corresponding positions in the spikelets. Within a spikelet, the two basal florets develop around 30-48 hrs ahead of the next floret up (Bennett *et al.*, 1973b). Pollen takes about ten days from meiosis to reach maturity (anthesis).

In Chapter 4, heat treatments applied at either 3 or 9 cm auricle interval length (AIL) caused substantial floret sterility in cvs. Waagan and Drysdale, and in a DH population derived from them, and treatment at either time revealed tolerance QTL. A step towards understanding the mechanistic basis of the tolerance difference between Waagan and Drysdale, and of the QTLs that determine this difference, could be to determine the stage of microsporogenesis that is most sensitive to heat induced floret sterility in cv. Drysdale (during the targeted stages occurring during 3-9 cm AIL).

Huang *et al.* (2000) found that in wheat cv. Wilgoyne, meiosis occurred in the most advanced florets of the spike (basal florets in central spikelets) at 9 days before anthesis. Based on that information and given the 3-4 day difference within a spike (see above), one should expect meiosis to complete across the spike from 9 to 4 days before anthesis of the most advanced florets. In Chapter 4 it was determined that heat-treated Drysdale plants took 12 and 7 days from 3 and 9 cm AIL respectively, to reach anthesis (Day.AILtoAnth trait), whereas Waagan took 9 and 7 days, respectively. Hence Drysdale may be susceptible at around meiosis.

The aim of the work in this chapter was to determine the stages of microsporogenesis where heat caused the greatest floret sterility in Drysdale in comparison to the tolerant genotype Waagan. This was done in reference to AIL, spike length and days before anthesis to provide more convenient indicators of the susceptible stages for use in future genetic and physiological studies of heat tolerance.

6.3. Materials and Methods

6.3.1. Plant materials and growth conditions

The experiment was conducted from July to November in 2016 using the Australian Plant Accelerator plant growth facilities, University of Adelaide, Waite Campus. Two single-plant selections for each of cvs. Drysdale (selections 187 and 188; susceptible) and Waagan (selections 192 and 194; tolerant) were chosen at random for this experiment. Although some selections within these varieties have been found to differ for major flowering time genes (reflecting some residual within-variety heterogeneity, which is not unusual for a wheat variety; data not shown), the selected plants all carried the winter allele at *Vrn-A1*, and the spring allele at both, *Vrn-B1* and *Vrn-D1*. The two varieties carry functionally identical alleles at each of the respective loci *Ppd-B1* and *Ppd-D1* (Shirdelmoghanloo *et al.*, 2016c).

Plants were initially grown in a naturally lit greenhouse compartment as described in chapter 3, where the average temperature and relative humidity was recorded as 23/20 ºC and 51/59 % day/night respectively (Appendix table 6.1). Temperature was 23/19 C day /night around booting stage (heat treatment stage).

6.3.2. Experimental design, treatments and anther sampling

Anthers were sampled to determine the stage of microsporogenesis in main stems of Waagan and Drysdale plants grown under non-stress conditions, at 11 tiller stages: 4 d and 2 d before flag leaf auricle emergence (BAE), at flag leaf auricle emergence (‘0 cm’), and at 1 cm, 3 cm, 6 cm, 9 cm, 11 cm ,13 cm AIL. 14 cm and 16 cm. In addition, 18 cm AIL was also considered for Drysdale. Heat susceptibility at each of these stages, except at the latter two

stages for both genotypes, was determined by subjecting plants as close as possible to the respective stages to a 3 d heat treatment and scoring floret fertility at maturity using the methods for heat treatment described in Chapter 3. A set of non-treated control plants were also scored for floret fertility.

Movement of plants for heat treatment was only done once a day after 3 pm, so there was some variation in AIL around the target length when anther sampling and heat treatment took place. To factor in this variation, the AIL of each plant (both those used for anther sampling and seed set assessment) was recorded to the nearest 0.5 cm, as close as possible to the time of sampling or treatment. Almost all heat treated plants also had their AILs measured immediately after taking the plants out of the heat treatment chamber (although AILs of control plants were not measured at the corresponding time).

The experiment was laid out in randomized complete block (RCB) design with three replications. For each of the 4 genotype selections × 19 purposes (anther sampling at each stage, heat treatment at each stage for assaying fertility, and control for assaying fertility), three plants were used as replicates.

To estimate the timing of the two stages before auricle emergence (BAE), which are 4 and 2 d BAE, six plants of each of the four genotypes were sown 15 days earlier than the main experiment. The average number of days from sowing to flag leaf auricle emergence (‘0 cm’) was determined for each genotype and used to estimate the day of 4 and 2 d BAE in each of the genotypes for the main experiment.

Acetocarmine is a basic dye derived from the insect *Coccus cacti*. It strongly stains chromatin and weakly stains the cytoplasm, providing good contrast between the nucleus and surrounding cellular material (Dundas *et al.*, 1981). The sampled anthers were squashed and stained with acetocarmine to determine the microsporogenesis stage. This stain was prepared in the Cereal Cytogenetics Laboratory on the Waite Campus through a series of steps: 2 g of carmine (certified by Biological Stain Commission) (Sigma Aldrich C6152-25G) was dissolved in 100 ml of boiling 45% acetic acid (45 ml glacial acetic acid, 55 ml deionized water), cooled to room temperature, then filtered into brown bottles and stored at room temperature. A 2% solution of carmine in 45% acetic acid was used to stain the samples.

At the designated sampling times, the main tiller was cut at about two-thirds of the way down from top of spike and tillers were kept in a water-filled beaker until further analysis. Spikes were dissected out from the flag leaf sheath (boot) using forceps and a razor blade, and spike length was measured from the bottom of the basal-most spikelet to the top of the glumes of the terminal spikelet using a ruler. Unintentionally, spike length was not recorded for stages before 3 and 3.5 cm AIL and after 10.5 and 9.5 cm AIL, in Drysdale and Waagan, respectively. Per plant, one anther was sampled from each of the top, middle and bottom thirds of the spike with the aid of a stereo dissecting microscope (Leica MZ6) and placed on a microscopic slide (one anther per microscope slide). The anther was taken only from one of the basal two florets positions in a spikelet. A drop of 2% acetocarmine stain was applied, the sample covered with a cover slip and the cover slip tapped gently with a needle at one end to remove bubbles. Then the anthers were quickly squashed with a thumb and release the pollen and observed under a light microscope.

6.3.3. Microscopy

Observations were performed using a compound microscope (LEICA DM1000). Images of each sample were taken using a LEICA DFC 295 camera fitted on top of the microscope. Leica application suite software (LAS V4.8) was used to view and capture images using the settings: 35% brightness, 1 saturation and 0.75 gamma.

Stages of microsporogenesis were defined as specified in Table 6.1, with reference to descriptions by other authors (Bennett *et al.*, 1973b; De Storme and Geelen, 2014; Huang *et al.*, 2000; Saini *et al.*, 1984; Song *et al.*, 2014). To provide a rough framework for data representation and statistical analysis, the stages were also defined by progressive number codes (Table 6. 1).

On each slide, a total of 40 to 200 cells (microsporocyte, microspore or pollen grain) from across four zones on the slide were individually classified for the developmental stage. The most commonly scored stage was then used to define an average stage for the sample (anther). The mean of the developmental stage and its variance across anthers sampled from the three replicates (for the same genotype × stage × treatment × spike position combination) were then used for data representation and statistical analysis.

Table 6. 1 Definition of wheat pollen development stages (non-heat treated plants) for this study

Code	Stage name	Criteria used
1	Pre meiosis	Does not have clear nuclear region.
2	Early meiosis	Distinct nuclear region surrounded by nuclear membrane, just until the onset of leptotene.
3	Meiosis	Condensed chromosomes, including clearly recognisable stages metaphase I and II (chromosomes line up in the middle of nucleus, and anaphase I and II (chromosome migrating to opposite sides of the cell).
4	Tetrad	Four nuclei cluster together within a callose wall.
5	Early uni-nucleate	Microspores are irregularly shaped with large nucleus.
6	Late uni-nucleate	Microspores are round with large nucleus.
7	Bi nucleate	Two nuclei visible. Microspore nucleus has just divided into two. Pollen grains begins to accumulate starch as indicated by granular appearance.
8	Tri nucleate	Three nuclei visible. Generative nucleus has just divided to form two ovoid sperm nuclei. Pollen grains assume spherical shape, pollen grains full of starch.

6.3.4. Data collection for fertility and architectural traits

At plant maturity, floret fertility traits (GrNoSplt. 1&2.Top, GrNoSplt.>2.Top, GrNoSplt.1&2.Mid, GrNoSplt.>2.Mid, GrNoSplt.1&2.Bot, GrNoSplt.>2.Bot, and GrNoSplt.Spk) and architectural traits (AwnL.Mat, SpL_Mat, AIL_Mat, PedL_Mat, and PH_Mat) were scored as described in Chapter 3.

The phenotypic data were subjected to analysis of variance (ANOVA) using GenStat version 16 software. The total variability for the traits was quantified using the following model:

$$P_{ijkl} = \mu + b_i + g_j + t_{k(j)} + s_{l(j)(k)} + gs_{jl} + e_{ijkl}$$

Where, P_{ijkl} is the phenotypic value of the jth genotype under ith replication in kth treatment and at lth stage with replication i, treatment k and stage l; b_i = ith replication; g_j = the effect of jth genotype; $t_{k(j)}$= the effect of kth treatment within jth genotype; $s_{l(j)(k)}$= the effect of lth stage with in kth treatment and jth genotype; $(gs)_{jl}$, the interaction effects and e_{ijkl} = random error.

6.4. Results

6.4.1. Aligning AIL, spike length and microsporogenesis stages

This study sought to define the stage that was most susceptible to heat induced floret fertility, using several tiller characteristics to define develomental stage. The definition of develomental stages was confounded by the growth that occurred during the 3d heat treatments. Hence, the amount of growth of AIL during the 3d heat treatment was quantified for plants heat treated at the various AIL stages. As expected, the growth rate of AIL during the 3d heat treatment was greatest for plants heat treated at 0 cm AIL (~ 2.5 cm/day) and decreased for plants treated at later stages (to ~1.5 cm/day; Figure 6.1).

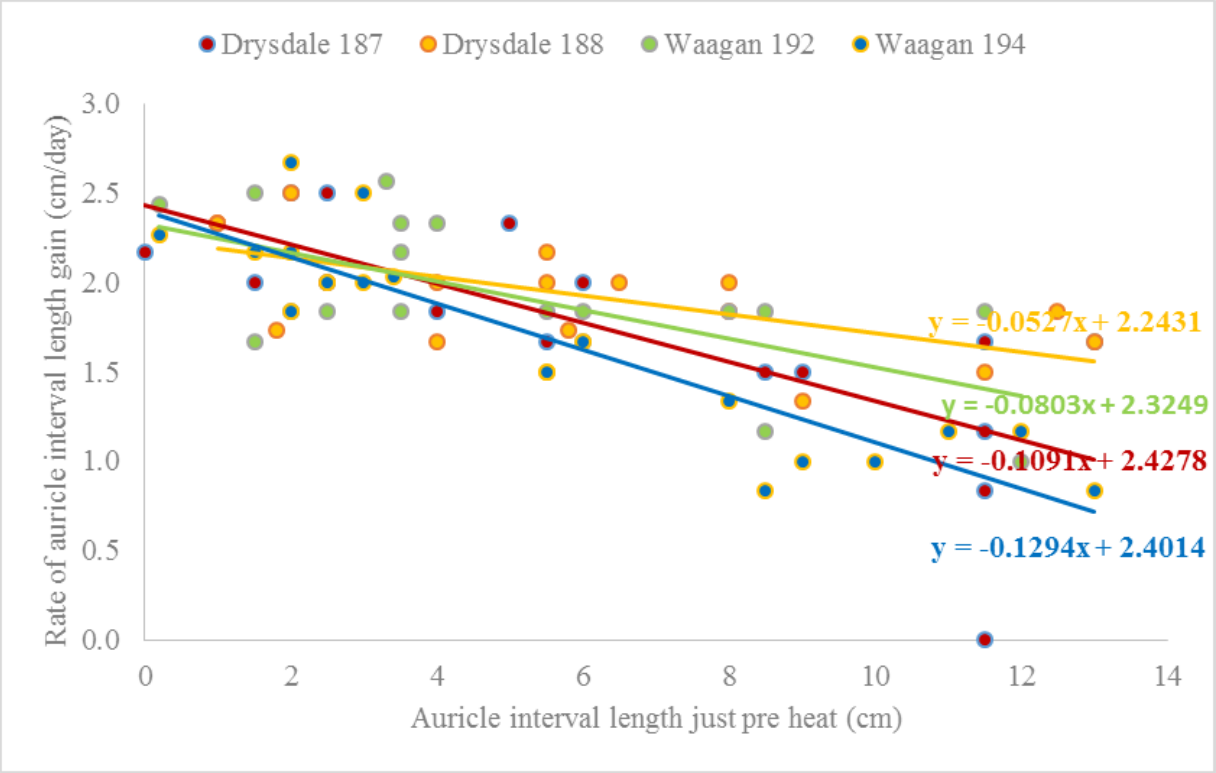

Figure 6. 1 Rate of auricle interval length gain for Waagan 192 and 194 (tolerant) and Drysdale 187 and 188 (intolerant) during three days of heat treatment at 37/27 ºC (day/night). Corresponding measurements were not taken on control plants.

To relate microsporogenesis stage to spike length, spike length was measured on the plants used for anther sampling, at the different AILs, from 0 cm AIL to maturity. Spikes grew ~5-fold over the sampled times and in both varieties final spike length averaged 9 to 10.5 cm (Figure 6.2; Table 6.2), which was similar to what was observed in Chapter 4. Past around 10

cm AIL, AIL and spike length became uncorrelated, probably because spikes stopped elongating at this stage while AIL continued extending.

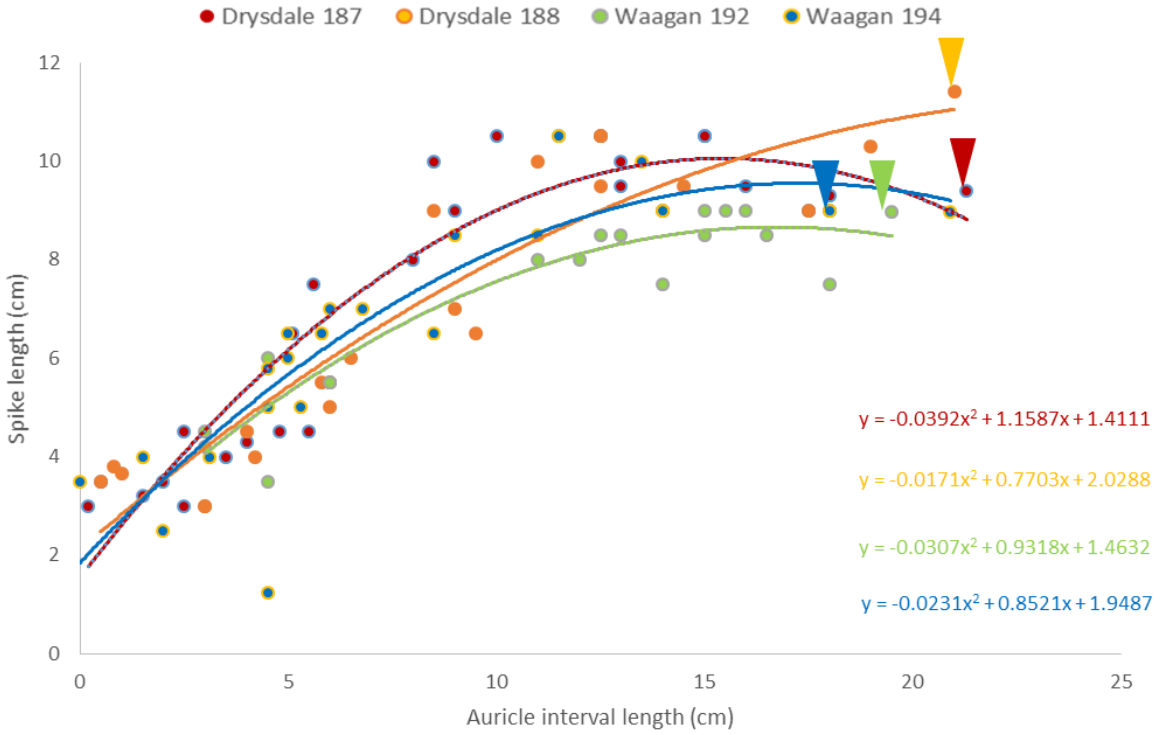

Figure 6. 2 Spike length plotted against auricle interval length for Waagan 192 and 194 (tolerant) and Drysdale 187 and 188 (intolerant) under control conditions. Triangles indicate measurements taken at maturity.

Pollen development stage was determined using anthers from the floret 1&2 positions in the spikelets, separately in the top, middle and bottom thirds of the spike. The data showed that at spike length 6.5 cm and 8 cm, meiosis in the middle part of the spike occurred earlier compared to the bottom and top part of the spike (Figure 6.3). This is consistent with the common knowledge that floret development begins first in the middle of the wheat spike, and then progresses upwards and downwards. In the middle of the spike, florets were at late meiosis (tetrad break up) stage when the spike was about 7 cm long and were at bi nucleate stage when the spike was 9.5 cm. Spikelets in the top and bottom of the spike reached these stages later than those from the middle of the spike. When spikes reached 9 cm length, meiosis was completed in all parts of the spike.

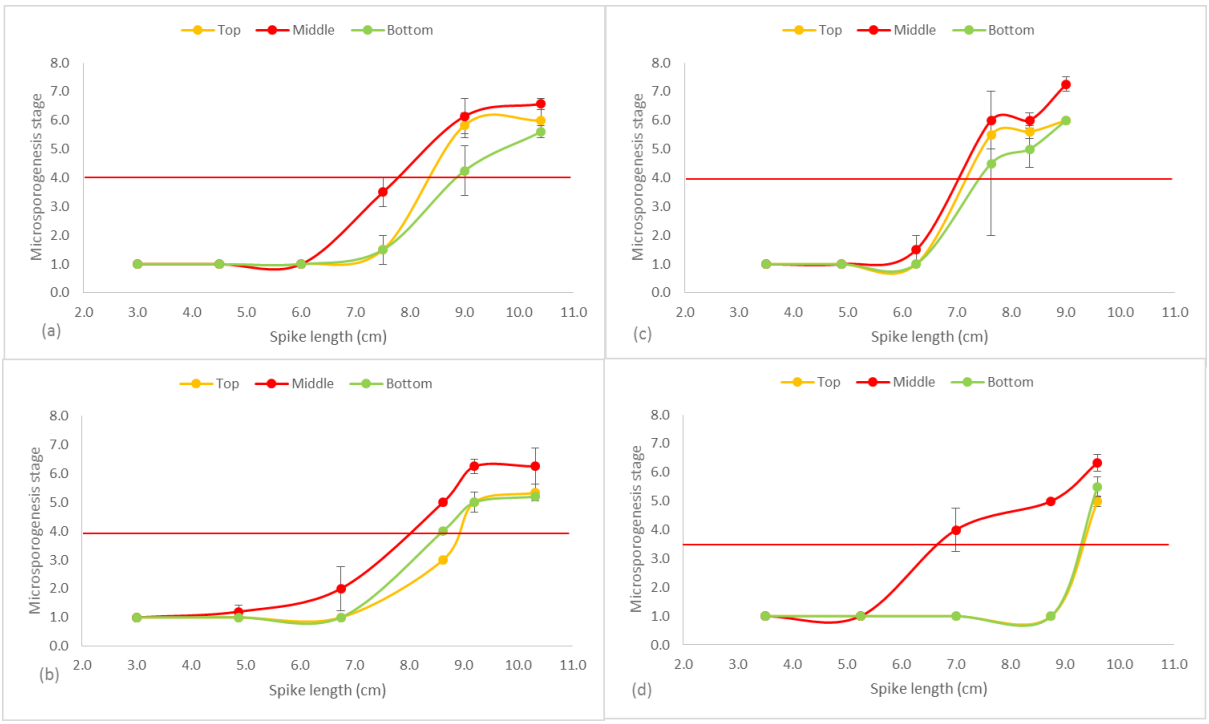

Figure 6. 3 Microsporogenesis stage vs. spike length. Data are based on the lowest two floret positions in spikelets from three positions within the spike (bottom, middle and top third). Shown are Drysdale selection 187 (a); Drysdale selection 188 (b); Waagan selection 192 (c) and Waagan selection 194 (d). Microsporogenesis stages are indicated by numbers 1: pre meiosis, 2: early meiosis, 3: meiosis, 4: tetrad, 5: early uninucleate, 6: late uninucleate, 7: after first pollen mitosis (bi nucleate stage), and 8: after second pollen mitosis (tri nucleate stage). The red horizontal lines indicate the end of meiosis at stage 4. The error bars indicate standard error.

Microsporogenesis stages at particular tiller stages as defined by AIL, are shown in Figure 6.4. As expected, the trend was for the middle of the spike to be more advanced in microsporogenesis than the top and bottom thirds, at a given AIL (Figure 6.4). Microsporogenesis stage for a given AIL was more advanced in Waagan than in Drysdale. For example, meiosis was reached in the middle part of the spike at around 6.5 and 8.0 cM AIL, in Waagan and Drysdale, respectively. Similarly, Waagan reached the early uninucleate stage at 9 to 11 AIL, while Drysdale reached it at 11 to 14 cm AIL. However, perhaps counter to this observation, Waagan took more time to reach meiosis from the sowing date than Drysdale (59 d vs. 54 d) (Chapter 4; Appendix table 6.2a).

6.4.2. Comparison of developmental stages most susceptible to heat induced floret sterility

Floret sterility resulting after heat treatment at the various stages (from 4d BAE to 13 cm AIL) are summarized in Figure 6.4 and Table 6.2. There was a significant treatment effect (heat vs. control) on GrNoSplt 1&2 across the whole spike, but not for GrNoSplt >2 across the entire spike. Treatment stage as indicated by AIL had an effect in both the 1&2 and >2 floret positions in the spikelets, across all three parts of the spike. Of the treatment stages, 9 cm AIL produced the greatest contrast in fertility between cvs. Waagan and Drysdale (with Waagan being most tolerant), particularly for florets 1&2 (Figure 6.4 and Table 6.2).

Among the fertility traits, only GrNoSplt.1&2.Bot and GrNoSplt.Spk exhibited a significant genotype by treatment-stage interaction effect (across both varieties; Table 6.2). In Drysdale, heat reduced fertility across all parts of the spike, for treatments beginning between 0 and 11 cm AIL (including non-significant trend for 0-1 cM AIL in Drysdale 187), while in Waagan effects of heat were observed in treatments beginning at 0 and 6 cm AIL (Figure 6.4). Heat reduced fertility more in Drysdale than Waagan, and the sterility in Waagan occurred mainly in the upper third of the spike. The highest sterility in Drysdale occurred for treatments beginning from 3-11 cm AIL, while in Waagan it tended to be for treatments beginning from 0 to 3 cm AIL, suggesting that Waagan became susceptible at shorter AILs than Drysdale.

In Drysdale 187, Drysdale 188 and Waagan 194, the data suggested two susceptibility peaks – an effect for the treatment beginning at 0 cm AIL, followed by less susceptibility for the treatment beginning at 1 cm AIL, followed by greater susceptibility for treatments beginning at 3 cm AIL and after.

Florets in the middle of the spike progressed through microsporogenesis earlier than those from the top and bottom parts of the spike (Figure 6.4), consistent with the known order of development of florets in the wheat spike. This difference was most extreme in selection Waagan 194. Florets from the middle of the spike were therefore expected to pass through the heat susceptible stage earlier than the florets from the bottom and top thirds of the spike. There was little evidence of such a trend, except in Drysdale 188, which showed greater sterility in the outer parts of the spike with the treatment applied at the end of the window of susceptibility (treatment beginning at 11 cm AIL) (Figure 6.4).

The ability to determine the microsporogenesis stage(s) sensitive to heat was limited by the following unkonwns: (a) the minimal time of heat treatment necessary to reduce pollen viability, and (b) whether high tempertures could affect the correspondence between AIL and microsporogenesis stage, since this relationship was only defined by sampling in plants that not been heat treated. Saini and Aspinall (1982a) reported that exposure of wheat plants to heat stress (30 °C) just for one day in the susceptible developmental window was sufficient to cause significant floret sterility. Taking the assumption that the effects of heat are instantaneous and that the AIL-stage relationship observed under control also held under heat, susceptibility in both Drysdale selections began at around 7 cm AIL (first peak) and intensified by 9 cm AIL (second peak). Susceptibility ceased before 13 cm AIL in Drysdale 187 and before 11 cm (in the middle of the spike) in Drysdale 188 (Figure 6.4). On this basis, sensitivity in Drysdale began at pre- to early- meiosis and finished by the late uninucleate microspore stage (Figures 6.4 and 6.5).

Stages that were most sensitive in Waagan were more difficult to determine due to the lower effect of heat on floret fertility (i.e., Waagan was classified as tolerant). Based mostly on sterility in the upper third of the spike, the window of heat sensitivity began at 0 cm AIL and ended before around 9 cm AIL (Figure 6.4). This corresponded to premeiosis to the late uninucleate microspore stage (Figure 6.5).

Comparing microsporogenesis stage to spike length (Figure 6.3) and to AIL (Figure 6.4), it can be seen that Drysdale was susceptible when its spike was 6.6 to 9.8 cm long. In Waagan, florets in the middle segment of the spike were sensitive when the spike length was from 2.5 to 7.8 cm.

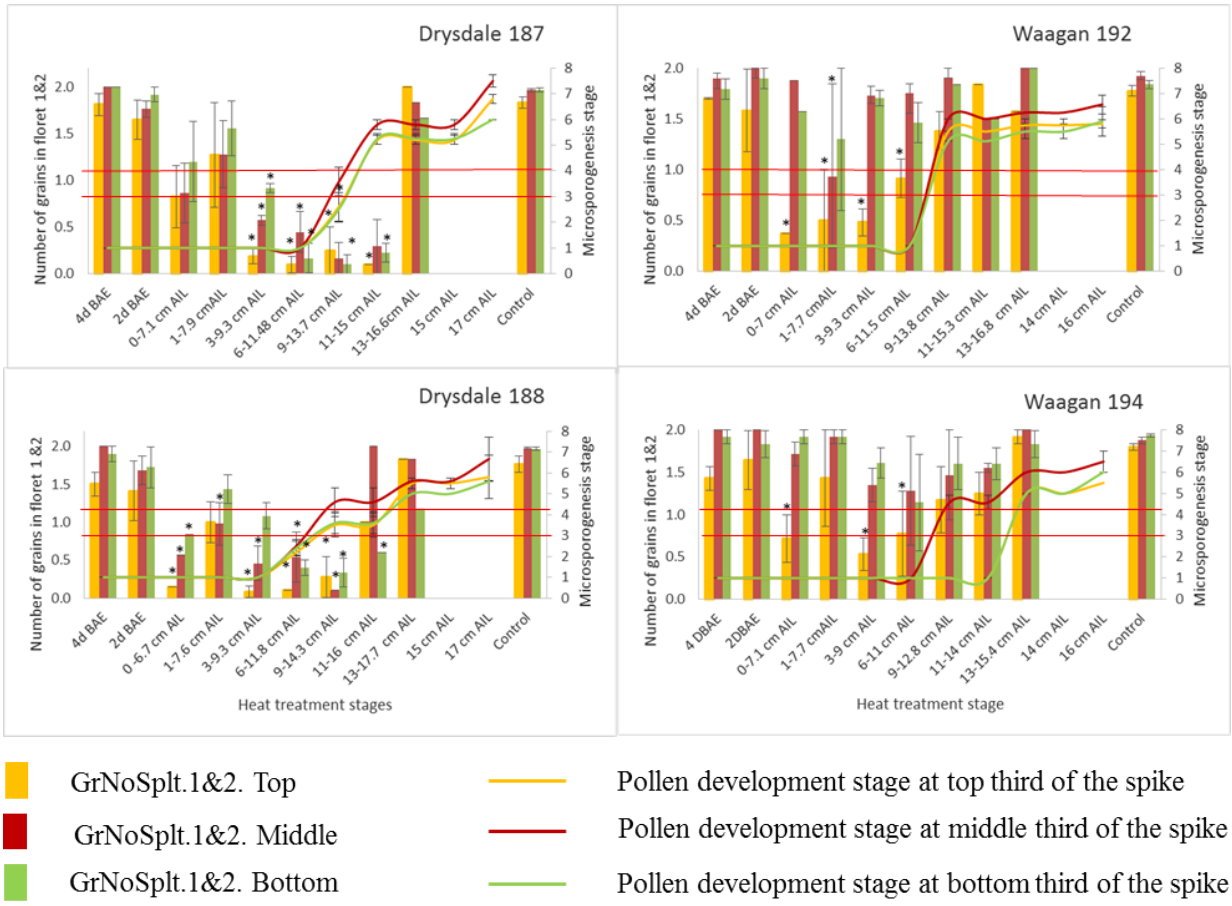

Figure 6. 4 Grain number at florets 1&2 after heat treatment at various tiller stages (auricle interval length, AIL, or days before auricle emergence, BAE), and microsporogenesis stages at the commencement of the heat treatment. Heat treatments were at 37/27 °C day/night for 3 d. Each point on the horizontal axis shows the range of AIL during the course of the heat treatment. Bars show number of grains set and lines indicate microsporogenesis stages; Microsporogenesis stages are indicated by numbers 1: pre meiosis, 2: early meiosis, 3: meiosis, 4: tetrad, 5: early uninucleate, 6: late uninucleate, 7: after first pollen mitosis (bi nucleate stage), and 8: after second pollen mitosis (tri nucleate stage). The red horizontal lines indicate the period of meiosis. Error bars are ± SE. Note that the green and yellow lines for Waagan 194 overlap for most of the stages.

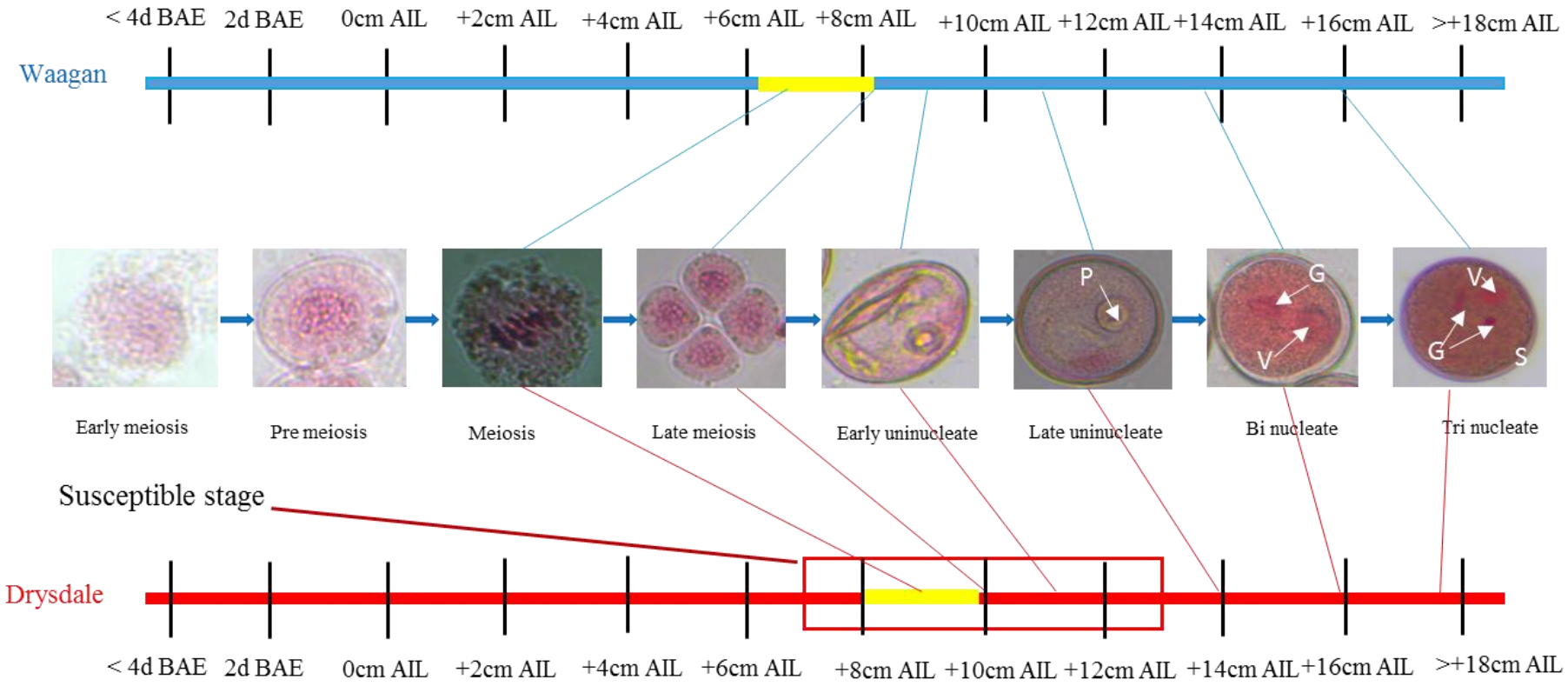

Figure 6. 5 Windows of heat induced floret sterility, and corresponding microsporogenesis stages, for the main stem in two Australian wheat cultivars Waagan and Drysdale, with respect to tiller developmental stage as defined by auricle interval length (AIL), or days before flag leaf auricle (BAE). Squashed anthers were stained with 2% acetocarmine. The period of meiosis is represented by the yellow bar. Abbreviations: P, germination pore; G, generative nuclei; V, vegetative nucleus

In general, microspores in the middle of the spikes in both Drysdale and Waagan were at tetrad stage while their spikes were about 7.3 cm. Waagan took around 58 days from sowing to reach this stage, while Drysdale took around 53 days. Both reached bi nucleate stage while their spikes were 9.3 cm long, which required 60 and 64 days from sowing for Drysdale and Waagan respectively. At this point (at 9.3 cm spike length), microspores located in the top and bottom segment of its spike were at late uni-nucleate stage. In Chapter 4, Drysdale and Waagan took 52 and 59 days from sowing to reach 3 cm AIL, respectively (Appendix table 4.2a).

6.4.3. Effects of treatment timing on final organ length, or gain in organ length from the commencement of treatment to maturity

The opportunity was taken to use the plants in the experiment to investigate how the timing of heat treatment could affect responses of various plant architectural traits measured at maturity.

In the analysis of variance, awn length, peduncle length and height (AwnL.Mat, PedL.Mat and Ht.Mat, respectively) showed significant genotype effects (across the four selections), while spike length and auricle interval length (SpkL.Mat and AIL.Mat, resepectively) showed no treatment effects (heat at all stages vs. control) (Table 6.2). PedL.Mat and Ht.Mat exhibited significant treatment effects, with heat at around 11-13 cm AIL significantly shortening PedL.Mat and Ht.Mat in both genotypes. Unexpectedly, treatments at later stages (6-11 cm AIL) tended to have a greater effect on AIL at maturity than those at earlier stages (4 d BAE to 3 cm AIL) (Table 6.2).

Table 6. 2 Means ± S.E. for traits measured in the nine stages of heat treatment in selections of Drysdale and Waagan genotypes. The highlighted values significantly differed ($p < 0.05$) from the value in control (treatment effect).

Genotype/selection	Trt/Stage	AwnL_Mat	SpL_Mat	AIL_Mat	PedL_Mat	Ht_Mat	GrNoSplt. 1&2.Top	GrNoSplt.>2.Top	GrNoSplt.1&2.Mid	GrNoSplt.>2.Mid	GrNoSplt.1&2.Bot	GrNoSplt.>2.Bot	GrNoSplt.Spk
Drysdale/187	-4	5±0	9.5±0.28	17.5±0.86	34.5±0.5	84.2±1.3	1.81±0.12	0.54±0.11	2±0	1.28±0.27	2±0	0.85±0.14	2.81±0.15
	-2	5.25±0.25	10±0	16.5±0.5	30.251.25	82±2	1.64±0.21	0.64±0.21	1.76±0.09	1.71±0.28	1.92±0.08	0.75±0.08	2.8±0.2
	0	5.3±0.44	9.6±0.44	15.3±0.66	31.1±0.83	82.3±2.94	0.82±0.33	0.40±0.21	0.86±0.32	1.45±0.31	1.2±0.43	1.3±0.25	1.99±0.41
	1	5.5±0.28	9.66±0.17	16.5±2.0	32.6±2.77	83.6±3.17	1.26±0.56	0.52±0.02	1.27±0.36	1.05±0.24	1.55±0.29	0.89±0.31	2.17±0.33
	3	5.75±0.14	9.63±0.24	15.6±1.02	28.7±1.58	79.6±3.69	0.18±0.08	0.36±0.17	0.57±0.05	1.17±0.19	0.91±0.05	0.97±0.09	1.37±0.15
	6	5.17±0.44	9.83±0.44	16.5±1.32	29.3±1.67	77.5±3.12	0.09±0.09	0.38±0.38	0.44±0.22	0.78±0.43	0.17±0.16	0.67±0.38	0.82±0.52
	9	6.75±0.25	9.5±0	15±0	28±0.5	76.5±1.5	0.25±0.25	0±0	0.17±0.17	0.33±0.17	0.1±0.1	0.1±0.1	0.32±0.20
	11	6±0.54	9.13±0.43	17.8±2.74	26.85±2.9	75.8±2.9	0±0	0±0	0.29±0.29	0.08±0.08	0.35±0.10	0.1±0.1	0.22±0.12
	13	6.5±0	10±0	19±0	27±0	80±0	2±0	0.57±0	1.83±0	1±0	1.67±0	0.33±0	2.47±0
	Control	5.67±0.15	9.39±0.21	21.3±0.44	34.6±0.95	92.5±1.83	1.83±0.06	0.38±0.06	1.96±0.02	1.16±0.12	1.96±0.02	0.86±0.09	2.72±0.09
Drysdale/188	-4	5.25±0.25	9.5±0.5	18.5±0.5	30.5±6.5	82±4	1.5±0.16	0.33±0.33	2±0	0.92±0.08	1.9±0.1	0.627±0.22	2.42±0.18
	-2	5.33±0.17	9.5±0.5	17.6±1.45	32.1±1.5	85±0.57	1.41±0.39	0.68±0.01	1.68±0.19	1.22±0.22	1.72±0.27	0.93±0.29	2.54±0.14
	0	5±0	10.5±0	17.5±0	30±0	78.5±0	0.14±0	0.43±0	0.57±0	2±0	0.83±0	1±0	1.65±0
	1	5.5±0.15	9.9±0.24	17.2±0.4	32.4±1.13	82.4±1.57	1±0.27	0.62±0.15	0.98±0.28	1.5±0.21	1.43±0.19	0.97±0.14	2.15±0.17
	3	5.37±0.31	9.37±0.31	16.7±0.75	30.2±1.45	81±2.34	0.08±0.08	0.20±0.08	0.46±0.23	1.25±0.33	1.08±0.17	1.3±0.09	1.44±0.22
	6	5.7±0.2	10.2±0.12	16.6±0.4	28.8±1.56	80.1±1.53	0±0	0.14±0.07	0.54±0.33	0.52±0.20	0.4±0.1	0.3±0.15	0.62±0.13
	9	5.33±0.17	5.83±2.68	15.3±1.36	30.1±2.1	77.6±3.17	0.28±0.27	0±0	0±0	0.44±0.36	0.33±0.19	0.39±0.24	0.47±0.13
	11	6.5±0	9.5±0	17±0	27±0	78±0	1±0	0±0	2±0	0±0	0.6±0	0±0	1.23±0
	13	6±0	10.5±0	19±0	29±0	82.5±0	1.83±0	0±0	1.83±0	0.67±0	1.16±0	0±0	1.83±0
	Control	5.97±0.20	11.4±2.14	21.0±0.38	33.5±2.07	92.8±1.78	1.76±0.11	0.38±0.06	1.97±0.01	1.21±0.10	1.96±0.02	0.93±0.08	2.74±0.09
Waagan/192	-4	5±0.28	8.83±0.17	19.3±1.36	30±1	82±3.62	1.69±0.01	0±0	1.89±0.05	0.04±0.04	1.79±0.10	0±0	1.81±0.01
	-2	4.75±0.25	8.5±0.5	16±2	25±3	73±7	1.58±0.41	0.07±0.07	2±0	0.42±0.42	1.9±0.1	0±0	1.99±0.35
	0	5±0	9±0	15.5±0	24±0	69±0	0.37±0	0.5±0	1.88±0	0.75±0	1.57±0	0.28±0	1.78±0
	1	4.75±0.75	9±0.5	17.7±0.25	25.7±2.25	70.5±6.5	0.5±0.5	0.13±0.125	0.93±0.92	0.439±0.43	1.3±0.7	0.21±0.21	1.13±0.96
	3	5±0.26	9±0.28	15.2±0.67	27.2±0.95	71.4±2.15	0.48±0.13	0.12±0.06	1.73±0.09	0.62±0.11	1.70±0.08	0.63±0.13	1.76±0.1
	6	5±0.28	8.83±0.17	14.5±0.28	27.6±1.17	74.3±0.44	0.91±0.19	0±0	1.75±0.09	0.33±0.26	1.46±0.2	0.27±0.28	1.56±0.24
	9	5.33±0.33	8.8±0.2	14.7±0.88	24.6±1.3	75±2.75	1.38±0.19	0.09±0.09	1.90±0.09	0.25±0.2	1.83±0	0.11±0.05	1.84±0.13
	11	5±0	9±0	17±0	26.5±0	73±0	1.83±0	0±0	1.5±0	1±0	1.5±0	0.67±0	2.16±0
	13	5.5±0	9.5±0	15±0	23±0	63±0	1.57±0	0±0	2±0	0.71±0	2±0	0±0	2.1±0
	Control	5.21±0.15	8.96±0.13	19.5±0.52	31.4±0.45	85.2±1.45	1.77±0.05	0.14±0.04	1.92±0.04	0.40±0.11	1.8±0.04	0.18±0.07	2.08±0.09
Waagan/194	-4	5±0	9.25±0.25	20.2±2.75	27.7±3.25	79.2±9.75	1.42±0.14	0.21±0.07	2±0	1±0	1.92±0.08	0.28±0.28	2.26±0.21
	-2	5±0	9.5±0	21.7±0.75	29.5±1.5	80.5±0.5	1.64±0.35	0±0	2±0	0.36±0.35	1.83±0.16	0±0	1.94±0.29
	0	5.5±0.5	9.25±0.25	19±1	28.7±1.25	79.5±0.5	0.71±0.28	0.07±0.07	1.71±0.14	0.43±0	1.92±0.08	0.58±0.08	1.77±0.07
	1	6.75±1.25	8.75±0.25	19.5±1.5	31.2±1.25	81.8±4.25	1.42±0.57	0±0	1.92±0.08	0.6±0.1	1.92±0.08	0.42±0.41	2.07±0.02
	3	5.08±0.15	8.91±0.27	15.4±1.17	27.3±0.99	74.8±3.18	0.53±0.19	0.02±0.02	1.34±0.20	0.62±0.2	1.61±0.18	0.53±0.13	1.52±0.25
	6	5.33±0.72	14.3±5.36	13±0.57	24.8±2.92	64.5±4.34	0.77±0.5	0±0	1.28±0.64	0.49±0.24	1.14±0.57	0.67±0.33	1.43±0.72
	9	5.16±0.6	8.5±0.57	13.6±1.01	24.1±0.92	62±1.5	1.17±0.39	0.38±0.38	1.47±0.53	0.83±0.37	1.6±0.31	0.72±0.23	2.05±0.68
	11	5.25±0.75	8.75±0.25	13±1	23.2±2.75	65±1.5	1.25±0.25	0.083±0.08	1.55±0.05	0±0	1.6±0.19	0±0	1.48±0.01
	13	6.5±0.5	8.75±0.75	18±0	24.7±1.25	75±2	1.91±0.08	0.14±0.14	2±0	0.41±0.41	1.83±0.16	0.5±0.5	2.25±0.42
	Control	6±0.16	8.96±0.14	20.9±1.02	29.5±1.52	85.2±1.25	1.79±0.04	0.07±0.03	1.87±0.04	0.77±0.06	1.93±0.02	0.28±0.07	2.23±0.05
Statistical test (significance level)	Replication										P<0.01		
	Genotypes (G)	P<0.05			P<0.001	P<0.001	P<0.05	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	
	Treatment (T)				P<0.05	P<0.01	P<0.001		P<0.001		P<0.001		P<0.001
	stage (S)			P<0.001	P<0.01	P<0.01	P<0.001	P<0.05	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
	GxS										P<0.01		P<0.05

6.5. Discussion

6.5.1. Staging methods

Architectural traits such as AIL may not be entirely accurate (transferrable between genotypes) for estimating spike developmental stage, because wheat genotypes may differ in their spike development relative to AIL. Indeed, assigning microsporogenesis stages to externally definable stages of the plant development (decimal growth stage) has been reported to be unreliable (Barber *et al.*, 2015). This factor was also addressed in Chapter 4, in relation to segregation of the semidwarf genes *Rht-B1* and *Rht-D1*, in the Drysdale × Waagan population. There is also plant to plant variation in the relationship between AIL and anther development stage.

The heat tolerant variety Waagan required longer from sowing to reach a particular AIL than Drysdale. Drysdale reached meiosis at 8 cm AIL, while Waagan reached it at about 6.5 cm AIL. The highest sterility in Drysdale occurred for treatments beginning from 3-11 cm AIL, while in Waagan it tended to be for treatments beginning from 0 to 3 cm AIL. These findings provided evidence that there were developmental differences between Drysdale and Waagan, which altered the relationship between AIL and spike developmental stage (microsporogenesis stage). Hence, external developmental features such as AIL are not entirely accurate as a means of identifying plants at a similar spike developmental stage (e.g. to determine when to apply heat treatments), among groups of different wheat genotypes.

The current study suggested that spike length as an indicator of microsporogenesis stage may be more transferrable between genotypes than AIL: Waagan and Drysdale showed similar spike length at a particular microsporogenesis stage (Figure 6.3), whereas Waagan and Drysdale differed for AIL at a particular microsporogenesis stage (Figure. 6.4). However, this trait requires destruction of the tiller, hence it is not very practical as a method to routinely stage plants for heat treatment.

Other wheat scientists sometimes judge the spike meiosis developmental stage by feeling the top of the boot, when the bulge of the top of the spike can be felt two finger widths below the flag leaf auricle (Margaret Pallotta and Ian Dundas, personal communication, Adelaide University). It is conceivable that this trait may be dependent on both boot length

(AIL) and spike length. As this method is non-destructive, it deserves to be tested for its accuracy relative to assessing the microsporogenesis stage of wheat tillers.

6.5.2. Floret position effects

There was some difference in sensitivity between florets from different positions within the spikelet, at a particular AIL. In the middle and bottom third part of the Drysdale spike, 11-13 cm AIL was the most susceptible stage for GrNoSplt.>2, while 9 cm was the most susceptible stage for GrNoSplt.1&2 (Table 6.2). These differences between florets in different positions within the spikelet is likely to be due to the fact that anther differentiation begins in the basal most florets of the spikelet and then proceeds up the spikelet (Bonnett (1936). Similarly, in the middle of the spike, meiosis started when the spikes were 6.5 cm long, and at 8 cm in the top and bottom thirds of the spike, consistent with the fact that spikelet differentiation begins in the middle of the wheat spike.

The variety Waagan is considered heat tolerant for floret fertility (Chapter 4), however, under the experimental conditions applied in this study, the top third of the spike showed some sterility after heat was applied at 0 to 6 cm AIL (Table 6.2). This was also the case in Chapter 4, where Waagan showed the greatest (and the only significant) heat induced sterility for the upper 3rd of the spike (for 3 cm AIL treatment, primary tillers, Table 4.2). Three QTLs for tolerance to heat induced floret sterility in the Drysdale × Waagan mapping population were detected in which Drysdale contributed the tolerance allele (QTL 2, 16 and 17). Of these, only QTL17 (likely to represent *Rht-D1*) showed strongest expression in the upper part of the spike, although this QTL seems likely to represent an escape artefact due to its co-location with *Rht-D1*, i.e., the locus affected the relationship between spike developmental stage and AIL due to its strong effects on plant architecture. Hence, the susceptibility seen in Waagan in the upper part of the spike did not appear to translate to a detected tolerance QTL effect.

The greatest susceptibily of Waagan in the upper part of the spike may also relate to observations made in Chapter 3, in which those hexaploid wheat and durum genotypes that showed some sterility under control conditions tended to show this sterility in the upper third of the spike, suggesting that this part of the spike was inherently less fertile and therefore perhaps more sensitive to sub-optimal growing conditions. It could also relate to the phenomenon observed in wheat crops referred to as ‘tipping’, whereby drought and/or heat

stress conditions around the reproductive stage cause sterility and/or shrivelling of the upper part of the spike (Alqudah and Schnurbusch, 2014; Fischer and Wood, 1979).

6.5.3. Staging for meiosis and the susceptible developmental window

In the middle of the spike, meiosis started when the spikes were 6.5 cm long, and at 8 cm in the top and bottom thirds of the spike. This finding was consistent with the study of Onyemaobi *et al.* (2016), who reported that 3 to 12 cm AIL in most of the wheat genotypes corresponded to meiosis. Onyemaobi *et al.* (2016), used 46 different wheat genotypes and observed in most of the genotypes that meiosis started from 3 cm AIL and concluded at 12 cm AIL. Ji *et al.* (2010), using four Australian commercial wheat varieties and six progeny of crosses between synthetic hexaploid wheats and Australian cultivars, reported that an AIL of 4 cm corresponded to the young microspore stage (early uninucleate).

Sensitivity in Drysdale began at around 7 cm AIL and ceased before 13 cm AIL (Figure 6.4, Figure 6.5). This corresponded to the young microspore (late uninucleate) stage. This was largely consistent with reported windows of susceptibility determined for cereals and other plant species. In various cereals and pulse crops, pre-anthesis (12 to 7d before anthesis) has been the most commonly reported stage to be the most sensitive for floret sterility (Singh *et al.*, 2012). The most sensitive developmental stages for heat induced sterility (failed seed set) in various crop species have been reported to be: for wheat, from the onset of meiosis in pollen microspores until the conclusion of tetrad break up and during the first pollen mitosis (Saini *et al.*, 1983); for cowpea, between 9 and 7 days before anthesis (after the release of tetrads) (Ahmed *et al.*, 1992); for bean (*Phaseolus vulgaris*), 7–12 days before anthesis (during microsporogenesis) (Porch and Jahn, 2001); for barley, during meiosis (Sakata *et al.*, 2000). Likewise, for fruit number effects in some horticultural crops: for tomato, between 10 and 7 days before anthesis (Pressman *et al.*, 2002; Sugiyama *et al.*, 1965); and for bell pepper, 4 days before anthesis (at microspore mother cell meiosis) (Erickson and Markhart, 2002).

The fact that fertility and pollen development stage was done on a separate set of plants (compared via the common trait of AIL) was a limitation for accurate determination of the sensitive stage of pollen development in cv. Drysdale in the current study. An improved estimate may be possible using the repeated anther sampling approach (Bennett *et al.*, 1971; Bennett *et al.*, 1974; Bennett and Smith, 1972; Draeger and Moore, 2017). In this method, a

window is cut out of the boot so that a single anther can be sampled for staging, the hole repaired, the plant heat treated, and then additional anther(s) sampled from the same floret to assess the effect of heat treatment. The advantage of this method lies in the fact that development of the three anthers of a floret are closely (although not perfectly) synchronous (Draeger and Moore, 2017).

Chapter 7: Mechanisms of heat-induced floret sterility tolerance and mode of expression of tolerance at the 2B locus

7.1. Abstract

A series of experiments were conducted to investigate mechanisms of heat-induced floret sterility tolerance and mode of expression of heat tolerance conferred by a QTL located on chromosome 2B. Two Drysdale × Waagan derived RIL lines that were heterogeneous for the 2B QTL and their parental lines were used to ascribe effects to the 2B locus. Staining of the nuclei with DAPI and pollen starch staining suggested that from the nine developmental stages analysed, heat stress reduced pollen starch in cv. Drysdale particularly when it was applied at 6 cm auricle interval length (AIL), indicating that heat stress disturbed carbohydrate metabolism in pollen grains. Incomplete dominance, tending towards dominance, was the mode of expression for heat-induced floret sterility tolerance at the 2B QTL, based on relative percentages of grain set and starchy pollen in heat treated plants of the three 2B QTL genotypic classes (homozygous tolerant, intolerant and heterozygous). Further, the mode of heat-induced floret sterility tolerance QTL expression was sporophytic (a result of gene expression in diploid cells). It was clear that the tolerance had a large effect on male reproduction, and crossing experiments also ruled out a similarly large effect on female reproduction. However, due to limitations in the crossing experiments, a small effect on female reproduction could not be ruled out. These results have implications for strategies to molecularly identify the 2B tolerance gene and to apply selection for heat tolerance in wheat breeding programs.

7.2. Introduction

The contrast in tolerance to heat induced floret sterility between wheat cvs. Waagan and Drysdale provides the opportunity to identify mechanisms of tolerance for heat induced floret sterility. Furthermore, the strong 2B QTL identified in Chapter 4, and RILs with residual heterozygosity for this chromosomal region identified in Chapter 5, provide the opportunity to examine mechanisms specifically relating to this QTL.

The male reproductive organs are considered to be the most heat sensitive reproductive structures in many crop species, including wheat Mark (2016); (Paupière *et al.*, 2014; Saini and Aspinall, 1982a), barley (Porch and Jahn, 2001), cowpea (Mutters *et al.*, 1989) and tomato

(Firon *et al.*, 2006). Pollen sterility is at least partly related to the availability of resources, i.e., starch filling of pollen grains (Firon *et al.*, 2006; Jain *et al.*, 2007; Prasad and Djanaguiraman, 2011; Pressman *et al.*, 2002). The presence and absence of starch in pollen can easily be assessed by staining anther squashes with iodine stain (I_2–KI) (Heidmann *et al.*, 2016; Lafleur *et al.*, 2015; Sheoran and Saini, 1996; Yang *et al.*, 2016).

Two distinct and successive developmental phases, microsporogenesis and microgametogenesis, lead to the production of the mature pollen grain. Microsporogenesis leads to the formation of the haploid unicellular microspores. Meiosis is immediately preceded by premeiotic interphase and begins with prophase, during which homologous chromosomes pair and recombine. Prophase can be further sub divided into the stages leptotene, zygotene, pachytene, diplotene and diakinesis (Bennett *et al.*, 1971). This is followed by metaphase I, then two cell divisions, resulting in four haploid daughter cells, also known as the microspores (Bennett *et al.*, 1973b; Bennett *et al.*, 1979).

Microgametogenesis comprises events in which the unicellular microspores develop into mature microgametophytes containing the gametes, i.e., the pollen grain with the two sperm cells. This phase begins with the expansion of the microspore. Then, the nucleus undergoes a first pollen mitosis (pollen mitosis I) which results in the formation of two unequal cells, a large vegetative cell and a small generative cell, each containing a haploid nucleus. The generative cell subsequently detaches from the pollen grain wall and is engulfed by the vegetative cell forming a unique 'cell within a cell' structure. The engulfed generative cell divides once more by mitosis (pollen mitosis II) to form the two sperm cells completely enclosed within the vegetative cell cytoplasm (tricellular pollen). Experiments in Chapter 6 and other previous studies (Alghabari *et al.*, 2014; Saini and Aspinall, 1982a) indicate that pollen meiosis and the early microspore stage is the susceptible stages to heat stress. A simple nuclear and chromosome stain with DAPI (4', 6-Diamidino-2-Phenylindole, Dihydrochloride) can be used to help determine pollen developmental stages (Schreiber *et al.*, 2004; Sepsi *et al.*, 2017; Tikhenko *et al.*, 2017; Vergne *et al.*, 1987).

The female reproductive organs in wheat have been reported to have some susceptibility to heat and drought stress. The female reproductive cells are more challenging to observe by microscopy than the male reproductive cells because they are deeply embedded within the ovule. However, female susceptibility can be tested by heat treating plants of

different heat tolerance levels and using them as female parents in crosses with non heat treated pollen donor plants.

As often referred to in research on self-incompatibility (Pandey, 1960), genetic effects on gametes can be classified as sporophytic or gametophytic. In sporophytic expression, the trait is determined by the genotype of the diploid parent, whereas in gametophytic expression, the trait is determined by the haploid genotype of the gametophyte (Forsthoefel and Vernon, 2011). If a significant part of the physiological processes controlling pollen development are under the control of the haploid gametophytic genome, it is to be expected that the genetic variability expressed in the pollen population of a single heterozygous plant would lead to important selection effects due to enhanced fitness of those pollen grains carrying tolerance alleles, increasing the frequency of the tolerance alleles in the next generation (Ottaviano *et al.*, 1988; Pfahler, 1975, 1983). Pollen development also requires the sporophytic tissue within the anther to contribute to the nutrition of the microspores, regulation of sporogenesis and pollen wall development (Scott *et al.*, 2004). The formation of microspores relies on the interaction of the microsporocytes with several types of somatic anther wall cells, including the tapetal cells (Ma *et al.*, 2008). Determining whether the 2B floret heat tolerance locus expression is gametophytic or sporophytic is important for defining breeding strategies and defining in which reproductive tissue (and at what time) the tolerance gene is expressed. The plan here is to heat treat heterozygotes of the 2B locus and measure the frequency of the tolerance (Waagan) 2B allele in the next generation. If the frequency is increased relative to that observed in progeny of non-heat-treated control heterozygotes, expression of the tolerance at the 2B locus must be sporophytic. If the expression is sporophytic, then it can be tested for dominance.

Since successful floret fertilization can result from only a small proportion of pollen grains being viable, percentage grain set may not be a very accurate way to quantify the effects of tolerance genes. Therefore, pollen starch staining was used to more directly quantify the effect of the 2B tolerance locus.

7.3 Materials and Methods

7.3.1 Plant materials

In addition to the cvs. Drysdale and Waagan, families segregating for the 2B heat tolerance QTL were used for this study. The latter represented the Drysdale × Waagan $F_{2:5}$ recombinant inbred line (RIL) families WW30647 and WW30784, as well as additional families derived by single plant selections within these families. WW30647 and WW30784 were heterogeneous for markers across the 2B QTL region (KASParMAS048, wsnp_JD_c3732_4781170, and wsnp_Ex_c944_1810245; Figure 5.4, Chapter 5) and were semi dwarf types (Figure 7.1).

7.3.2. Marker analysis

Six KASP markers spanning the chromosome 2B floret sterility heat tolerance QTL were used to follow segregation of the tolerance locus (KASParMAS048, wsnp_JD_c3732_4781170, AHW_DW_032, AHW_DW_037, AHW_DW_053 and AHW_DW_054) (Figure 7.1). Leaf samples were collected from two-week-old seedlings, stored at −80 °C until DNA was extracted as described in Chapter 5. DNA concentrations were determined by absorbance at 260 nm using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies) and diluted to 5ng/µL for KASP analysis.

7.3.3. Plant growth and heat treatments

The experiments were conducted in the Australian Plant Accelerator plant growth facilities (University of Adelaide, Waite Campus). Plants were grown individually in 8 cm wide pots, initially in a naturally lit greenhouse compartment under a non-stressed temperature range. The dates at which the experiments were conducted and conditions are shown in Appendix table Tables 3.1, 5.1, 6.1 and 7.1 and highlighted in the result section under each experiment.

Heat treatments were conducted similarly to the procedures described in Chapter 3. Briefly, heat was applied for 3 days in a growth chamber set at 37°C/27°C day/night, before moving the plants back to the greenhouse to complete development.

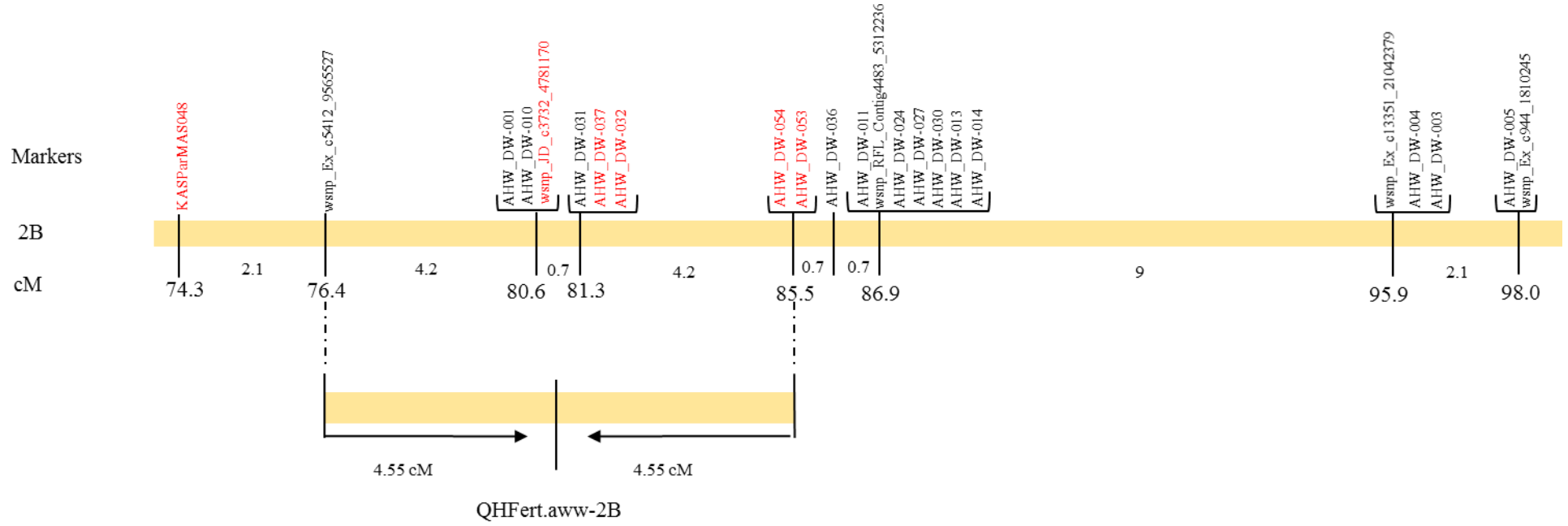

Figure 7. 1 Genetic map of the chromosome 2B heat-induced floret sterility tolerance QTL region from Chapter 5, with markers used in this study highlighted in red.

7.3.4. Pollen starch-staining

Florets from the middle part of wheat spike were removed just before dehiscence and put in 1.5 mL Eppendorf tubes containing 70% ethanol and stored in the dark at 4 ºC until further analysis. Sampling strategies for anthers and sample units for data analysis are described in the respective figure captions in Results. For microscopy, florets were dabbed with tissue to remove excess ethanol and extracted anthers were transferred to 1.5 mL Eppendorf tubes containing 40 mL of Lugol's solution (I_3K-potassium triiodide). This stain reacts with the coil structure of the polysaccharide starch, forming a dark-blue/black color.

The whole anther was crushed in the tube with a knitting needle and debris was removed using the knitting needle. A 20 µL volume of the solution containing the pollen was transferred to a microscope glass slide and covered with the cover slip, starting from one side and supported with forceps to avoid trapping air bubbles. The samples were observed under a compound microscope (LEICA DM1000) and an image of each sample was taken with a LEICA DFC 295 camera fitted on the microscope. Leica application suite (LAS V4.8) software was used to view the images, using settings 35% for brightness, 1 for saturation and 0.75 for gamma.

7.3.5. DNA staining

For staining of the pollen vegetative and sperm cell nuclei, DAPI was used (4'-6-Diamidino-2-phenylindole-2H Cl, Sigma Lot D9542-5MG, Batch: 035M4029V) which is stored at 4ºC as a powder. A stock solution was prepared by dissolving 1 mg of DAPI into 1 mL of distilled water with the help of a sonicator. A DAPI working solution was prepared by mixing 100 µL of DAPI stock solution with 100 mL of citrate-phosphate buffer, pH 4.0. The citrate-phosphate buffer was prepared by combining 19.3 mL of 0.2M dibasic sodium phosphate (Na_2HPO_4), 30.7 mL of 0.1 M citric acid and 50 mL of deionized water (Milli-Q). Finally 1 mL of Triton X-100 (Sigma) was added to the working solution (Vergne *et al.*, 1987). Anthers taken from the same spikelets that were used for starch staining, stored in 70% ethanol, were squashed using forceps under a Stereo dissecting microscope (Leica MZ6). Sample units for data collection are defined in the respective figure captions in Results. Anther debris were removed and pollen mixed with 20 µL of DAPI working solution and covered with a coverslip.

The samples were incubated in the dark for 5-10 min then visualized using a Nikon Optical Microscope with a DAPI epifluorescence filter set (UV, main wavelength 365 nm)

7.3.6. Crossing

Magnifying lenses mounted on a visor (OptiVISOR; Donegan Optical Company Inc) were used to assist during emasculation and pollination. Emasculation was performed when one-third of the spike had emerged from the flag leaf sheath, when anthers were still green. Spikelets at the very top and bottom of the spike were removed and discarded, as were florets from the third-and-above positions in the remaining spikelets. The top of the lemma and palea of the remaining florets were cut off and anthers then removed using forceps, taking care not to damage the stigmas. The spike was then covered with a translucent bag, folded tightly at the bottom onto the peduncle and fastened using a paper clip. About 4 to 6 days later, non-heat-stressed Waagan plants were used to obtain anthers immediately prior to dehiscence and used to pollinate the emasculated florets. Anthers were transferred using forceps and the pollen tipped over the florets. Pollination was done on three consecutive days to maximize the chance for successful pollination. Spikes were covered again, and at maturity the numbers of grain bearing florets and empty florets were counted.

7.4 Results

7.4.1 Experiments to identify manifestations of the Waagan vs. Drysdale tolerance difference in the pollen, and to more precisely define the susceptible tiller stage.

Two experiments were performed with the parent cvs. Waagan and Drydale with the objectives to (a) identify how the tolerance difference was manifested in the pollen (using iodine and DAPI staining of mature pollen), and (b) more precisely locate the susceptible window with respect to AIL stage. In both experiments, plants were heat treated at a range of tiller AIL stages and the pollen sampled and examined just prior to dehiscence. The design of both of these experiments was a randomized complete block design with 4 replications.

In the first experiment, plants were heat treated at 1 and 6 cm AIL and effects on pollen were examined using iodine staining. Plants were grown in a naturally lit greenhouse

compartment and the average temperature and relative humidity was as described in chapter 3 (Appendix table 3.1).7 The staining showed qualitative differences between pollen grains, allowing the general distinction of two classes: dark-blue/black starch-filled (starchy) pollen and yellow pollen, with no or less starch (non-starchy) (Figure 7.2e and f).

Counting of the pollen revealed that Drysdale plants showed a slightly reduced proportion of starchy pollen when heat treated at 1 cm AIL, and this effect drastically increased when heat was applied at the 6 cm AIL stage (Figure 7.3). Waagan plants showed a non-significant decrease (33%) in the proportion of starchy pollen when heat was applied at 1 cm AIL, and was unaffected by the heat treatment applied at 6 cm AIL (Figure 7.3). This result indicated that the tolerance difference between Waagan and Drydale was associated with a reduced proportion of starchy pollen in heat-treated plants of the susceptible variety Drysdale, and that susceptibility to this effect started at 1 cm AIL and increased until 6 cm AIL, which was the final developmental stage assessed in this experiment.

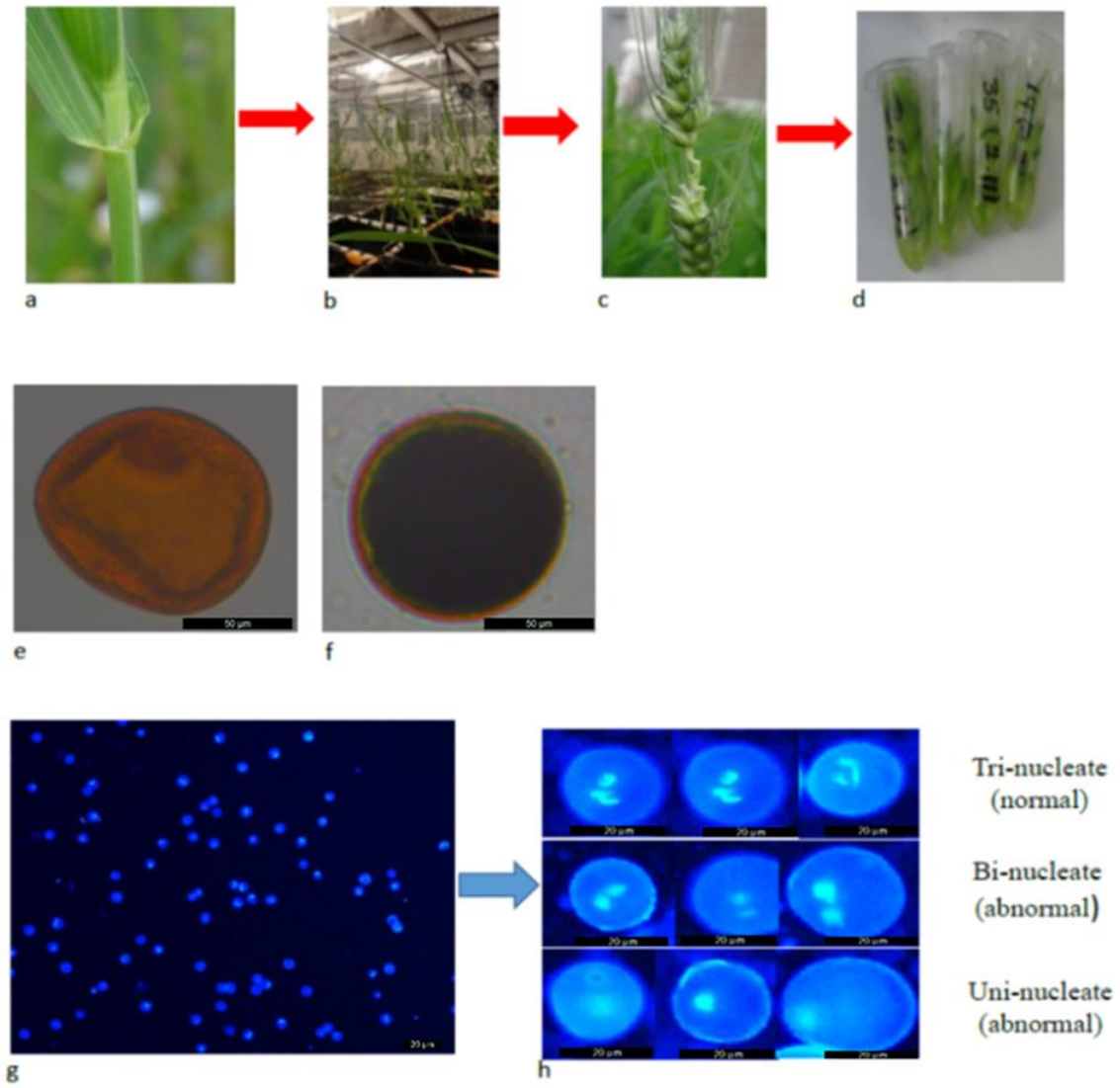

Figure 7. 2 Illustration of procedures used to investigate the effects of heat treatment and genotype on pollen starch or number of nuclei in mature pollen. a, plant in the greenhouse ready for heat treatment; b, plants in a growth chamber undergoing the 3 d heat treatment; c, sampling of spikelets from the central part of the spike, which were stored in 70% ethanol in Eppendorf tubes in the dark at 4 ºC, prior to staining for starch or chromatin; or squashed anthers were stained for starch using Lugol's iodine stain directly after collection (not shown); Drysdale and Waagan pollen stained for starch (e and f respectively); g. Pollen grains stained with DAPI; h, close up of selected pollen grains in g, showing three examples of each, of tri-nucleate pollen, binucleate and uninucleate pollen.

As 1 cm AIL was the earliest heat treatment stage used in the above first experiment, and as Waagan appeared to show some susceptibility at this stage, another experiment was performed to assess the effect of heat on pollen starch filling at earlier developmental stages (Figure 7.4). In addition, DAPI stain was used in this experiment to investigate effects of heat on pollen nuclear division. To determine the time required from sowing to auricle appearance, twelve plants each for Waagan and Drysdale were sown two weeks before the main experiment. This information was then used to determine the sampling time points (days after sowing) for the before auricle emergence (BAE) stages in the main experiment.

Plants were initially grown in a naturally lit greenhouse compartment, where the average temperature and relative humidity was recorded as 24/20 ºC and 59/67 % day/night respectively (Appendix table 7.1). Temperature was 24/20 day /night and 4 days over 30 °C around booting stage (heat treatment stage) due to high outside temperatures.

In this experiment, no significant reductions in the proportion of pollen grains containing starch were observed in Waagan, for any of the heat treatment stages, which ranged from 10 d BAE to 6 cm AIL (Figure 7.4). In contrast and in agreement with the first experiment, Drysdale showed a peak in susceptibility at 6 cm AIL. Hence, Waagan can be considered tolerant of the effects of heat on pollen starch filling at all analysed developmental stages. As pollen grains lacking starch would be expected to be non-viable, failure of pollen grains to fill with starch was identified here as a likely reason why Drysdale was more prone to heat induced floret sterility than Waagan.

7.4.2. Determining the effect of heat on pollen mitosis

In developing anthers, each microsporocyte undergoes meiosis to produce four haploid microspores. These undergo two rounds of mitosis to produce pollen grains containing three nuclei - two generative nuclei (that form the two sperm cells) and one vegetative nucleus.

In wheat, both rounds of mitosis complete by the time that pollen matures (at anther dehiscence) (Kihara and Hori, 1966). To test whether heat susceptibility of cv. Drysdale involves disruption of these mitotic divisions, mature pollen from the plants in the second aforementioned experiment were sampled just prior to anther dehiscence and nuclei stained with DAPI.

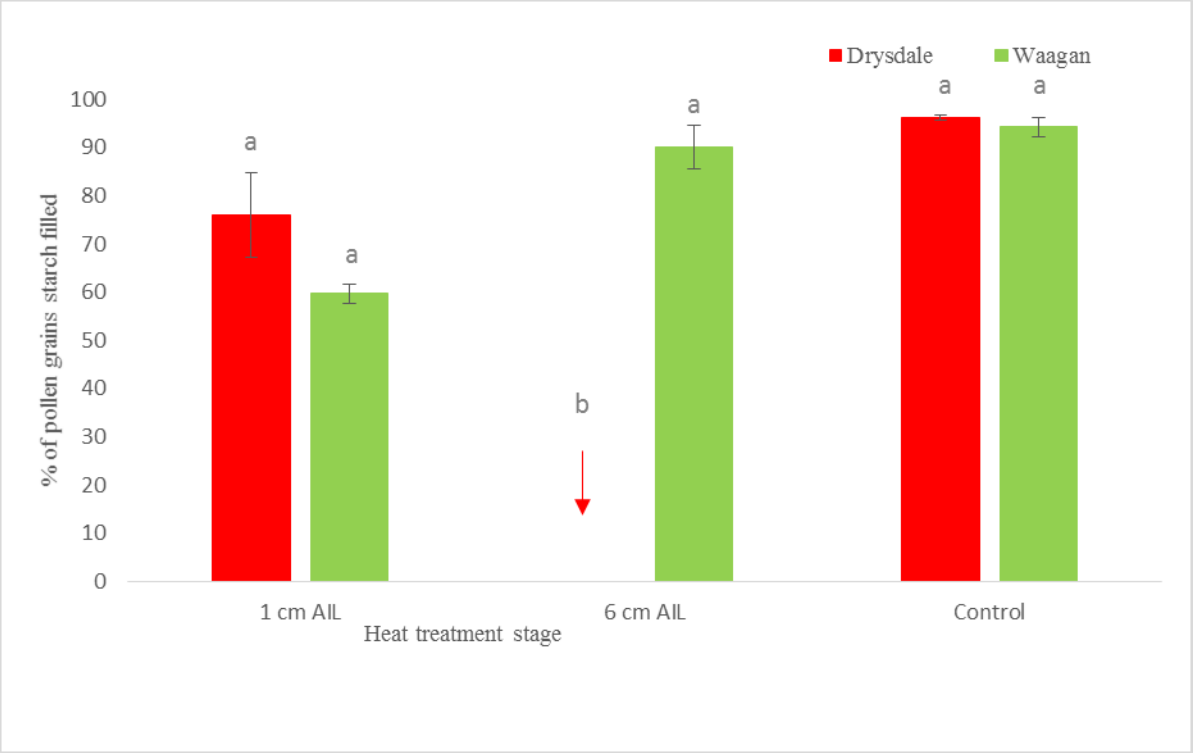

Figure 7. 3 Effects of heat treatment at two developmental stages (based on the main stem) on the proportion of starchy pollen grain in two wheat cvs. Drysdale and Waagan. Means with same letter above the bars were not significantly different at $p > 0.05$ (t-statistics). All examined pollen grains from Drysdale plants that were heat stressed at 6 cm AIL lacked starch.

For all genotype × treatment combinations, roughly three-quarters of the pollen were found to be 'normal', in that they contained three visible nuclei. Of the remaining ('defective') pollen grains, roughly two-third were binucleate and one third was uninucleate (Figure 7.2h, Figure 7.4b; Additional file table 7.3). There was no significant effect of the heat treatment relative to the control for treatments applied at any stage (from 10 d BAE to 6 cm AIL), or of the genotype, on the proportion of pollen with < 3 nuclei. Thus, no evidence was obtained that heat treatment affected nuclear divisions in pollen, or that such an effect was involved in the differential heat susceptibility of cvs. Drysdale and Waagan. This data therefore suggests that the main effect of heat stress is on pollen starch filling, rather than pollen meiosis or mitosis.

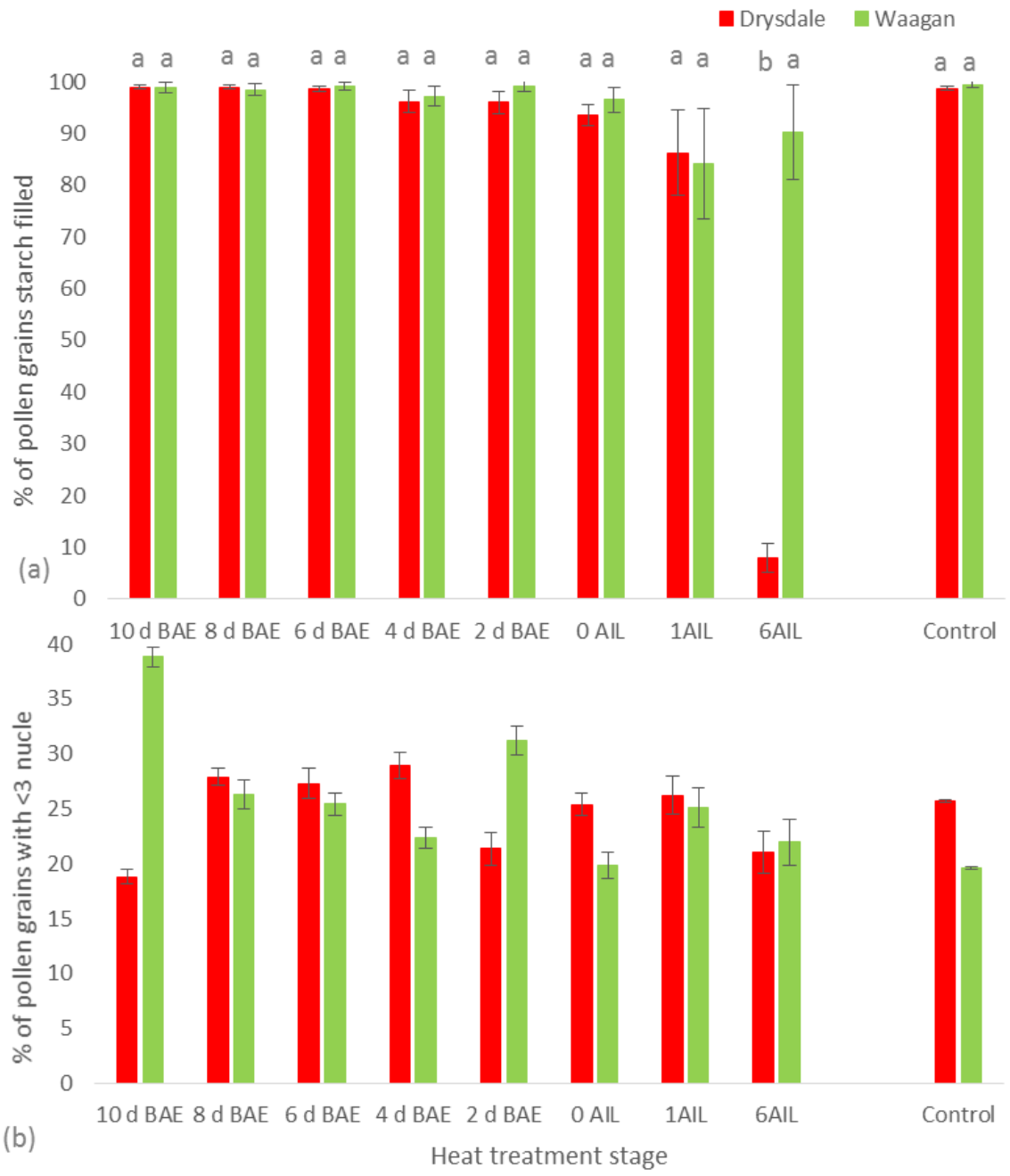

Figure 7. 4 Effects of heat treatment at various developmental stages (based on the main stem) on the proportion of pollen grains containing starch (a), and the proportion of pollen containing < 3 nuclei (b), in two wheat varieties Drysdale and Waagan. Means with the same letter were not significantly different within and across treatment stages (t-tests). Absence of letters in (b) indicates that there were no significant treatment differences within a given stage. The error bar indicates standard error. For starch analysis and DAPI staining, one anther was sampled from a floret from one of the lower two florets in the spikelet, from the middle of the spike, per plant, and the score for that anther was treated as the sampling unit for statistical analysis.

7.4.3. Other traits affected by heat stress applied during booting stage

To derive additional information from the second experiment, various other plant traits were measured to investigate their responses to heat and how these were influenced by the timing of heat treatment. These traits comprised: days from sowing to anthesis (Day.Sow to Anth), AIL at anthesis (AIL Anth) and peduncle length at anthesis (PdL. Anth).

Table 7. 1 Mean ±standard error of traits in plants exposed to a 3-day heat treatment at various developmental stages.

Heat treatment stage	**Day.Sow to Anth**		**AIL. Anth**		**PdL. Anth**	
	Drysdale	Waagan	Drysdale	Waagan	Drysdale	Waagan
Control	57.3±0.87	62±0.91	18.3±0.24	17.8±0.14	23±0.4	23.1±0.37
10 d BAE [a]	58±1.2	62±0.87	22.1±0.13	19.4±0.43	22.3±1.3	21.9±1.1
8 d BAE	58±0.29	59±0.48	21.8±0.78	18±0.54	19.4±0.85	21.1±0.66
6 d BAE	58±0.89	62±0.64	19.6±1.2	19.4±0.85	23.4±1.7	22.5±1.7
4 d BAE	59±1.38	61±0.85	17.6±0.83	18±0.41	20.4±1.2	20.8±0.43
2 d BAE	59±0	61±0	17±1.1	18.3±0.32	23.5±0.26	21.1±1.6
0 cm AIL	58±0	61±0.75	17.1±1.3	16.7±0.17	21.1±1.3	18.8±0.63
1 cm AIL	59±0.71	62±1.5	16±0.82	15.8±1.2	21±0.96	22±1.1
6 cm AIL	56±0.29	62±0.48	17.8±0.63	16.3±0.25	22.4±1.2	19.9±1.1
Genotype [b]	***		*		ns	
Stage [c]	ns		***		ns	

[a], d BAE, days before flag leaf auricle emergence; Day.Sow to Anth: days from sowing to anthesis; AIL Anth: auricle interval length at anthesis; and PdL. Anth: peduncle length at anthesis; * and *** indicate significant difference in ANOVA between Waagan and Drysdale ([b]) and significant effects of heat-treatment stages ([c]) at $p < 0.05$ and $p < 0.001$, respectively; and [ns], indicates non-significant difference.

For anthesis date, there was no overall effect of the stage of heat treatment, however, there was a genotype effect with only Drysdale tending to respond to the heat treatment with delayed anthesis (Table 7.1). In both genotypes, AIL at anthesis was dependent on the stage when heat was applied, with treatments applied earlier in development tending to increase it and those applied later tending to reduce AIL (Table 7.1). There was also a genotype effect on AIL at anthesis, with Drysdale tending to have a longer AIL at anthesis after most of the treatments. Peduncle length at anthesis was not significantly affected by the stage of heat

treatment nor genotype, although there was a trend was the peduncles to be shorter in the heat treated plants.

7.4.4. Mode of expression of heat-induced floret sterility tolerance at the 2B locus

Experiments were conducted to investigate the mode of expression of the major heat-induced floret sterility tolerance QTL detected on chromosome 2B in the Drysdale × Waagan DH mapping population. In the previous section of this chapter, 6 cm AIL was identified as a sensitive stage in Drysdale, hence heat treatments were applied at this stage. Staining of pollen for starch was again used to assess mode of the tolerance at the level of the individual pollen grains.

Experiments using Drysdale × Waagan RILs WW30647 and WW30784 to determine dominance of expression at the 2B tolerance locus

An initial experiment to investigate dominance of expression of tolerance at the 2B locus was carried out using field-multiplied seed of two Drysdale × Waagan $F_{2:5}$ RILs WW30647 and WW30784, which had been identified in Chapter 5 as being heterogeneous across the 2B tolerance locus, and showing strong (WW30647) and weak (WW30784) tolerance association with markers in individual plants (Figure 5.5). Seeds were sown (91 per line) and the plants genotyped for the six markers covering the QTL that are indicated in Figure 7.1. Information on the two markers AHW_DW_037 and AHW_DW_053 were not presented, as they segregated with markers AHW_DW_032 and AHW_DW_054, respectively. Marker alleles derived from the respective parents tended to segregate together (i.e., in coupling phase) in both the WW30647 and WW30784 RILs (Figure 5.5, Chapter 5), allowing the classification of plants as homozygous Waagan or Drydsale allele or heterozygous at the 2B tolerance locus. Plants resulting from recombination in the interval were not used. Two thirds of the plants were heat treated at 6 cm AIL and one-third was used as control, and the plants were scored for floret fertility at maturity.

Plants were initially grown in a naturally lit greenhouse compartment, where the average temperature and relative humidity was recorded as 25/21 ºC and 56/65 % day/night respectively (Appendix table 5.1). Temperature was 24.6/20.2 day /night and 7 days over 30 °C around booting stage (heat treatment stage) due to high outside temperatures.

Plants from the two RIL families both showed similar high fertility under control conditions, but after heat, family WW30647 showed greater heat induced floret sterility than family WW30784 (Figure 7.5). This was also observed in Chapter 5 and might be explained by allelic differences in the backgrounds of the two RIL families at additional, minor tolerance loci. For all markers and in each family, the homozygous Drysdale (DD) class of plants showed significantly lower fertility under heat than the homozygous Waagan (WW) class, whereas neither homozygous classes showed significant fertility differences from the heterozygous (DW) class. Hence, these data suggesting an intermediate tolerance level in the heterozygotes, suggested that tolerance at the 2B locus was expressed in an incompletely dominant manner. Incompletely dominant means that, in the heterozygote, the tolerance allele is able to be expressed in the presence of the intolerance allele but not to the same level that it is in homozygous tolerant individuals.

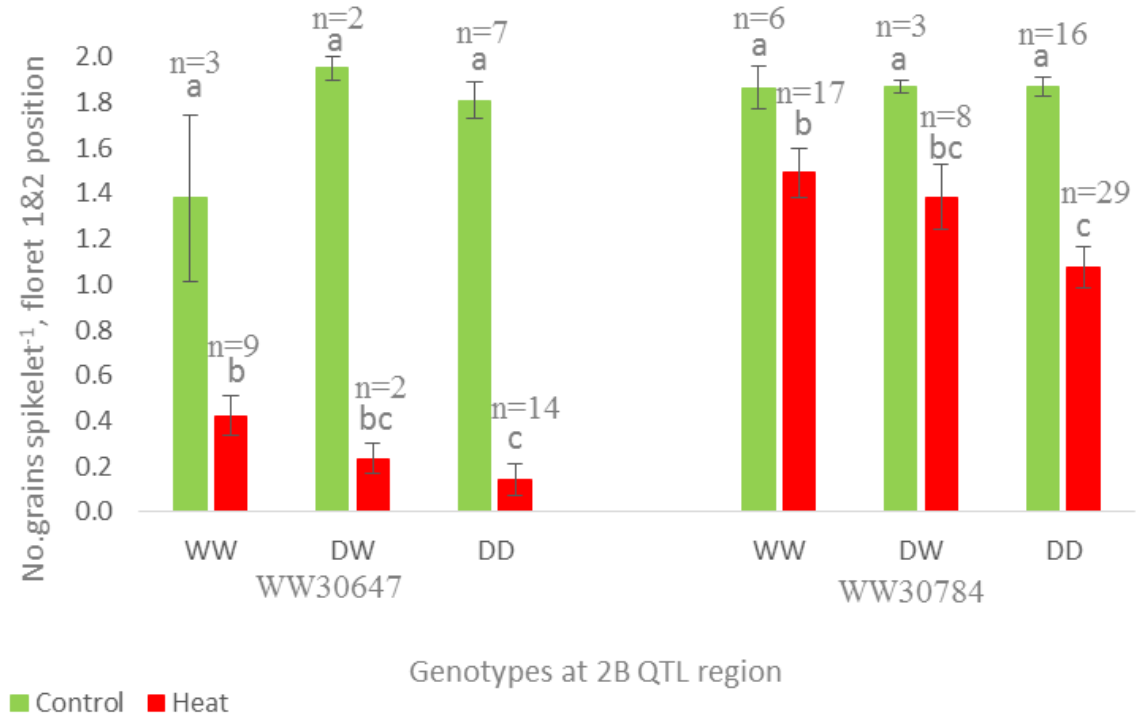

Figure 7. 5 Mean and SE of grains per spikelet at floret 1&2 positions, in the middle and bottom third of the main stem spike (combined), under heat and control condition, for plants from RIL families WW30647 and WW30784. Plants were classified using molecular markers as being homozygous for Waagan or Drysdale alleles or heterozygous (WW, DD and DW, respectively), at the 2B tolerance locus. The same letter code indicates means that were not significantly different at $p > 0.05$ (t-test) for comparisons within a family. The number (n) of plants of each genotype class that were sampled are also shown.

Using Drysdale × Waagan RILs WW30647 and WW30784 to test for gametophytic or sporophytic expression of the 2B tolerance locus.

Progeny of heterozygous plants from the aforementioned experiment were used to test if the 2B tolerance was the result of expression in haploid cells (gametophytic expression) or diploid cells (sporophytic expression). If the heat tolerance at the 2B QTL exerts its effect through expression in the haploid reproductive cells (i.e., after the first meiotic division, either on the male or female side), one would expect heat treatment of heterozygotes at the 2B locus to enrich for the Waagan (tolerance) allele in their progeny.

In total, ten Drysdale × Waagan RIL plants that were heterozygous across all six of the marker loci were used: two heat treated plants and one control plant from the WW30647 family, and five heat treated plants and two control plants from the WW30784 family. Seed resulting from self-pollination of these plants were sown (43 and 100 from the WW30647 and WW30784 families, respectively) and the resulting plants were analysed with the markers to classify each plant for its genotype at the 2B tolerance locus (homozygous Waagan or Drysdale allele or heterozygous); plants resulting from recombination in the interval were not used. The percent frequency of the Waagan allele in the progeny was calculated as: [number of heterozygotes + (2× number of homozygous Waagan plants)] × 100 /(the total number of plants × 2). Drysdale marker allele frequency was not presented but is simply 100 minus the Waagan allele frequency.

No enrichment of the tolerance (Waagan) allele was observed in the progeny as a result of heat treatment of the heterozygous parents (Figure 7.6). In fact, the Waagan allele was significantly depleted in the two WW30647 sub-families derived from heat treated heterozygotes, although the frequencies of alleles were not significantly different in these sub-families compared to the progeny of non-heated plants within the WW30647 family. This indicated the tolerance was not the result of gene expression in the haploid gametophytic cells, but instead, it must be due to gene expression in the diploid sporophytic cells influencing the viability of the gametes.

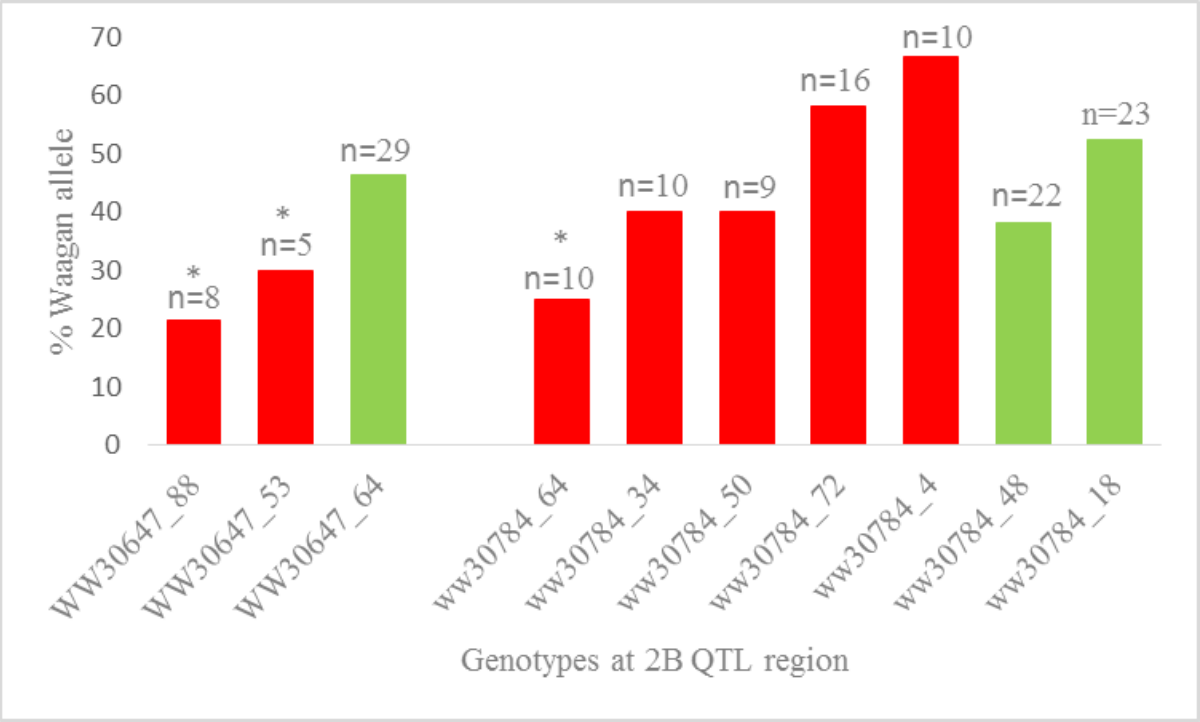

Figure 7. 6 Frequency of Waagan alleles at the 2B heat tolerance QTL region, in progeny of heat treated plants heterozygous for the 2B QTL region (resulting from self-pollination), from two Drysdale × Waagan RIL families, WW30647 and WW30784. Means for progeny of control plants (green bars), and for progeny of plants heat treated at 6 cm AIL (red bars), are shown. n= number of individual plants in each sub family. * Number of Waagan alleles was significantly fewer than that of Drysdale alleles within the sub-family ($p<0.05$, t-test). Allele frequencies were not significantly different between progeny of heat treated plants, compared to progeny of control plants, for all pairwise heat-control sub-family comparisons within a family ($p > 0.05$, t-tests).

Experiments using Drysdale × Waagan single-plant derived RILs WW30647_64, WW30784_18 and WW30784_48 – dominance at the level of pollen grains and grain set, and tests for effects on female reproduction.

Seed collected from three non-heated 2B-heterozygous control plants from the aforementioned experiment (WW30647_64, WW30784_18 and WW30784_48; Fig 7.6) were used for further experiments. One subset of progeny plants was used for both grain set and pollen starch analysis, while the other subset was used as female parents in a crossing experiment (Table 7.2).

Molecular marker and heat tolerance data from the progeny of these plants revealed that plant WW30647_64 was the result of two single recombination events within the 2B QTL region; the family was still segregating for tolerance and the phases of the tolerance-marker allele associations were determined (Figure 7.7). Plants WW30784_18 and WW30784_48 each carried two non-recombinant (parental) chromosomes. With this knowledge, the genotype of each progeny plant with respect to the 2B tolerance locus (WW, DD or DW) were defined from the marker data; plants resulting from (further) recombination were not used. Unfortunately, due to limited seed, the numbers of plants used in these experiments was lower than desired, and not enough plants were available to have non-heat treated controls for the crossing experiment.

In this experiment, plants were initially grown in a naturally lit greenhouse compartment, where the average temperature and relative humidity was as described in chapter 6 and appendix table 6.1. Temperature was 23/19 ºC day /night around booting stage (during the heat treatment stage).

Table 7. 2 Numbers of plants of each marker genotype class at the 2B heat tolerance locus, from three single-plant-derived Drysdale × Waagan RIL families, used in experiments to investigate modes of expression of the 2B locus.

Sub family	**Genotype**	**Used as female crossing parent**	**Floret fertility and pollen starch**	
		Heat	Heat	Control
WW30647_64	DD	3	2	2
	DW	2	2	1
	WW	2	1	1
WW30784_18	DD	2	2	2
	DW	2	2	1
	WW	3	2	2
WW30784_48	DD	3	3	2
	DW	3	3	3
	WW	2	1	1

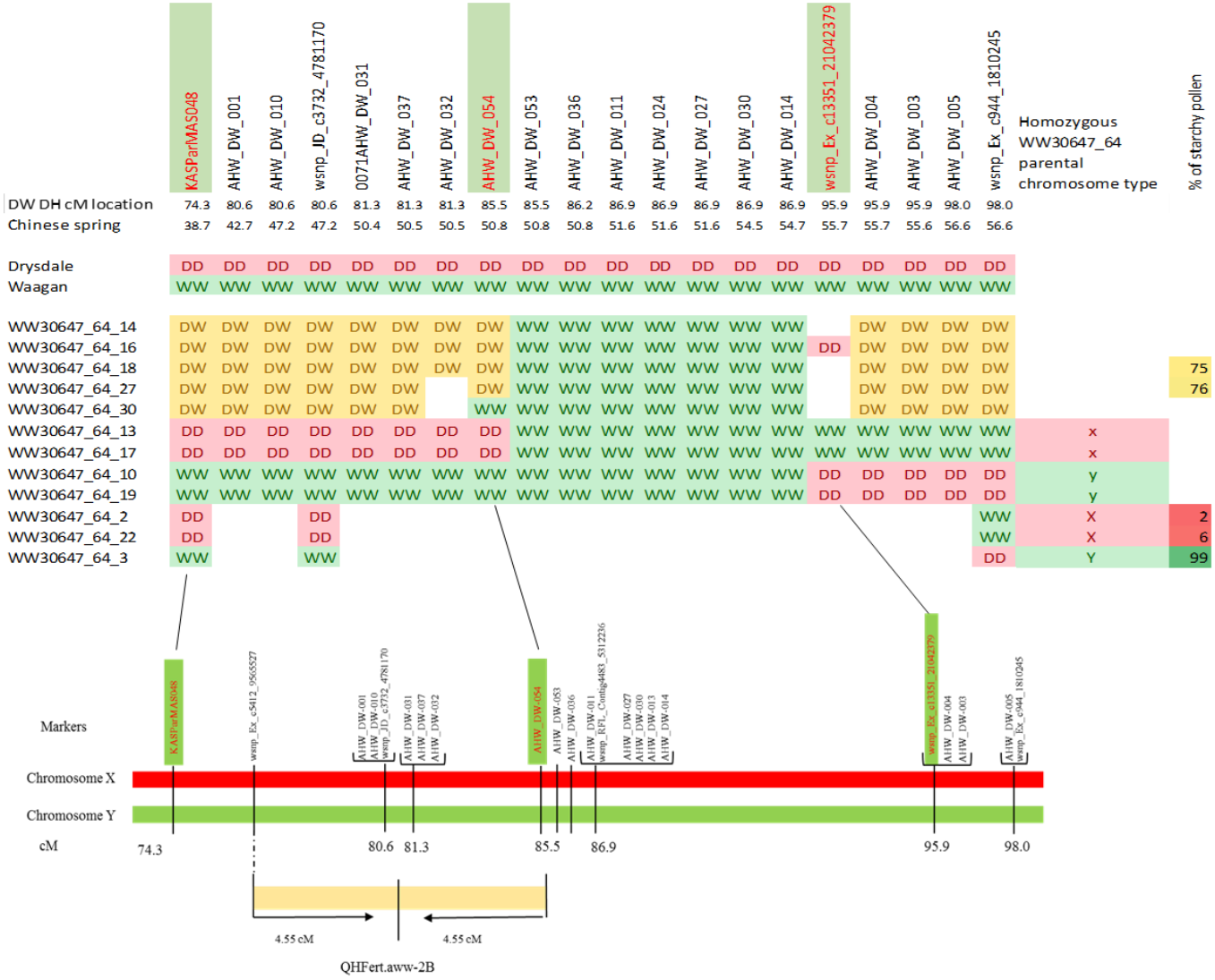

Figure 7. 7 Data establishing the makeup of the two recombinant 2B chromosomes present in plant WW30647_64. The data on the right shows the pollen starch from heat treated plants used in the experiment described in this chapter. The nine plants at the top were genotyped for all new KASP makers from Chapter 5 (exercise not otherwise described in this thesis) showing that the family segregated for two recombinant chromosomes, herein defined as X and Y type. Red and green denote Drysdale and Waagan segments/alleles, respectively. Comparison of the phenotype and genotype data showed that the X and Y chromosomes carried Drysdale and Waagan alleles at the 2B tolerance locus, respectively. The markers highlighted in green on the genetic map from Chapter 5 were used to follow the inheritance of the X- and Y-type chromosomes in the study described in the current chapter.

For the ‘seed-set/pollen starch’ experiment, the marker genotyped plants were heat treated at 6 cm AIL (or left as control). Just prior to anther dehiscence, anthers were sampled for starch analysis as previously described, and the same plants were also analysed for seed set at maturity. The florets that had anthers removed for pollen analysis were not used in the quantification of seed set at maturity.

The proportion of pollen that was starchy did not significantly differ between heat treated WW and DW progeny plants, but in DD plants it was drastically and significantly reduced compared to the other two classes of plants (Figure 7.8a). This confirmed that the difference in pollen starch observed in heat treated plants of cvs. Waagan and Drysdale (Figure 7.3 and 7.4) was at least partly (and probably mainly) attributable to the effect of the 2B heat tolerance locus, and that the level of heat induced pollen inviability was the basis for the tolerance effect at this locus. Furthermore, the data showed that the dominance of the 2B tolerance was manifested at the level of the pollen grains. This could not be completely addressed using grain set data, because close to 100% fertility may be achievable with only a small proportion of pollen being viable, potentially masking incomplete dominance at the level of the pollen when using seed set as a read-out.

The pattern of seed set in this experiment was also consistent with a dominant mode of expression of the 2B locus tolerance (Figure 7.8b). The trends were also consistent with the idea that the WW30647 family was more prone to sterility than the WW30784 family – as observed in the other experiments - due to possible genetic background effects. However, overall spikelet fertility of the WW30647 family was higher than in the previous experiment (Figure 7.5).

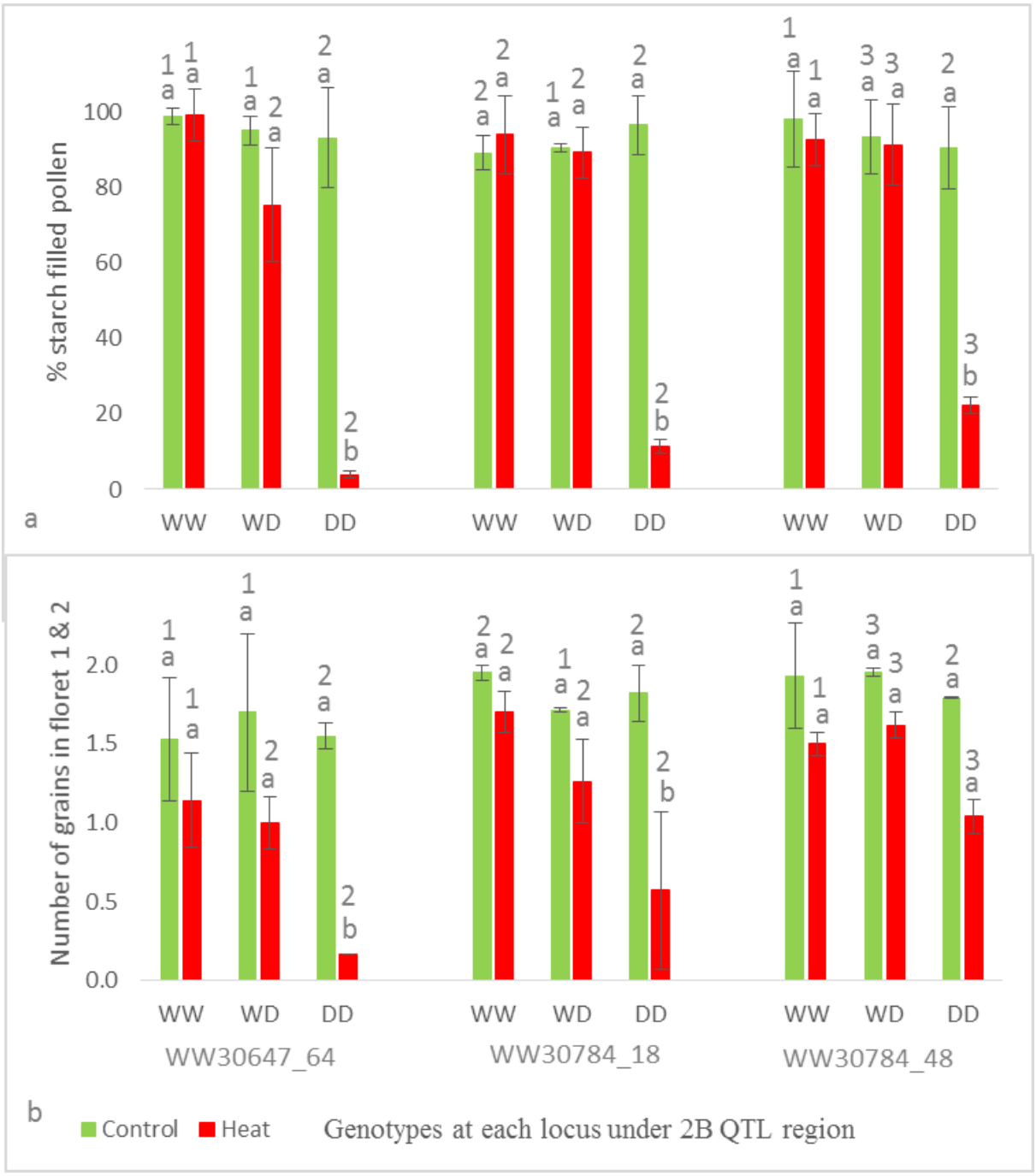

Figure 7. 8 Percent starchy pollen grains (a) and grains per spikelet at floret 1&2 positions (b), in control and heat treated plants of three genotypic classes for the 2B tolerance locus in three RIL sub-families. Plants were classified using molecular markers as homozygous for Waagan or Drysdale alleles or heterozygous (WW, DD and DW, respectively) across the 2B heat tolerance locus. The 3 d heat treatment was applied when the main tiller was at 6 cm AIL. The number of plants used to derive each mean are shown on top of each bar. For pollen starch analysis, per plant, one anther was sampled from the middle of the spike and the starchy pollen count on each anther was regarded as the sampling unit for the purposes of calculating the SEs

and statistics. Grain set was based on florets in the bottom two positions of the spikelets, from the middle and bottom third of the main stem spike. Grain set for the two thirds of the spike were treated as separate sampling units for calculating SEs and performing statistics. Means with the same letter above the bars were not significantly different at $p > 0.05$ (t-test).

For the crossing experiment to determine the effect of heat on the female reproductive organs, genotyped plants were heat treated at 6 cm AIL, and then emasculated and crossed with pollen from non-heat-treated Waagan plants. The fertility of the emasculated and cross-pollinated florets was low (0.06 to 0.87%; Figure 7.9) even in the WW class of plants that typically shows near-complete seed set after heat. Therefore, either the heat treatment made the emasculated florets more prone to sterility (independent of the 2B effect), or the emasculation/pollination technique was sub-optimal. If there were heat treated controls, it would have been possible to distinguish between these two possibilities. The low overall fertility in the emasculated/pollinated florets compromised the experiment by lowering the numbers of set grain upon which statistical analysis could be done, and probably also by introducing additional variability into the data.

Notwithstanding these factors, the DD plants showed no consistent or drastic reduction in grain set relative to the other two classes. Therefore, there was no evidence to suggest that the 2B locus influenced the heat susceptibility of the female gametes, at least to the same drastic extent that it affected pollen viability. This could mean that heat did not affect female viability (something that couldn't be tested, due to the lack of control plants), and/or that the 2B tolerance gene did not protect female reproduction against heat damage.

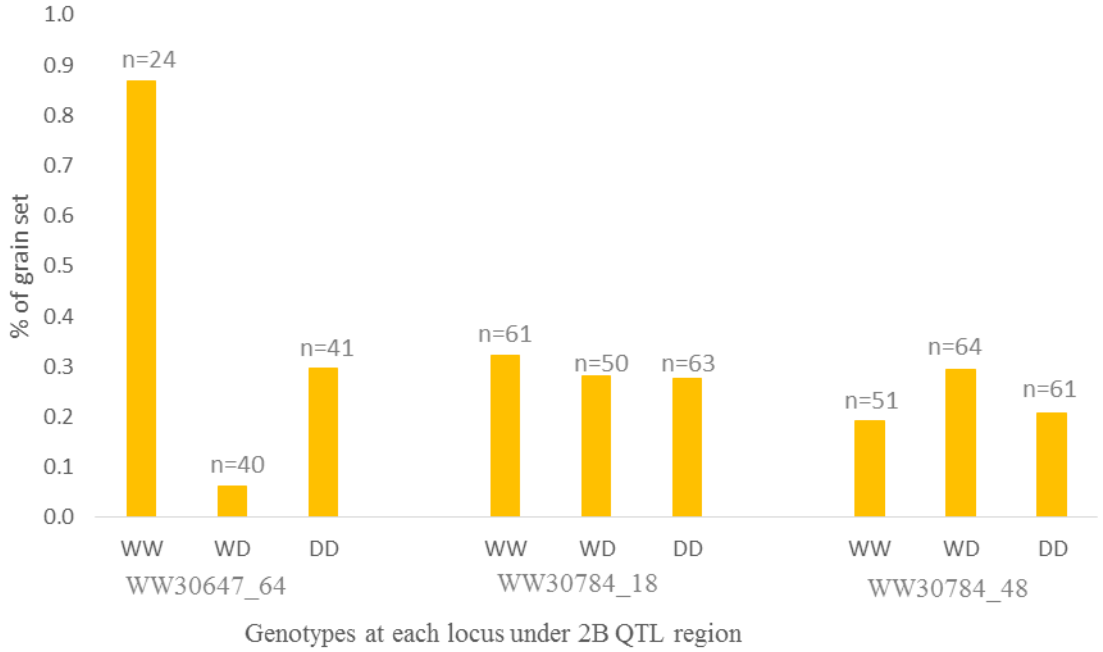

Figure 7. 9 Percent grain set in crossed-pollinated florets in heat treated plants of the three genotypic classes for the 2B tolerance locus in three Drysdale × Waagan RIL families. The 3 d heat treatment was applied when the main tiller was at the 6 cm AIL stage, plants emasculated and the florets pollinated with pollen from Waagan plants grown under control conditions; n= number of florets that were emasculated and then pollinated. Numbers of female plants used are indicated in Table 7.2.

7.5 Discussion

Effects of heat applied during pre-anthesis stages of tiller development (from 10 days BAE to 6 cm AIL) on floret fertility were investigated using wheat cvs. Waagan (tolerant) and Drysdale (sensitive) to identify the susceptible window of development and mechanisms behind differences in heat-induced floret sterility.

7.5.1. Relationships to nuclear division in developing pollen

When the pollen lands on the stigma and germinates, the sperm cells move down into the growing pollen tube until it reaches the ovule where one sperm cell fertilizes the haploid egg cell to produce the diploid zygote and the other fertilizes the central cell, containing two haploid nuclei, to give rise to the triploid endosperm. The vegetative nucleus is assumed to have a role during pollen germination and pollen-tube growth. Thus, functional differentiation

of the sperm cell and vegetative cell is pre-requisite for double fertilization in angiosperms, such as wheat (Tanaka, 1997).

Pollen grains with only one nucleus visible at anther dehiscence may represent microspores that did not proceed to pollen mitosis. Similarly, binucleate pollen at dehiscence may represent microspores that were unable to proceed from pollen mitosis I to mitosis II. In the current study, pollen with only one or two nuclei visible were observed by DAPI staining, but the frequency of these pollen grains, and that of normal pollen, was unaffected by the heat treatment or genotype (cvs. Waagan or Drysdale). Hence, there was no evidence to suggest the tolerance difference between Drysdale and Waagan was related to the process of nuclear division, or that heat affected this process.

In contrast to the current study, Harsant *et al.* (2013) observed that pollen of heat treated *Brachypodium distachyon* plants produced just one nucleus, suggesting that pollen development had ceased at the uni-nucleate stage. They also observed that the affected pollen became swollen and misshapen. However, the heat treatment was very different from the one used here – plants were heat treated at the reproductive meristem initiation stage for 1h, the plants moved back to control conditions for 24 h, and the plants then heat treated again until maturity. Draeger and Moore (2017) found that wheat cv. Chinese Spring lines lacking chromosome 5D (nullisomic 5D-tetrasomic 5B) were more susceptible to floret sterility induced by a relatively mild heat treatment (35 ºC for 20 h) than wild-type cv. Chinese Spring, that the sensitivity occurred from premeiotic interphase to late leptotene and that this sensitivity resulted in failure of meiosis to progress. This seemed to contrast to the current findings - failure to progress through meiosis would most likely prevent the later pollen mitotic divisions and result in abnormal number of nuclei in mature pollen, but pollen appeared normal for final nucleus number in the current study. The contrasting heat responses of pollen observed in the current study vs. the study of Draeger and Moore (2017) may reflect a fundamental difference in the nature of sensitivity of the genetic materials that were used. i.e., Chinese Spring may possess the tolerance allele at 2B, making it tolerant to the effects of heat on pollen starch filling, with loss of a gene on 5D (present in both Waagan and Drysdale), due to the nulli-tetrasomic substitution, making its meiosis become heat-sensitive.

7.5.2. Relationships to starch in mature pollen

The young anther consists of an epidermis, endothecium, middle layer and a tapetum of diploid cells surrounding the pollen mother cells (De Storme and Geelen, 2014). Under non stress conditions, the tapetum in wheat anthers commences programmed cell death (PCD) and breakdown at the early uni nucleate stage (2.5 days after meiosis) and tapetal cell walls break down at the binucleate stage (Saini *et al.*, 1984). However, in heat stressed wheat anthers, tapetal degradation starts earlier, at meiosis (Figure 7.10). In addition, the tapetum and outer layers (epidermis, endothecium and middle layer) of the anther wall display severe subcellular alterations due to heat, such as increased vacuolization (hypertrophy), chloroplast overdevelopment and mitochondrial swelling. Starch grains were also larger and more numerous in the lodicule walls of stressed anthers compared with control anthers (Oshino *et al.*, 2007). High temperatures during meiosis was also reported to lead to abortion of development and differentiation of tapetum cells and pollen mother cell development in barley (Abiko *et al.*, 2005).

The timing of degeneration of the tapetum is important, since the tapetal cells and the process of their degeneration provides nutrition or signals for the developing pollen grains (Figure 7.10). During the daytime, much of the carbon that is fixed by photosynthesis remains in the chloroplast and enters the starch biosynthesis pathway. In wheat, starch is deposited in the anther wall during meiosis, from where it disappears after meiosis, coincident with starch deposition in the maturing pollen grains (Saini *et al.*, 1984). Partitioning of carbon into starch reserves is determined by both assimilate supply and sink demand (Jenner, 1982).

Stress could therefore inhibit starch deposition in pollen by lowering assimilate availability to the pollen (e.g., due to early PCD of tapetum) and/or impairing the activities of enzymes of metabolic pathways leading to starch biosynthesis in the pollen (e.g., ADP-glucose pyrophosphorylase) (Preiss, 1988). At least in the developing wheat grains, soluble starch synthase has been found to be both a rate limiting part of starch synthesis, and highly heat sensitive (Hawker and Jenner, 1993; Keeling *et al.*, 1993; Keeling *et al.*, 1994; Rijven, 1986). Starch is a major source of energy for pollen development and germination and its absence severely reduces fertility (Clément *et al.*, 1994; Kumar *et al.*, 2015; Miki-Hirosige and Nakamura, 1983; Pacini and Franchi, 1988; Sheoran and Saini, 1996). A major reason for heat (Kumar *et al.* 2015) and drought (Sheoran and Saini, 1996) stress-induced reduction in fruit or

grain set is therefore likely to be decreased pollen starch before anthesis, resulting in a decreased amounts of soluble sugars in the mature pollen grains at anthesis, which impairs pollen tube growth and fertilization. Lalonde *et al.* (1997) observed absence of starch deposition in stressed pollen grains and endothecium 9 days after drought stress (when pollen were nearly mature) in the wheat genotype cv. Katepwa.

In the current study, heat stress drastically reduced the amount of pollen starch in the intolerant wheat cv. Drysdale and RILs carrying the Drysdale allele at the 2B tolerance locus, particularly when heat stress was applied at the 6 cm AIL stage, showing that the heat susceptibility (and tolerance) related to processes of carbohydrate metabolism in anthers. The absence of starch in these pollen was expected to render them non-functional and explains the lack of seed set.

As a next step, microscopy of anther tissue sections should be carried out to determine if the 2B tolerance locus exerts its effect by altering the timing of tapetal degeneration and/or timing of appearance/disappearance of starch in the cells surrounding the developing pollen.

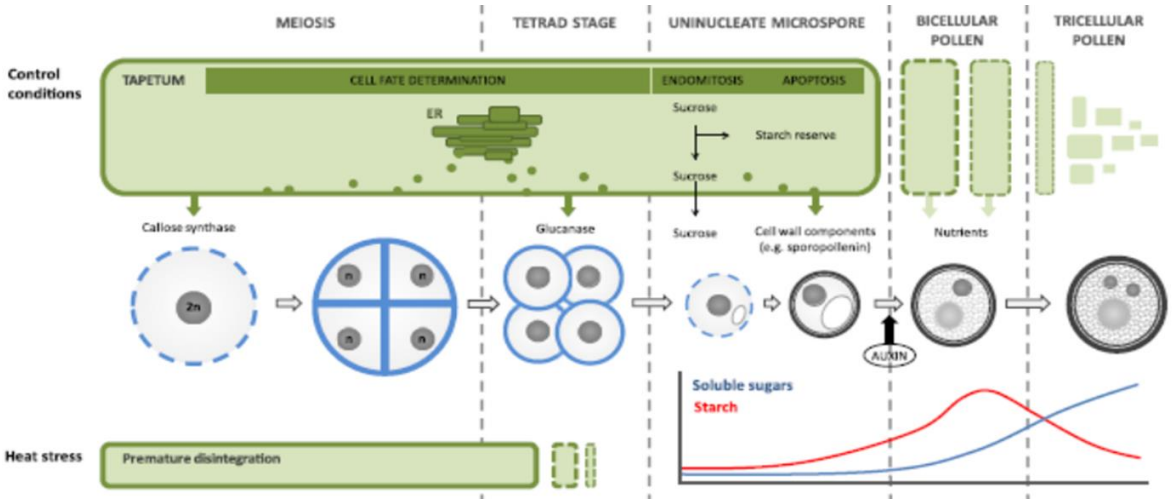

Figure 7. 10 Schematic diagram of microsporogenesis (from the microsporocyte (2n) to mature pollen (n)), illustrating the impact of heat stress on the timing of tapetal cell disintegration (adapted from De Storme and Geelen (2014)). The plant species this is based on was not specified.

7.5.3. Mode of expression of tolerance at the 2B locus

The 2B locus tolerance allele was not enriched in progeny of the heat treated 2B locus heterozygotes, indicating that the tolerance allele was the result of gene expression in diploid cells and not haploid cells (i.e., sporophytic, not gametophytic), despite the fact that the tolerance was manifested through the (haploid) male gametes. This result seems compatible with the observed lack of starch in the pollen, since sugars for starch formation in the pollen grains are provided by the diploid tapetal cells. Hence, the 2B tolerance gene might be expressed in the tapetal cells and contribute to processes that facilitate sugar provision to the developing pollen.

Alternatively, the 2B tolerance gene may encode starch biosynthetic enzymes or sugar importers that are synthesized in the diploid pollen mother cells prior to the first meiotic division, which then persist through to the pollen mitosis I stage when the starch is normally synthesized in the pollen. i.e., in heat treated plants of susceptible genotypes, the pollen may still receive sugars from the tapetum but be unable to convert them into starch.

The pattern of floret fertility and pollen starch content across heat treated plants of the three classes 2B-locus genotypes revealed that the tolerance effect at the 2B locus was expressed in an incompletely dominant manner over the intolerance (heterozygote has intermediate tolerance), although tending more towards complete dominance (heterozygote tolerance level is closer to that of the homozygous tolerant class).

The dominant mode of expression of the 2B tolerance effect in the pollen of heterozygotes is also consistent with a sprorophytic mode of expression of the tolerance, as gametophytic expression should have otherwise resulted in only half the pollen of heat treated heterozygotes being starchy, not close to 100%, as was observed (Figure 7.8a).

Either incomplete dominance, or complete dominance as shown by the pollen starch staining (Figure 7.8a), would be consistent with the intolerance allele at the 2B locus having a defective/null gene function. An expectation that the gene is functional in Waagan but defective in Drysdale could help guide efforts to identify the 2B tolerance gene from among a list of candidates using gene sequence or expression data from cvs. Waagan and Drysdale.

The crossing experiment suggested that the heat tolerance effect of the 2B QTL was not manifested through female reproduction, or at least not to the same extent that it was manifested in pollen. Although, this experiment needs to be repeated as general fertility in the emasculated florets (e.g., those pollinated with pollen from non stressed plants) was abnormally high. Saini and Aspinall (1982a) reported that heat stress during meiosis in wheat rendered 80% of the florets male sterile but caused female sterility in only about 15%, supporting the notion that female fertility in cereals may be less sensitive to heat stress than male fertility. One explantion may be the timing of sugar requirements - sink strength of anthers is thought to be high from the microspore mother cell to vacuolated microspore stages, but high in the ovary during anthesis to grain filling stages (Ji *et al.*, 2010). The different nature of the tissues (e.g., ovules well surrounded by other tissues, the thin anther filaments) may make anthers more prone to dehydration effects during heat treatment.

7.6 Conclusion and implications

The mechanism of tolerance to heat-induced floret sterility in cv. Waagan relative to cv. Drysdale, and of the tolerance controlled by the chromosome 2B locus, was found to relate to starch deposition in pollen grains. Pollen starch staining can be done earlier in plant development than scoring of grain set, and reveals more extreme differences between tolerant/intolerant genotypes than grain set. Results based on grain set may also be obscured by the effects of cross fertilization of male sterile florets from stray viable pollen, unless spikes are bagged. Hence, pollen starch staining could provide a more effective way of following the inheritance of the 2B tolerance allele (e.g., in a positional cloning effort) than grain set, and might be applicable as a scoring technique in breeding, if it can be shown to relate to tolerance loci that are present across wheat germplasm more broadly. Image analysis software could be used to count starchy vs. non-starchy pollen grains to perform high-throughput screening. It was found that heat induced floret sterility tolerance at the 2B locus related to starch deposition, was expressed as in an incompletely dominant to dominant manner, was of a sporophytic nature, and manifested through the male and not (at least to a large extent) the female reproductive structures. This guides future work for the cloning of the 2B tolerance gene(s). Because pollen grains carrying the 2B tolerance allele under heat stress have no advantage over those carrying the intolerance allele, exposing hybrids to pre-anthesis heat stress at would not enrich for tolerant genotypes in the next generation during a breeding program, at least in relation to the 2B locus.

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

Heat stress is a major concern for wheat crop production and productivity. Heat-induced male sterility is a critical problem in wheat that significantly compromises its yield. Therefore, wheat responses to elevated temperature, and the mechanisms underlying the development of heat tolerance, needs to be investigated. To this end, the present work sought to contribute to the understanding of the genetic and physiological bases of heat-induced floret sterility tolerance in wheat. This chapter summarizes the major findings of this study and discusses possibilities for further research.

Knowledge of the extent and pattern of genetic diversity present within a given crop is essential for further improvement. Bread and durum wheat genotypes were therefore screened for heat-induced floret sterility tolerance (Chapter 3). Parents of existing bread wheat populations contrasting for heat-induced floret sterility were identified, i.e., Drysdale (intolerant) vs. Waagan (tolerant), Drysdale (intolerant) vs. Gladius (tolerant), Excalibur (tolerant) vs. Kukri (intolerant), Young (tolerant) vs. Reeves (intolerant), Sunco (intolerant) vs. Tasman (tolerant), Westonia (intolerant) vs.*2/Janz (tolerant), and Westonia (intolerant) vs. Kauz (tolerant) (Chapter 3; Figure 3.8). Therefore, these populations could be useful in discovering new heat induced floret sterility tolerance QTL. Targeted crosses could also be made between some of the most tolerant and intolerant genotypes to produce new populations for genetic studies. The Drysdale × Waagan population was chosen for the genetic analysis of heat tolerance for this study as described in Chapter 4.

Australian durum wheat is considered sensitive to heat-induced floret sterility, so introgressing alleles from new tolerance sources into locally adapted Australian varieties could be useful as a strategy for variety improvement. Genotyping of the phenotyped lines with a dense SNP marker array, and comparison with the tolerance phenotype data generated in Chapter 3, could be used to perform genome wide association mapping of tolerance QTL, to identify markers that could be used for selection in breeding. Molecular markers could also be identified by crossing genotypes that contrasted for tolerance to heat induced floret sterility and by performing QTL analysis in these biparental populations. These avenues offer a good chance for genetic improvement. The most tolerant tetraploid genotypes were TERBOL97-3, CIMMYT-67 - PLATA 16, BIGOST-1, MERIDIANO, and Altar 84, and these could be used sources of tolerance in breeding.

In Chapter 4, the Drysdale × Waagan population, which contrasted for heat tolerance in Chapter 3, was used to map floret sterility heat tolerance QTL. Six tolerance QTL were identified. To our knowledge, these are the first tolerance QTLs identified in wheat. A major QTL for floret sterility tolerance was detected on chromosome 2B and five minor QTL on 1B, 3B, 4B, 4D and 7A, explaining 5.4 to 49% of the phenotypic variance. The QTL on 3B, 4B and 4D co-located with QTL for absolute organ length traits or heat responses of these other traits, suggesting that these tolerance effects represented a confounded effect with, which is escape artefacts (chapter 4). The QTL on 1B, 2B and 7A were not associated with such traits and thus appeared to represent genuine tolerance loci.

The major fertility tolerance QTL on chromosome 2B (QTL 7) mapped close to the *Yr27* yellow rust resistance gene. Since the heat tolerance and yellow rust resistance alleles both derived from the Waagan parent, it might be possible to select for both traits simultaneously.

The 2B locus, while it mapped close to the photoperiod sensitivity gene *Ppd-B1*, was separated from it by recombination (Chapter 5). This showed that *Ppd-B1* was not the basis for the heat tolerance effect and that it will be possible for breeders to select for heat tolerance independently of flowering time.

Shirdelmoghanloo *et al.* (2016c) had previously used the Drysdale × Waagan DH mapping population and the same phenotying platform as in the current study, except that heat stress was applied during grain filling to identify QTL for tolerance to the effects of heat on final grain size. Two tolerance QTL were identified, located on chromosomes 3B and 6B. Neither of the grain filling tolerance QTL co-located with any of the heat-induced floret sterility tolerance QTL identified in this study (Chapter 4). Thus, tolerance for the two yield components (grain size and grain number) are controlled independently, and therefore need to be selected for separately in breeding programs. QTL 7, with its large effect on fertility tolerance (expressed 49% its phenotypic variance), could be useful to breeders, if its beneficial effect can be shown to be expressed under field conditions. To our knowledge, there are no diagnostic molecular markers for tolerance to heat induced floret sterility yet being used for selection in breeding programs.

In Chapter 5, an attempt at fine mapping the fertility heat tolerance QTL on chromosome 2B (QHFert.aww-2B) was made but because several critical recombinants could not be genotyped for the 2B QTL with confidence, this only delimited the locus to a relatively large interval (9.1 cM; 31.5 Mb) containing 203 predicted genes. However, RIL families showing clear marker-trait association were identified and seeds propagated for future fine mapping. Also, high throughput KASP markers were developed for the region, and links established to the IWGS cv. Chinese Spring genomic reference sequence v1 and other wheat genomics tools, such as DAWN (Ute Baumann, unpublished), providing the platform for the facile generation of more KASP markers, and identification of candidate genes, in the region. Thus, the work reported here provides a sound platform for further fine mapping, and eventual positional cloning of the 2B QTL. Cloning the underlying gene(s) and subsequent identification of the molecular function will improve our understanding of the mechanisms regulating tolerance to heat induced floret sterility. Cloning the gene should also pave the way to generating diagnostic molecular markers for use in selection of heat tolerance in breeding programs.

For the 2B QTL, a subset of 32 NILs was developed and multiplied at Waaga Waaga (Chapter 5), with the objective to enable quantification of the yield benefit under field conditions. Similarly, it will be important to verify if the tolerance effect can be observed in different genetic backgrounds. The closely linked KASP markers developed in this study could be directly used for breeding provided the allele phase with tolerance can be verified in breeding material (e.g., by phenotyping and genotyping a subset of individuals).

In Chapter 6, the floret developmental stage from pre-meiosis to late uni-nucleate microspore stage, corresponding to an AIL of 7 to 13 cm, was identified as the sensitive stage of heat-induced floret sterility tolerance, in the cv. Drysdale. Unfortunately, the study didn't provide enough temporal resolution to determine which observable stage within this range was the critical one. Nonetheless, it showed at what general stage further studies should focus on. It should be possible to define the sensitive stage more accurately by applying shorter heat treatments and/or by assessing the same florets for anther development stage both pre- and post- heat treatment, using the anther sampling technique described by Draeger and Moore (2017).

In Chapter 7, heat-induced floret sterility tolerance mechanisms were investigated using contrasting genotypes Waagan and Drysdale. Nuclear division in the pollen, which occurs after meiosis, was unaffected by heat. Therefore, it seems unlikely that the process of meiosis itself was affected, as failure to satisfy a meiosis checkpoints would be expected to prevent further progression of the developmental program.

Heat did however dramatically reduce pollen starch accumulation in the susceptible genotype Drysdale, suggesting that this was the basis for the difference in susceptibility between the genotypes. This finding was in line with that of Jain *et al.* (2007), who reported that heat induced floret sterility in sorghum resulted from alteration of starch accumulation in the microspores. Further, these authors could not detect sucrose in the microspores of heat-stressed sorghum plants. Further study could therefore be done to determine if tolerance in wheat relates to sugar profiles of wheat anthers. The three enzymes sucrose synthase, soluble starch synthase and granule bound starch synthase are considered limiting for starch biosynthesis in wheat grains (Hawker and Jenner, 1993). Soluble starch synthase of the developing grain is also heat sensitive (Keeling *et al.*, 1993; Keeling *et al.*, 1994; Rijven, 1986). Therefore, further work could test if there these three enzyme activities in developing anthers relate to differences in tolerance to heat induced floret sterility in wheat.

In Chapter 7, tolerance at the 2B locus was found to be expressed in a sporophytic (diploid) manner, suggesting that the tolerance could relate to gene action in the diploid tapetum tissue. This would also be consistent with the subcellular alterations in tapetum tissue observed with heat stress (Oshino *et al.*, 2007) and the known key role that the tapetum plays in facilitating microspore development, including supplying sugars for starch synthesis. Microscopy could be performed on sections of heat treated anther tissues, to look for any visible signs of the involvement of tapetal tissue in tolerance, e.g., degradation of tapetal tissue under heat which might occur earlier in susceptible genotypes than tolerant genotypes. Alternatively, the tolerance may relate to the ability of the microspores to convert sugars to starch, although this seems less likely, as it would require carry over of gene effects from the diploid microsporocytes, through meiosis, to the binucleate microspores, when starch is synthesised.

Tolerance at the 2B locus was also shown to be expressed in an incomplete dominance and towards mainly a dominant fashion, based at least on the effect on starch accumulation in the pollen (Chapter 7). Also, no evidence was obtained for a major effect of the locus on

tolerance in the female reproductive organs, although additional experiments would be required to test for more subtle effects.

The aforementioned observations provide clues as to where and when the 2B tolerance gene might be expressed and about the nature of the gene product, and could thereby help in identifying the underlying gene. Based on the current data, the gene would be expected to be expressed within the period from pre-meiosis to late uni-nucleate microspore stage, in the anthers, and more specifically in the tapetum, but not necessarily in the female reproductive organs, the allele in intolerant genotypes might be absent or non-functional (suggested by the dominant nature of the tolerance), and the gene product may be involved in starch biosynthesis, sugar transport, or regulation of tapetum programmed cell death. Once the gene is cloned, it will allow more direct tests of its temporal and spatial patterns of expression (e.g., by in-situ hybridisation) and its sequence should shed light on the nature of its molecular function.

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Appendices

Appendix table 3. 1 Measured temperatures (ºC) and humidity in greenhouse during durum wheat genotypes screening (chapter 3) and first experiment of manifestation of Waagan vs Drysdale (pollen starch) (chapter 7) experimental period. Booting occurred during end of April and May. Columns indicate daily average (Day), nightly average (Night), average daily maximum (Maximum average), average daily minimum (Minimum average), maximum, minimum and number of days where temperature greater or equal to 30ºC.

		Day	Night	Maximum average	Minimum average	Maximum	Minimum	Days ≥ 30ºC
Temperature (ºC)								
	March	24.1	19.9	27.8	18.3	31.7	15.8	5
	April	20.7	17.6	23.6	16.5	28.0	14.6	-
	May*	19.5	16.7	22.1	15.6	25.9	14.3	-
	June	19.4	16.7	21.4	15.6	24.5	14.2	-
	July	19.8	17.1	21.5	15.8	22.8	14.6	-
2015	August	22.2	17.5	25.6	16.1	34.8	14.9	9
% relative humidity								
	March	59.1	66.7	74.0	47.3	85.9	29.8	
	April	66.6	73.6	82.2	55.6	88.8	38.8	
	May*	73.7	81.2	88.5	60.4	90.2	42.2	
	June	69.1	76.7	85.8	54.5	94.6	42.9	
	July	61.3	70.2	81.2	49.2	88.3	36.8	
2015	August	53.0	65.8	73.9	41.5	94.3	22.2	

Appendix table 3. 2 Means ± S.E. of traits measured at two heat treatment stages (1 and 6 cm AIL) along with the control on primary tillers of durum wheat genotypes

Traits	1 cm AIL stage		6 cm AIL stage	
	Control	Heat	Control	Heat
Traits measured pre-heat				
Day.AIL	61.0±0.91	60.1±0.88	61.6±0.76	61.4±0.83
AIL.PreH	1.60±0.06	1.45±0.05	5.59±0.19	5.38±0.04
Ht.PreH	35.6±0.66	35.1±0.7	40.2±0.58	39.9±0.53
Chl0	33.4±0.33	33.6±0.31	36.8±0.32	37.0±0.30
Duration of developmental processes				
Day.AwnEm	63.2±0.96	62.4±0.94	62.8±0.88	61.9±0.83
Day.Anth	76.3±0.96	72.2±1.03	74.5±0.82	70.5±0.92
Day.AILtoAwnEm	3.17±0.26	2.37±0.43	1.93±0.53	1.16±0.19
Day.AILtoAnth	16.0±0.32	12.6±0.49	13.6±0.48	10.1±0.33
Actual and rate of stay green (flag leaf chlorophyll retention)				
Chl3	38.3±0.32	40.2±0.32	39.6±0.31	40.0±0.37
Ch114	41.7±0.39	44.5±0.39	41.6±0.39	42.6±0.54
Ch128	42.1±0.45	45.1±0.46	41.2±0.49	42.1±0.64
RChChl3_0	1.52±0.11	2.1±0.13	0.92±0.04	0.83±0.12
RChChl14_3	0.24±0.02	0.29±0.02	0.15±0.03	0.18±0.04
RChChl28_14	0.03±0.01	0.05±0.02	-0.02±0.02	-0.04±0.01
Final organ length, or gain in organ length from the commencement of treatment				
AwnL.Mat	12.0±0.23	11.5±0.21	12.4±0.18	11.9±0.18
SpkL.Mat	6.32±0.07	6.29±0.07	6.29±0.07	6.21±0.06
AIL.Mat	16.1±0.24	14.3±0.2	16.1±0.22	13.5±0.16
PedL.Mat	28.3±0.57	25.2±0.47	29.5±0.46	26.7±0.45
Ht.Mat	70.9±1.1	64.7±1.0	70.7±0.92	64.5±0.71
AIL.GroPostH	13.4±0.36	11.8±0.32	10.6±0.21	8.26±0.16
PlHt.GroPostH	37.0±1.5	33.8±1.41	31.1±0.68	25.3±0.56
Spikelet number and development of basal spikelets				
NoDevSplt.Spk	15.9±0.14	16.1±0.14	16.1±0.12	16.1±0.14
UndvSplt.Spk	1.7±0.11	1.55±0.10	1.48±0.09	1.45±0.10
NoDevSplt.Top	5.68±0.05	5.67±0.05	5.67±0.04	5.72±0.04
NoDevSplt.Mid	5.31±0.05	5.35±0.05	5.31±0.04	5.35±0.05
NoDevSplt.Bot	4.94±0.05	5.02±0.05	5.05±0.04	5.05±0.05
Grain number per spikelet				
GrNoSplt.1&2.Top	1.74±0.03	0.57±0.04	1.78±0.02	0.99±0.05
GrNoSplt.1&2.Mid	1.77±0.04	0.61±0.05	1.82±0.03	1.09±0.05
GrNoSplt.1&2.Bot	1.70±0.04	0.42±0.04	1.82±0.03	0.80±0.05
GrNoSplt.>2.Top	0.46±0.03	0.12±0.02	0.56±0.03	0.14±0.02
GrNoSplt.>2.Mid	0.90±0.04	0.24±0.03	0.98±0.04	0.31±0.03
GrNoSplt.>2.Bot	0.64±0.04	0.21±0.03	0.73±0.03	0.18±0.02
GrNoSplt.Spk	2.39±0.06	0.73±0.05	2.52±0.05	1.22±0.13
GrNo.Spk	38.3±1.0	11.6±0.84	41.0±0.73	18.8±0.92

Appendix table 4. 1 Measured temperatures (ºC) and humidity in greenhouse during QTL mapping (chapter 4) and hexaploid wheat genotypes screening (chapter 3) experimental period. Booting occurred during end of April and beginning of May. Columns indicate daily average (Day), nightly average (Night), average daily maximum (Maximum average), average daily minimum (Minimum average), maximum, minimum and number of days where temperature greater or equal to 30ºC.

		Day	Night	Maximum average	Minimum average	Maximum	Minimum	Days ≥ 30ºC
Temperature (ºC)								
2014	March	22.8	19.8	25.3	17.7	27.9	14.9	-
	April	21.0	17.6	24.0	16.0	28.1	14.8	-
	May*	20.5	17.7	23.1	16.9	27.0	15.5	-
	June	18.9	16.0	20.7	15.1	21.5	14.6	-
	July	19.2	16.1	21.7	15.3	24.1	14.3	-
	August	20.2	15.9	23.0	14.9	26.9	14.2	-
% relative humidity								
2014	March	63.1	71.4	79.3	51.0	88.6	36.0	
	April	64.7	75.0	83.3	53.1	90.3	46.7	
	May*	73.4	77.3	84.3	63.2	90.2	46.8	
	June	78.1	81.7	90.2	61.4	92.4	49.6	
	July	75.8	79.9	88.6	61.4	93.1	47.4	
	August	56.7	71.0	77.1	44.4	83.2	35.0	

Appendix table 4. 2 a. Means ± S.E. of traits measured at two heat treatment stages (3 and 9 cm AIL) along with the control on primary tillers of Drysdale × Waagan DH mapping population and parental lines.

Stage/Trait	**Drysdale**		**Waagan**		**DH**	
3 cm AIL	Control	Heat	Control	Heat	Control	Heat
Traits measured pre-heat						
Day.AIL	52.8±0.11	52.3±0.22	58.8±2.02	58.8±2.47	56.1±3	56±2.9
AIL.PreH	4.5±0.86	2.68±0.01	4.38±0.87	4±0.17	4±1.78	3.52±1.4
Ht.PreH	38.4± 1.39	39.3± 2.82	37.2± 2.85	32.5±2.78	37.2±5.8	36.8±4.6
Duration of developmental processes (d)						
Day.AwnEm	59±0.71	56.8±1.04	62.8±0.22	62.5±4.05	62.1±3.6	59.9±3.1
Day.Anth	67.8±0.22	65±0.71	70.3±1.75	67.7±2.47	69.6±4.1	66.6±3.6
Day.AILtoAnth	15±0.24	12.8±0.9	11.5±0.86	9±0	13.5±0	10.5±2.5
Day.AILtoAwnEm	6.25±0.6	4.5±0.4	4±0.71	3.75±0.21	6±2.4	3.9±1.65
Final organ length, or gain in organ length from the commencement of treatment (cm)						
AwnL.Mat	5.98±0.07	4.83±0.06	5.3±0.13	4.75±0.1	5±0.7	4.74±0.77
SpkL.Mat	9.23±0.12	8.98±0.02	8.5±0.18	7.87±0.23	8.3±0.58	8.69±0.61
AIL.Mat	21.1± 0.62	17.5±4.09	19±0.24	15.5±0.86	18.7±1.7	16.1±2.06
PedL.Mat	34.4±0.26	28.3±8.76	30.4±1.3	26.8±8.79	30±3.7	27.7±4.2
Ht.Mat	86.8± 3.21	77.3±9.5	78±6.84	66.4±2.24	77.1±6.8	71.8±6.3
AIL.GroPostH	16.6±0.86	14.8±3.5	14.6±0.26	11.5±0.81	14.7±1.9	12.6±2.4
PlHt.GroPostH	48.4±1.48	38±8.1	40.8±3.12	33.9±15	39.6±5.6	35±6.1
Spikelet number and development of basal spikelets						
NoDevSplt.Top	6±0	6.5± 0.29	5.75±0.11	5.5±0.43	5.7±0.66	6.35±0.68
NoDevSplt.Mid	5.5± 0.29	6.5± 0.29	5.75±0.11	5±0.23	5.4±0.62	6.02±0.7
NoDevSplt.Bot	5.5± 0.28	5.75±0.11	5.75±0.11	4.75±0.11	5±0.62	5.69±0.67
NoDevSplt.Spk	17± 0.57	18.8± 0.75	17.3±0.97	15.3±2.02	16±1.7	18.1±1.9
UndvSplt.Spk	5.25±0.22	3.75± 0.75	6.5± 0.28	6.75±3.48	6.4±1.58	4.45±1.5
ProUndsplt	0.24±0.02	0.16±0.0	0.25±0.02	0.30±0.09	0.28±0.09	0.20±0.06
NoSplt.Spk	22.3±0.5	22.5±0.58	23.8±1.9	22±1.41	22.4±2.38	22.5±2.37
Grain number per spikelet						
GrNoSplt.1&2.Top	1.79±0.04	0.93±0.12	1.53± 0.48	0.89±0.04	1.6±0.35	0.68±0.42
GrNoSplt.1&2.Mid	2.0±0.0	1.04±0.09	1.92± 0.41	1.79±0.07	1.8±0.27	1.0±0.47
GrNoSplt.1&2.Bot	1.92± 0.29	1.46±0.02	1.83±0.02	1.45± 1.37	1.8±2.9	1.09±0.42
GrNoSplt.>2.Top	0.46±0.02	0.15±0.01	0.04±0.0	0.12±0.03	0.1±0.19	0.19±0.19
GrNoSplt.>2.Mid	1.03±0.05	0.6±0.04	0.44±0.01	0.29±0.01	0.7±0.36	0.68±0.42
GrNoSplt.>2.Bot	0.58±0.03	0.47±0.02	0.18±0.01	0.25±0.11	0.4±0.3	0.48±0.32
GrNo.Spk	44±4.10	28±6.17	34±1.65	24±5.4	34.6±8.33	24.8±9.2
GrNoSplt.Spk	2.58±0.04	1.54±0.12	1.98±0.01	1.58±0.15	2.1±0.39	1.36±0.48

Appendix table 4.2a. Continuied…

Stage/Trait	**Drysdale**		**Waagan**		**DH**	
9 cm AIL	Control	Heat	Control	Heat	Control	Heat
Traits measured pre-heat						
Day.AIL	53.5±0.4	56.3±2.08	60.7±1.04	60.5±5.74	58.3±2.7	58.2±4.1
AIL.PreH	8.75±0.1	8.05±0.12	8±0.06	8.6±0.28	8.7±1.3	8.53±0.94
Ht.PreH	46.1±6.63	49.9±5.14	42.3±1.01	44.4±8.54	44.1±4.1	44.3±5.3
Duration of developmental processes (d)						
Day.AwnEm	57.3±0.22	58.3±2.48	61.7±0.0	62.3±6.42	60.5±3.0	59.7±4.8
Day.Anth	65.5± 0.28	63±0.71	70.7±2.62	68±3.06	68.2±2.9	64.8±4.2
Day.AILtoAnth	12±0.71	6.75±1.04	10± .057	7.5±1.2	9.99±1.7	6.62±2.2
Day.AILtoAwnEm	3.75±0.6	2±0.24	1.67± 1.15	1.75±0.11	2.18±1.9	1.46±2.6
Final organ length, or gain in organ length from the commencement of treatment (cm)						
AwnL.Mat	5.37±0.03	5.38±0.05	5.38±0.05	5.38±0.23	5.34±0.64	5.21±0.7
SpkL.Mat	9.13±0.03	9.05±0	9±0.06	8.5±0.41	8.47±0.59	8.41±0.56
AIL.Mat	22.9±0.23	15.2± 1.86	18.8±0.86	17.1±4.19	19.2±2.3	15.6±1.8
PedL.Mat	36.1± 0.97	30±2.18**	30.6±8.99	30.1±2.68	30.4±3.4	27±3.5
Ht.Mat	90.1±1.06	78±9.19**	78±6.96	73.1± 2.78	78.1±5.7	71±6.8
AIL.GroPostH	14.1±0.21	7.15± 1.75**	10.9±0.86	8.53±2.24	10.4±2.6	7.09±1.9
PlHt.GroPostH	43.9±2.89	28.1±12.3**	35.8±7.34	28.8±15.1	33.8±4.7	26.6±5.2
Spikelet number and development of basal spikelets						
NoDevSplt.Top	6±0	6.25±0.11	6.5± 0.29	6.25±0.11	5.82±0.74	5.91±0.65
NoDevSplt.Mid	5.75±0.11	5.75±0.11	6.25±0.11	6±0.24	5.45±0.78	5.59±0.63
NoDevSplt.Bot	5.5± 0.28	5.25±0.11	6±0.24	5.5±0.43	5.12±0.75	5.25±0.65
NoDevSplt.Spk	17.3±0.22	17.3±0.6	18.8±0.9	17.8±2.02	16.4±2.1	16.8±1.7
UndvSplt.Spk	4.75±0.22	5±0.24	6.75± 1.6	6.75±1.4	5.94±1.87	5.59±1.7
ProUndsplt	0.2±0.01	0.24±0.03	0.27±0.01	0.27±0.07	0.26±0.08	0.24±0.07
NoSplt.Spk	22.3±0	22.3±0.95	25.5±2.08	24.5±1	22.3±2.33	22.3±2.19
Grain number per spikelet						
GrNoSplt.1&2.Top	1.63± 0.17	0.9±0.06	1.45±0.02	1.31±0.01	1.63±0.3	0.87±0.5
GrNoSplt.1&2.Mid	1.96±0.0	1.12±0.09	2±0	1.83± 0.1	1.83±0.2	1.25±0.48
GrNoSplt.1&2.Bot	1.96±0.0	0.8±0.08	1.9±0.02	1.8±0.03	1.78±0.3	1.1±0.44
GrNoSplt.>2.Top	0.5± 0.09	0.00±0	0.07±0.04	0.04±0	0.21±0.19	0.11±0.96
GrNoSplt.>2.Mid	1.21±0.02	0.42± 0.25	0.32±0.08	0.45±0.02	0.8±0.33	0.49±0.37
GrNoSplt.>2.Bot	0.85±0.02	0.25± 0.15	0.38±0.05	0.31±0.02	0.54±0.29	0.37±0.29
GrNo.Spk	46.5±15.3	19.8±5.36	38.5±5.12	34.3±21.9	37.5±8.8	23.3±8.9
GrNoSplt.Spk	2.69±0.04	1.16±0.18	2.03± 0.19	1.92±0.03	2.25±0.38	1.38±0.48

Appendix table 4. 2b. Means ± S.E. of traits measured at two heat treatment stages (1.6 and 6.5 cm AIL) along with the control on secondary tillers of Drysdale × Waagan DH mapping population and parental lines.

Stage/Trait	**Drysdale**		**Waagan**		**DH**	
1.6 cm AIL	Control	Heat	Control	Heat	Control	Heat
Traits measured pre-heat						
AIL.PreH	1.75±1.03	1.38±1.38	2.3±1.45	0.8±0.8	1.69±0.14	1.63±0.11
Ht.PreH	30.3±4.8	25.3±5.06	30±4.45	31.1±2.27	30.7±0.81	30.6±0.78
Final organ length, or gain in organ length from the commencement of treatment (cm)						
AwnL.Mat	5.25±0.25	5±0.5	4.5±0	5.63±0.13	5.03±0.08	4.66±0.07
SpkL.Mat	9.25±0.75	10.3±0.25	7.75±1.25	8.25±0.75	7.86±0.06	8.44±0.07
AIL.Mat	21.8±2.25	18.2±0.45	18.8±1.75	16.4±0.41	17.9±0.43	16.1±0.5
PedL.Mat	29.8±4.25	25.6±3.88	26.5±0	25.25±2.25	29.6±0.8	27.5±0.73
Ht.Mat	76.5±10	70±6	74±5	69±6	73.9±1.94	68.9±1.70
AIL.GroPostH	18.3±1.77	15.6±2.09	18.6±1.56	14.9±2.03	16.2±0.44	14.3±0.51
PlHt.GroPostH	38.5±6	43.8±6.25	49.3±0.75	37.5±0.5	42.7±1.27	38.2±1.01
Spikelet number and development of basal spikelets						
NoDevSplt.Top	5.5±0.29	6±0.71	5.5±0.65	6.5±0.65	5.21±0.06	5.82±0.07
NoDevSplt.Mid	5.5±0.28	5.5±0.65	5.25±0.63	5.75±0.63	4.9±0.06	5.54±0.07
NoDevSplt.Bot	5±0.58	5±0.71	4.75±0.63	5.75±0.63	4.47±0.07	5.22±0.07
NoDevSplt.Spk	16±1.16	16.5±2.02	15.5±1.85	18.8±2.02	14.5±0.18	16.6±0.19
UndvSplt.Spk	6.25±0.48	5.75±1.03	8.25±0.25	5.75±0.85	7.12±0.18	5.15±0.14
ProUndsplt	0.29±0.01	0.26±0.0	0.34±0.04	0.23±0.05	0.32±0.0	0.24±0.0
NoSplt.Spk	22.3±0.75	22.3±1.25	23.8±2.06	24.5±1.19	21.6±0.15	21.7±0.17
Grain number per spikelet						
GrNoSplt.1&2.Top	1.63±0.16	1.1±0.40	1.34±0.20	1.15±0.16	1.49±0.03	0.92±0.03
GrNoSplt.1&2.Mid	1.96±0.04	1.83±0.12	1.96±0.04	1.56±0.25	1.8±0.03	1.15±0.04
GrNoSplt.1&2.Bot	1.90±0.06	1.49±0.23	1.91±0.05	1.79±0.21	1.69±0.03	1.24±0.04
GrNoSplt.>2.Top	0.08±0.08	0.08±0.08	0±0	0.03±0.03	0.06±0.01	0.17±0.01
GrNoSplt.>2.Mid	0.68±0.31	0.13±0.13	0.05±0.05	0.20±0.16	0.51±0.03	0.61±0.03
GrNoSplt.>2.Bot	0.54±0.24	0.29±0.11	0±0	0.15±0.09	0.36±0.02	0.42±0.02
GrNo.Spk	36.3±5.92	25.75±3.01	26.5±1.94	29±5.11	28.1±0.79	24.6±0.88
GrNoSplt.Spk	2.26±0.28	1.57±0.10	1.74±0.09	1.57±0.24	1.96±0.04	1.49±0.04

Appendix table 4.2b. Continuied…

Stage/Trait	**Drysdale**		**Waagan**		**DH**	
6.5 cm AIL	Control	Heat	Control	Heat	Control	Heat
Traits measured pre-heat						
AIL.PreH	4.75±1.96	7.13±1.16	7.83±0.72	5.63±0.83	6.6±0.17	6.52±0.22
Ht.PreH	40.5±2.33	37.3±1.3	44.6±2.16	41.1±2.50	39.3±0.95	39±0.99
Final organ length, or gain in organ length from the commencement of treatment (cm)						
AwnL.Mat	5.13±0.32	4.88±0.38	5.88±0.13	5.25±0.14	5.22±0.07	5.08±0.06
SpkL.Mat	9±0.2	8±0.46	9±0.41	8±0	8.13±0.05	8.11±0.06
AIL.Mat	21.3±0.83	17.1±0.42	18.3±0.95	17.3±3.03	18.6±0.41	15.3±0.27
PedL.Mat	33.6±0.72	29.5±1.57	29.1±0.77	26.6±0.43	30.3±0.78	27±0.68
Ht.Mat	86.8±1.65	73.1±3.69	76.5±3.52	74.9±2.50	76.51±2.01	68.5±1.61
AIL.GroPostH	16.5±1.53	9.81±1.3	11.0±0.87	12.2±1.39	11.9±0.45	8.76±0.34
PlHt.GroPostH	46.3±3.2	35.9±4.1	31.9±2.36	33.8±1.94	36.9±1.17	29.3±0.87
Spikelet number and development of basal spikelets						
NoDevSplt.Top	5.75±0.25	5±0.71	5.5±0.5	5.75±0.48	5.45±0.06	5.57±0.06
NoDevSplt.Mid	5±0	5±0.71	5.25±0.25	5.75±0.48	5.09±0.05	5.21±0.06
NoDevSplt.Bot	4.75±0.25	4.75±0.48	5.25±0.25	5.25±0.25	4.75±0.05	4.93±0.06
NoDevSplt.Spk	15.5±0.5	14.8±1.89	16±1	16.8±1.18	15.3±0.15	15.7±0.18
UndvSplt.Spk	5.75±0.48	9±1.78	5±0.71	7.25±1.49	6.51±0.18	5.94±0.16
ProUndsplt	0.25±0.02	0.24±0.04	0.39±0.01	0.31±0.04	0.29±0.01	0.27±0.01
NoSplt.Spk	21.3±0.25	23.8±0.63	21±0.41	24±1	21.76±0.146	21.66±0.156
Grain number per spikelet						
GrNoSplt.1&2.Top	1.69±0.08	1.17±0.25	0.5±0.24	1.06±0.23	1.6±0.02	0.82±0.04
GrNoSplt.1&2.Mid	2±0	1.95±0.05	0.95±0.36	1.95±0.05	1.88±0.02	1.13±0.04
GrNoSplt.1&2.Bot	2±0	2±0	1.03±0.24	1.95±0.05	1.77±0.02	1.07±0.04
GrNoSplt.>2.Top	0.38±0.07	0.04±0.03	0.12±0.07	0.07±0.07	0.15±0.01	0.12±0.01
GrNoSplt.>2.Mid	1.1±0.06	0.17±0.10	0.33±0.05	0.32±0.24	0.68±0.03	0.48.03
GrNoSplt.>2.Bot	0.99±0.15	0.27±0.16	0.32±0.14	0.38±0.13	0.48±0.02	0.38±0.02
GrNo.Spk	41.8±2.5	28.25±6.05	16.8±2.69	31.5±2.33	33.6±0.72	21.1±0.83
GrNoSplt.Spk	2.69±0.09	1.86±0.16	1.08±0.21	1.89±0.15	2.17±0.03	1.32±0.04

Appendix table 4. 3 Summary of tolerance QTL detected in the Drysdale × Waagan DH population sorted by Linkage group, assigned QTL number, positon of each QTL, closest marker(s), traits/QTL name, tiller, stage (AIL in cm), treatment (in control, heat-treated or tolerance, their LOD score, percentage of explained variation (R^2), additive effect, and high value allele (Drysdale, D; Waagan, W) are presented.

Linkage group	Assigned QTL number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
B	QTL2	26.24	wsnp_Ex_c14273_22230844	QGrNoSplt.>2.Bot.aww-1B	Primary	3	Heat	2.44	3.46	0.03	D
						9	Heat	5.37	11.35	0.05	D
		53.21	wsnp_Ku_c34980_44256215	QGrNo.Spk.aww-1B	Primary	3	Tolerance	3.58	8.05	1.15	D
		62.46	wsnp_Ex_c22377_31571527	QGrNoSplt.Spk.aww-1B	Secondary	3	Tolerance	3.74	8.33	0.12	D
		69.00	wsnp_Ex_c23235_32471358		Primary	3	Heat	4.02	7.80	0.12	D
2A	QTL4	70.54	wsnp_CAP12_c1269_649827	QSpkL.Mat.aww-2A	Primary	9	Heat	5.23	10.77	0.16	D
						3 and 9	Control	5.07	10.39	0.16	D
		74.99	wsnp_Ex_c42720_49228237	QGrNoSplt.>2.Bot.aww-2A	Primary	3	Heat	4.20	6.86	0.04	W
					Secondary	3	Heat	4.77	10.12	0.04	W
						9	Heat	4.68	10.25	0.04	W
					Primary	3 and 9	Control	4.26	7.17	0.05	W
		77.95	wsnp_Ex_rep_c102538_87682273	QGrNoSplt.>2.Mid.aww-2A	Primary	3	Heat	3.64	8.52	0.06	W
						3 and 9	Control	3.94	7.91	0.05	W
		93.58	wsnp_Ex_rep_c105158_89662129	QUndvSplt.Spk.aww-2A	Secondary	3	Tolerance	3.71	9.56	0.09	D
						9	Tolerance	3.52	9.03	0.14	D
		96.55	wsnp_Ku_c8927_15048149	QProUndsplt.aww-2A	Secondary	3	Heat	3.48	6.146	0.017	D
				QNoDevSplt.Spk.aww-2A			Tolerance	4.12	10.65	0.22	W
		97.29	wsnp_Ku_c4271_7774388	QNoDevSplt.Bot.aww-2A	Secondary	3	Tolerance	3.66	9.35	0.07	W
	QTL5	101.00	wsnp_JD_c15127_14676522	QAIL.GroPostH.aww-2A	Primary	3	Heat	4.16	2.90	0.53	D
		108.44	wsnp_Ex_c3808_6924802	QAIL.Mat.aww-2A	Primary	3	Heat	4.57	2.27	0.46	D
				QPlHt.GroPostH.aww-2A	Primary	3	Heat	4.70	1.59	1.16	D
							Control	2.64	1.08	1.35	D
						9	Control	4.22	1.24	1.26	D
							Heat	3.77	1.62	0.97	D

Appendix table 4.3. Continued…

Linkage group	Assigned QTL number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
2A	QTL5	110.66	wsnp_RFL_Contig4779_5764326	QHt.Mat.aww-2A	Primary	9	Heat	6.43	0.91	1.78	D
		146.98	wsnp_CAP11_c360_282889		Secondary	9	Tolerance	3.91	10.18	0.20	D
2B1	QTL7	49.10	wsnp_Ra_rep_c106119_89961852/wsnp_Ex_c10441_17078853	QGrNoSplt.1&2.Bot.aww-2B1	Primary	3	Heat	7.64	20.16	0.49	W
				QAwnL.Mat.aww-2B1	Secondary	9	Heat	3.51	10.32	0.16	D
				QGrNoSplt.>2.Bot.aww-2B1	Primary	9	Heat	5.50	18.01	0.06	W
		56.78	wsnp_Ex_c10441_17078853	QGrNoSplt.1&2.Mid.aww-2B1	Primary	3 and 9	Control	6.17	19.24	0.17	W
				QGrNoSplt.>2.Bot.aww-2B1	Primary	3	Tolerance	5.15	18.03	0.01	W
						9	Tolerance	5.17	18.11	0.05	W
		74.25	Ppd-B1	QAwnL.Mat.aww-2B1	Primary	3	Heat	4.68	9.50	0.15	D
						9	Heat	4.76	9.63	0.16	D
						3 and 9	Control	4.37	9.65	0.18	D
		79.52	wsnp_JD_c3732_4781170	QGrNoSplt.1&2.Top.aww-2B1	Primary	3	Tolerance	9.96	26.00	0.33	W
				QGrNoSplt.1&2.Mid.aww-2B1	Primary	3	Heat	12.61	32.15	0.63	W
				QGrNo.Spk.aww-2B1	Primary	9	Tolerance	22.25	48.75	4.96	W
				QGrNoSplt.1&2.Bot.aww-2B1	Primary	9	Heat	19.99	44.53	0.86	W
							Tolerance	17.74	42.74	0.74	W
				QGrNoSplt.1&2.Mid.aww-2B1	Primary	3	Tolerance	12.19	31.21	0.44	W
						9	Heat	12.22	29.51	0.65	W
							Tolerance	10.08	24.88	0.47	W
				QGrNoSplt.1&2.Top.aww-2B1	Primary	3	Heat	9.21	24.17	0.33	W
						9	Heat	11.54	26.29	0.37	W
							Tolerance	11.66	25.11	0.35	W
				QGrNo.Spk.aww-2B1	Secondary	3	Heat	3.81	4.98	1.47	W
					Primary	3	Tolerance	15.94	38.53	2.51	W
					Secondary	3	Tolerance	10.10	26.34	0.99	W
					Primary	9	Heat	21.35	46.70	5.32	W
					Secondary	9	Heat	10.51	23.90	3.07	W
							Tolerance	10.98	28.42	2.69	W
				QGrNoSplt.1&2.Bot.aww-2B1	Secondary	3	Heat	6.26	9.80	0.31	W
						9	Heat	9.81	20.22	0.47	W

Appendix table 4.3. Continued…

Linkage group	Assigned QTL number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
2B1	QTL7	79.52	wsnp_JD_c3732_4781170	QGrNoSplt.1&2.Bot.aww-2B1	Secondary	9	Tolerance	8.95	20.97	0.29	W
				QGrNoSplt.1&2.Top.aww-2B1	Secondary	9	Heat	7.07	18.70	0.38	W
							Tolerance	7.04	18.62	0.38	W
				QGrNoSplt.>2.Mid.aww-2B1	Primary	9	Heat	6.62	17.53	0.08	W
							Tolerance	8.88	23.34	0.08	W
					Secondary	9	Tolerance	4.01	10.36	0.04	W
				QGrNoSplt.Spk.aww-2B1	Secondary	3	Heat	6.04	15.07	0.16	W
					Primary	9	Heat	20.68	45.57	0.33	W
					Secondary	9	Heat	9.23	21.36	0.43	W
					Primary	9	Tolerance	21.39	47.23	0.32	W
					Secondary	9	Tolerance	11.89	30.54	0.40	W
		84.80	wsnp_RFL_Contig4483_5312236	QGrNoSplt.1&2.Bot.aww-2B1	Primary	3	Tolerance	9.52	24.77	0.22	W
				QGrNo.Spk.aww-2B1	Primary	3	Heat	7.53	15.04	3.06	W
				QGrNoSplt.1&2.Top.aww-2B1	Secondary	3	Tolerance	3.58	9.10	0.07	W
				QGrNoSplt.Spk.aww-2B1	Primary	3	Heat	9.17	21.72	0.20	W
							Tolerance	12.80	32.38	0.17	W
		93.92	wsnp_Ex_c13351_21042379	QGrNoSplt.1&2.Bot.aww-2B1	Primary	3 and 9	Control	3.68	5.91	0.15	W
2D4	QTL9	7.58	wsnp_Ra_c4712_8489753	QSpkL.Mat.aww-2D4	Secondary	3	Heat	5.34	14.03	0.16	D
						3	Tolerance	3.61	9.24	0.01	D
						9	Heat	4.89	12.81	0.16	D
						3 and 9	Control	4.89	12.81	0.14	D
		13.58	wsnp_RFL_Contig3286_3338919/wsnp_Ku_c30494_40319867	QSpkL.Mat.aww-2D4	Primary	9	Heat	5.35	12.34	0.17	D
						3 and 9	Control	5.29	12.21	0.18	D
		18.84	wsnp_Ku_c30494_40319867	QSpkL.Mat.aww-2D4	Primary	3	Heat	3.19	7.11	0.14	D
3B2	QTL12	80.92	wsnp_Ex_c9594_15882022	QGrNoSplt.1&2.Bot.aww-3B2	Secondary	3	Tolerance	4.07	10.65	0.12	W
		96.32	wsnp_Ex_rep_c101457_86818160	QHt.Mat.aww-3B2	Primary	9	Heat	4.34	0.59	1.43	W
						3 and 9	Control	3.07	0.51	1.63	W
						3	Heat	3.31	0.57	1.44	W
				QHt.PreH.aww-3B2	Primary	3	Control	2.41	0.58	0.76	W
		105.86	wsnp_Ex_rep_c101457_86818160	QGrNoSplt.1&2.Bot.aww-3B2	Secondary	9	Tolerance	5.03	14.46	0.24	W

Appendix table 4.3. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
4A2	QTL13	18.55	wsnp_Ex_c24474_33721784/wsnp_Ku_c1205_2398925	QGrNo.Spk.aww-4A2	Secondary	3	Heat	3.94	6.76	1.71	W
						3 and 9	Control	4.16	7.53	1.41	W
		31.43	wsnp_Ex_c4068_7351806	QAIL.PreH.aww-4A2	Primary	9	Heat	2.48	3.55	0.95	W
				QNoDevSplt.Spk.aww-4A2	Primary	3 and 9	Control	3.60	7.10	0.37	W
					Secondary	3 and 9	Control	5.17	8.64	0.25	W
				QNoDevSplt.Bot.aww-4A2	Secondary	3	Heat	4.58	7.83	0.11	W
				QNoDevSplt.Mid.aww-4A2	Secondary	3	Heat	3.96	8.21	0.12	W
						3 and 9	Control	3.86	6.32	0.07	W
				QNoDevSplt.Top.aww-4A2	Secondary	3	Heat	4.21	7.44	0.10	W
						3 and 9	Control	3.90	5.96	0.07	W
				QNoDevSplt.Spk.aww-4A2	Primary	3	Heat	3.58	6.68	0.45	W
		38.68	wsnp_BE442666A_Ta_2_1	QHt.PreH.aww-4A2	Secondary	9	Heat	7.18	2.67	1.75	W
		39.39	wsnp_Ex_c829_1621908	QPlHt.GroPostH.aww-4A2	Primary	3	Heat	3.63	1.17	1.00	W
				QAIL.Mat.aww-4A2	Primary	3	Heat	4.12	2.02	0.44	W
		41.55	wsnp_Ex_rep_c68569_67411985	QHt.Mat.aww-4A2	Primary	9	Heat	7.73	1.15	2.00	W
						3 and 9	Control	5.86	1.05	2.35	W
				QAIL.GroPostH.aww-4A2	Primary	3	Heat	3.91	2.70	0.51	W
				QAIL.Mat.aww-4A2	Primary	9	Heat	4.27	1.52	0.34	W
							Tolerance	4.27	1.52	0.00	D
				QAIL.Mat.aww-4A2	Primary	3 and 9	Control	4.27	1.52	0.58	W
					Secondary	3	Heat	4.09	2.09	2.81	W
							Tolerance	4.09	2.09	0.00	D
						9	Heat	4.09	2.07	2.64	W
						3 and 9	Control	4.09	2.09	3.33	W
					Primary	3	Heat	5.83	1.08	1.98	W
		42.27	wsnp_RFL_Contig25_2082245	QHt.PreH.aww-4A2	Primary	3	Control	5.52	1.56	1.25	W
							Heat	5.33	1.62	1.29	W
						9	Control	5.33	1.62	1.43	W
							Heat	6.33	1.48	1.35	W
		42.99	wsnp_Ex_c1373_2628597	QHt.PreH.aww-4A2	Secondary	3	Control	6.60	2.39	1.39	W
							Heat	6.60	2.39	1.33	W
						9	Control	6.60	2.39	1.64	W
		45.87	wsnp_BE403900A_Ta_2_1	QProUndsplt.aww-4A2	Secondary	3	Heat	4.083	7.327	0.019	D
		69.99	wsnp_RFL_Contig2771_2524880	QPlHt.GroPostH.aww-4A2	Primary	9	Control	3.72	1.34	1.31	W

Appendix table 4.3. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
4B	QTL15	62.94	wsnp_CAP7_c1723_854530/wsnp_Ex_c18433_27269748	QAIL.PreH.aww-4B	Primary	3	Heat	3.92	8.15	1.41	W
		71.55	wsnp_Ex_c18433_27269748	QNoDevSplt.Top.aww-4B	Secondary	3	Heat	6.23	13.37	0.13	D
				QGrNo.Spk.aww-4B	Secondary	9	Heat	6.68	14.43	2.39	D
				QGrNoSplt.1&2.Bot.aww-4B	Secondary	9	Heat	8.31	17.35	0.43	D
				QGrNoSplt.1&2.Top.aww-4B	Secondary	3	Heat	4.44	12.22	0.13	D
						3 and 9	Control	3.97	9.42	0.13	D
				QGrNoSplt.>2.Mid.aww-4B	Primary	3	Heat	3.62	8.93	0.06	D
						3 and 9	Control	1.94	3.88	0.03	D
				QGrNoSplt.Spk.aww-4B	Secondary	9	Heat	6.12	13.65	0.34	D
		77.72	wsnp_Ex_c18433_27269748/Rht-B1	QGrNoSplt.1&2.Bot.aww-4B	Primary	3	Heat	10.99	24.09	0.53	D
				QAIL.PreH.aww-4B	Primary	3	Control	4.24	7.90	1.36	W
				QNoDevSplt.Spk.aww-4B	Secondary	3 and 9	Control	5.67	11.31	0.28	D
				QNoDevSplt.Bot.aww-4B	Secondary	3	Heat	4.64	9.86	0.12	D
						3 and 9	Control	6.29	13.74	0.11	D
				QNoDevSplt.Mid.aww-4B	Secondary	9	Heat	2.08	5.22	0.09	D
						3 and 9	Control	5.07	10.30	0.08	D
				QProUndsplt.aww-4B	Secondary	3	Heat	10.39	26.44	0.036	W
								9.844	26.05	0.04	W
				QGrNoSplt.1&2.Bot.aww-4B	Primary	3 and 9	Control	11.77	26.67	0.32	D
				QGrNo.Spk.aww-4B	Primary	3	Heat	6.27	13.60	2.91	D
					Secondary	3	Heat	13.08	26.09	3.37	D
					Primary	3 and 9	Control	8.25	18.40	2.56	D
					Secondary	3 and 9	Control	11.97	24.29	2.53	D
				QGrNoSplt.1&2.Bot.aww-4B	Secondary	3	Heat	12.92	27.24	0.52	D
						3 and 9	Control	12.06	29.89	0.32	D
				QGrNoSplt.>2.Bot.aww-4B	Primary	3	Heat	1.93	3.59	0.03	D
						3 and 9	Control	2.77	5.58	0.04	D
				QGrNoSplt.Spk.aww-4B	Secondary	3	Heat	8.90	22.81	0.45	D
					Primary	3 and 9	Control	8.06	20.25	0.12	D
					Secondary	3 and 9	Control	7.69	18.15	0.43	D
				QPlHt.GroPostH.aww-4B	Secondary	3	Control	11.80	29.13	2.04	D
		83.90	Rht-B1	QAIL.GroPostH.aww-4B	Primary	9	Control	43.23	47.52	2.86	D
				QHt.PreH.aww-4B	Primary	9	Control	45.14	32.84	6.46	D
				QPlHt.GroPostH.aww-4B	Primary	3	Heat	50.69	47.47	6.35	D

Appendix table 4.3. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
4B	QTL15	83.90	Rht-B1	QPedL.Mat.aww-4B	Primary	3	Heat	33.30	44.16	4.89	D
				QHt.Mat.aww-4B	Primary	9	Heat	70.84	42.26	12.12	D
						3 and 9	Control	64.95	42.31	14.90	D
					Secondary	3	Heat	27.36	40.65	2.25	D
						9	Heat	27.76	40.39	1.78	D
						3 and 9	Control	31.30	42.99	3.05	D
				QAIL.GroPostH.aww-4B	Secondary	3	Control	38.42	49.32	3.22	D
					Primary	3	Heat	31.13	40.51	1.99	D
					Secondary	3	Heat	37.21	49.47	2.41	D
						9	Control	38.42	49.32	3.16	D
					Primary	9	Heat	45.05	48.37	1.76	D
					Secondary	9	Heat	15.64	28.26	1.49	D
				QAIL.Mat.aww-4B	Primary	3	Heat	38.05	40.67	1.96	D
						9	Heat	47.02	44.51	1.82	D
							Tolerance	47.01	44.51	0.00	W
						3 and 9	Control	47.02	44.51	3.13	D
				QDay.AILtoAnth.aww-4B	Primary	9	Control	10.76	23.48	0.51	D
							Heat	10.62	23.27	0.51	D
				QHt.PreH.aww-4B	Primary	3	Control	46.97	33.05	5.77	D
					Secondary	3	Control	42.27	33.20	5.16	D
					Primary	3	Heat	45.14	32.84	5.81	D
					Secondary	3	Heat	42.27	33.20	4.97	D
						9	Control	42.27	33.20	6.11	D
					Primary	9	Heat	49.37	45.32	7.47	D
					Secondary	9	Heat	43.25	34.95	6.33	D
				QPlHt.GroPostH.aww-4B	Primary	3	Control	44.62	49.90	9.17	D
						9	Control	53.08	45.64	7.66	D
							Heat	43.07	45.03	5.13	D
				QPedL.Mat.aww-4B	Secondary	3	Heat	34.49	45.63	5.46	D
					Primary	9	Heat	26.27	39.21	4.25	D
					Secondary	9	Heat	32.67	44.91	4.89	D
					Primary	3 and 9	Control	33.27	44.24	5.81	D
					Secondary	3 and 9	Control	34.19	45.35	5.99	D
				QHt.Mat.aww-4B	Secondary	3	Heat	37.45	41.17	12.47	D

Appendix table 4.3. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
4B	QTL15	83.90	Rht-B1	QHt.Mat.aww-4B	Secondary	3	Tolerance	37.45	41.17	0.00	W
						9	Heat	37.57	40.94	11.74	D
						3 and 9	Control	37.45	41.17	14.80	D
					Primary	3	Heat	64.07	41.93	12.30	D
				QUndvSplt.Spk.aww-4B	Primary	3	Heat	16.89	26.34	0.51	W
							Tolerance	16.89	26.34	0.00	D
						9	Heat	16.89	26.34	0.84	W
							Tolerance	16.88	26.33	0.00	D
						3 and 9	Control	16.89	26.34	0.92	W
					Secondary	3	Heat	14.18	25.30	0.55	W
						9	Heat	13.23	23.74	0.66	W
						3 and 9	Control	10.67	19.13	0.68	W
				QProUndsplt.aww-4B	Primary	3	Heat	11.28	26.33	0.033	W
		84.69	wsnp_Ex_c14026_21924297	QNoDevSplt.Top.aww-4B	Secondary	3 and 9	Control	6.98	13.02	0.10	D
				QPlHt.GroPostH.aww-4B	Secondary	9	Control	17.56	31.36	2.66	D
				QProUndsplt.aww-4B	Primary	3	Control	11.72	22.87	0.041	W
						9	Heat	12.27	25.9	0.04	W
					Secondary	9	Control	13.97	31.27	0.045	W
		86.26	wsnp_RFL_Contig4151_4728831	QProUndsplt.aww-4B	Primary	9	Control	6.919	16.02	0.034	W
		108.33	wsnp_CAP12_rep_c4278_1949864		Secondary	3	Control	6.18	22.2	0.038	W
4B	QTL16	117.19	wsnp_Ex_c39876_47057394	QHt.PreH.aww-4B	Primary	9	Heat	5.80	2.83	1.87	W
		127.49	wsnp_Ku_c11570_18860306	QGrNoSplt.1&2.Mid.aww-4B	Primary	3 and 9	Control	6.00	13.59	0.14	D
					Secondary	3	Heat	4.29	9.32	0.04	D
							Tolerance	4.28	9.35	0.00	D
						3 and 9	Control	4.29	9.32	0.03	D
				QGrNoSplt.>2.Bot.aww-4B	Primary	3	Heat	8.25	17.36	0.06	D
					Secondary	3	Heat	9.13	21.77	0.06	D
						9	Heat	7.92	18.94	0.05	D
					Primary	3 and 9	Control	8.01	17.29	0.07	D
					Secondary	3 and 9	Control	7.48	19.26	0.07	D
				QGrNoSplt.>2.Mid.aww-4B	Primary	3 and 9	Control	3.94	8.59	0.05	D
				QPlHt.GroPostH.aww-4B	Primary	9	Tolerance	4.68	11.94	0.55	D
				QUndvSplt.Spk.aww-4B	Secondary	3 and 9	Control	6.95	11.52	0.53	W
				QProUndsplt.aww-4B	Primary	9	Control	3.649	7.176	0.022	W

Appendix table 4.3. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
4B	QTL16	127.49	wsnp_Ku_c11570_18860306	QNoSplt.Spk.aww-4B	Primary	3	Heat	8.993	21.88	1.11	W
					Secondary	3	Control	12.64	28.87	1.174	W
						9	Control	12.58	29.59	1.189	W
		135.47	wsnp_Ex_c4148_7495656	QAIL.PreH.aww-4B	Primary	9	Control	17.44	41.14	3.13	W
							Heat	17.10	38.69	3.14	W
				QDay.AwnEm.aww-4B	Primary	9	Heat	17.43	39.64	3.03	W
				QDay.Anth.aww-4B	Primary	3	Heat	14.66	33.47	2.86	W
						3 and 9	Control	15.54	35.34	2.83	W
				QAIL.PreH.aww-4B	Primary	3	Control	15.43	33.43	2.79	W
							Heat	14.77	32.54	2.82	W
				QDay.AwnEm.aww-4B	Primary	3	Heat	17.10	39.02	2.88	W
						3 and 9	Control	17.33	39.46	2.90	W
				QDay.Anth.aww-4B	Primary	9	Heat	14.86	36.11	2.86	W
						3 and 9	Control	15.54	35.34	2.83	W
				QNoDevSplt.Bot.aww-4B	Secondary	3	Heat	5.70	10.76	0.13	W
						3 and 9	Control	5.04	9.10	0.09	W
				QNoDevSplt.Top.aww-4B	Secondary	3	Heat	5.17	10.06	0.12	W
						3 and 9	Control	5.48	9.70	0.09	W
				QNoDevSplt.Spk.aww-4B	Primary	3	Heat	3.60	6.71	0.45	W
						9	Heat	3.49	6.76	0.35	W
				QPedL.Mat.aww-4B	Secondary	3	Tolerance	5.87	15.14	0.07	D
				QUndvSplt.Spk.aww-4B	Primary	3	Heat	8.71	11.58	0.34	W
							Tolerance	8.71	11.57	0.00	D
						9	Heat	8.71	11.58	0.56	W
							Tolerance	8.71	11.58	0.00	D
						3 and 9	Control	8.71	11.58	0.61	W
					Secondary	3	Heat	9.03	14.12	0.41	W
						9	Heat	8.99	14.41	0.51	W
				QNoSplt.Spk.aww-4B	Primary	3	Control	11.5	26.19	1.216	W
						9	Control	13.46	33.2	1.343	W
							Heat	16.39	35.56	1.308	W
					Secondary	3	Heat	8.989	23.04	1.235	W
						9	Heat	12.86	31.91	1.286	W
				QProUndsplt.aww-4B	Primary	3	Control	3.342	5.07	0.019	W

Appendix table 4.3. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
4B	QTL16	141.25	wsnp_BE403378B_Ta_2_1	QAIL.Mat.aww-4B	Primary	9	Heat	4.85	1.82	0.37	D
							Tolerance	4.85	1.82	0.00	W
						3 and 9	Control	4.85	1.82	0.63	D
				QGrNoSplt.>2.Bot.aww-4B	Primary	9	Heat	5.32	11.33	0.05	D
4D	QTL17	0.00	Rht-D1	QAIL.GroPostH.aww-4D	Primary	9	Control	40.44	40.40	2.64	W
				QNoDevSplt.Bot.aww-4D	Primary	3	Heat	5.10	13.11	0.19	W
				QGrNoSplt.1&2.Bot.aww-4D	Primary	3	Heat	8.57	15.15	0.42	W
				QHt.PreH.aww-4D	Primary	9	Control	59.97	59.96	8.73	W
				QPlHt.GroPostH.aww-4D	Primary	3	Heat	46.31	39.05	5.76	W
				QNoDevSplt.Spk.aww-4D	Primary	3 and 9	Control	8.82	21.03	0.63	W
				QPedL.Mat.aww-4D	Primary	3	Heat	29.46	36.00	4.42	W
				QHt.Mat.aww-4D	Primary	9	Heat	76.90	51.70	13.40	W
						3 and 9	Control	70.00	51.31	16.41	W
				QAIL.Mat.aww-4D	Secondary	3	Heat	25.53	36.44	2.13	W
						9	Heat	26.58	37.62	1.71	W
						3 and 9	Control	29.44	38.80	2.90	W
				QAIL.GroPostH.aww-4D	Secondary	3	Control	32.93	37.69	2.82	W
					Primary	3	Heat	26.99	32.48	1.78	W
					Secondary	3	Heat	30.94	36.13	2.06	W
					Secondary	9	Control	32.93	37.69	2.76	W
					Primary	9	Heat	41.46	39.74	1.59	W
					Secondary	9	Heat	16.96	31.35	1.57	W
				QAIL.Mat.aww-4D	Primary	3	Heat	36.14	36.64	1.86	W
						9	Heat	46.05	41.95	1.76	W
							Tolerance	46.04	41.94	0.00	D
						3 and 9	Control	46.05	41.95	3.04	W
				QNoDevSplt.Spk.aww-4D	Secondary	3 and 9	Control	6.82	11.99	0.29	W
						3 and 9	Control	6.82	11.99	0.29	W
				QNoDevSplt.Bot.aww-4D	Primary	9	Heat	5.87	15.17	0.18	W
						3 and 9	Control	5.88	15.20	0.17	W
					Secondary	3 and 9	Control	6.65	12.07	0.11	W
				QNoDevSplt.Mid.aww-4D	Primary	3	Heat	5.72	14.78	0.22	W
						9	Heat	6.74	17.44	0.19	W

Appendix table 4.3. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
4D	QTL17	0.00	Rht-D1	QNoDevSplt.Mid.aww-4D	Primary	3 and 9	Control	6.78	17.54	0.19	W
					Secondary	3 and 9	Control	7.40	13.88	0.10	W
				QNoDevSplt.Top.aww-4D	Primary	3	Heat	5.42	13.98	0.22	W
						9	Heat	5.77	14.91	0.16	W
					Secondary	9	Heat	4.01	8.56	0.12	W
					Primary	3 and 9	Control	5.64	14.55	0.18	W
					Secondary	3 and 9	Control	8.63	15.64	0.11	W
				QGrNoSplt.1&2.Bot.aww-4D	Primary	3 and 9	Control	8.99	16.51	0.25	W
				QGrNoSplt.1&2.Top.aww-4D	Primary	9	Tolerance	6.81	12.43	0.25	D
				QGrNo.Spk.aww-4D	Primary	3	Heat	6.30	11.94	2.73	W
					Secondary	3	Heat	12.29	20.53	2.99	W
					Primary	3 and 9	Control	13.08	28.03	3.16	W
					Secondary	3 and 9	Control	15.09	27.64	2.70	W
				QGrNoSplt.1&2.Bot.aww-4D	Secondary	3	Heat	6.81	10.67	0.33	W
						3 and 9	Control	8.91	17.51	0.25	W
					Primary	3	Heat	4.74	7.67	0.04	W
						3 and 9	Control	5.07	8.59	0.05	W
				QGrNoSplt.>2.Mid.aww-4D	Secondary	3	Tolerance	5.90	15.24	0.00	W
					Primary	3 and 9	Control	3.85	7.45	0.05	W
					Secondary	3 and 9	Control	6.10	15.78	0.04	W
				QGrNoSplt.Spk.aww-4D	Secondary	3	Heat	7.79	16.57	0.39	W
					Primary	3 and 9	Control	8.55	18.62	0.11	W
					Secondary	3 and 9	Control	10.89	23.85	0.50	W
				QHt.PreH.aww-4D	Primary	3	Control	61.54	59.33	7.73	W
					Secondary	3	Control	54.21	55.28	6.66	W
					Primary	3	Heat	59.97	59.96	7.85	W
					Secondary	3	Heat	54.21	55.28	6.41	W
						9	Control	54.21	55.28	7.89	W
					Primary	9	Heat	66.95	60.78	8.65	W
					Secondary	9	Heat	52.27	51.90	7.71	W
				QPlHt.GroPostH.aww-4D	Primary	3	Control	38.95	38.44	8.05	W
						9	Control	51.44	41.96	7.34	W
					Secondary	9	Control	15.60	26.56	2.45	W
					Primary	9	Heat	39.73	38.32	4.74	W

Appendix table 4.3. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
4D	QTL17	0.00	Rht-D1	QNoDevSplt.Spk.aww-4D	Primary	3	Heat	7.97	17.54	0.72	W
						9	Heat	9.09	21.68	0.63	W
				QPedL.Mat.aww-4D	Secondary	3	Heat	31.36	38.81	5.04	W
					Primary	9	Heat	25.35	37.00	4.13	W
					Secondary	9	Heat	29.34	37.59	4.47	W
					Primary	3 and 9	Control	29.35	35.90	5.24	W
					Secondary	3 and 9	Control	31.23	38.88	5.55	W
				QHt.Mat.aww-4D	Secondary	3	Heat	39.29	44.85	13.01	W
							Tolerance	39.29	44.85	0.00	D
						9	Heat	39.76	45.31	12.35	W
						3 and 9	Control	39.29	44.85	15.45	W
					Primary	3	Heat	69.24	51.06	13.58	W
				QUndvSplt.Spk.aww-4D	Primary	3	Heat	10.55	13.43	0.36	D
							Tolerance	10.54	13.42	0.00	W
						9	Heat	10.55	13.43	0.60	D
							Tolerance	10.54	13.42	0.00	W
						3 and 9	Control	10.55	13.43	0.66	D
					Secondary	3	Heat	8.31	12.11	0.38	D
						9	Heat	8.28	12.38	0.47	D
						3 and 9	Control	7.07	9.64	0.48	D
				QProUndsplt.aww-4D	Primary	3	Control	8.363	13.92	0.032	D
							Heat	6.01	12.09	0.022	D
						9	Control	6.698	12.41	0.03	D
							Heat	10.27	20.51	0.035	D
					Secondary	9	Control	7.582	14.41	0.03	D
							Heat	6.423	13.22	0.028	D
		2.90	wsnp_CAP11_c356_280910	QGrNoSplt.1&2.Top.aww-4D	Primary	3 and 9	Control	3.77	9.46	0.14	W
				QGrNoSplt.1&2.Mid.aww-4D	Secondary	3	Heat	6.70	15.95	0.06	W
							Tolerance	6.44	15.33	0.00	W
						3 and 9	Control	6.70	15.95	0.04	W
				QGrNoSplt.1&2.Top.aww-4D	Secondary	3 and 9	Control	5.73	13.59	0.15	W
				QGrNoSplt.>2.Mid.aww-4D	Secondary	3	Heat	5.96	15.35	0.06	W
				QPlHt.GroPostH.aww-4D	Secondary	3	Control	9.72	19.48	1.67	W
		7.95	wsnp_Ex_rep_c107564_91144523	QDay.AILtoAnth.aww-4D	Primary	9	Control	9.26	19.32	0.47	W

Appendix table 4.3. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
4D	QTL17	7.95	wsnp_Ex_rep_c107564_91144523	QDay.AILtoAnth.aww-4D	Primary	9	Heat	9.09	19.03	0.46	W
				QGrNoSplt.1&2.Top.aww-4D	Primary	9	Heat	4.93	9.05	0.22	D
5D2	QTL20	40.23	wsnp_JD_c12424_12667585	QAwnL.Mat.aww-5D2	Primary	3	Tolerance	3.51	11.32	0.01	W
6A	QTL21	66.03	wsnp_Ex_c1104_2118684	QAwnL.Mat.aww-6A	Primary	3	Heat	4.42	8.71	0.14	D
					Secondary		Heat	3.80	8.12	0.14	D
					Primary	9	Heat	4.33	8.46	0.15	D
					Secondary		Heat	3.90	7.69	0.13	D
					Primary	3 and 9	Control	3.76	7.85	0.16	D
		69.95	wsnp_JD_c10969_11530496	QPedL.Mat.aww-6A	Primary	3	Heat	3.99	2.69	1.21	D
						3 and 9	Control	3.94	2.66	1.42	D
				QHt.Mat.aww-6A	Primary	3 and 9	Control	4.42	0.77	2.01	D
						3	Heat	4.80	0.88	1.78	D
		70.74	wsnp_JD_rep_c62949_40140212	QHt.Mat.aww-6A	Primary	9	Heat	5.66	0.81	1.68	D
				QProUndsplt.aww-6A	Primary	3	Control	2.621	3.608	0.016	W
		74.67	wsnp_CAP8_c5350_2554478	QAIL.GroPostH.aww-6A	Primary	9	Control	6.32	3.07	0.73	D
						3	Heat	3.34	2.26	0.47	D
						9	Heat	6.05	2.73	0.42	D
				QAIL.Mat.aww-6A	Primary	3	Heat	3.89	1.90	0.42	D
				QPlHt.GroPostH.aww-6A	Primary	3	Heat	6.17	2.24	1.38	D
						9	Control	6.92	2.28	1.71	D
							Heat	6.05	2.84	1.29	D
				QUndvSplt.Spk.aww-6A	Primary	3	Heat	4.14	4.51	0.21	W
							Tolerance	4.14	4.52	0.00	D
						9	Heat	4.14	4.51	0.35	W
							Tolerance	4.14	4.51	0.00	D
						3 and 9	Control	4.14	4.51	0.38	W
		87.71	wsnp_Ex_c6870_11844501	QUndvSplt.Spk.aww-6A	Secondary	3 and 9	Control	3.75	6.29	0.39	W
7A2	QTL25	24.33	wsnp_Ku_c139_279238	QAIL.Mat.aww-7A2	Primary	3	Heat	5.31	3.85	0.60	D
				QHt.Mat.aww-7A2	Primary	9	Heat	6.85	1.43	2.23	D
				QPlHt.GroPostH.aww-7A2	Primary	3	Heat	4.08	1.84	1.25	D
							Control	3.80	2.32	1.98	D

Appendix table 4.3. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
7A2	QTL25	33.74	wsnp_Ex_c2268_4251636	QAIL.GroPostH.aww-7A2	Primary	9	Control	4.32	2.61	0.67	D
							Heat	5.68	3.47	0.47	D
				QAIL.Mat.aww-7A2	Primary	9	Heat	5.80	3.04	0.47	D
							Tolerance	5.80	3.04	0.00	W
						3 and 9	Control	5.80	3.04	0.82	D
						3 and 9	Control	7.36	2.01	3.24	D
				QPlHt.GroPostH.aww-7A2	Primary	9	Control	5.40	2.25	1.70	D
							Heat	3.67	2.14	1.12	D
	QTL26	43.15	wsnp_Ex_c2268_4251636	QGrNoSplt.1&2.Top.aww-7A2	Primary	9	Tolerance	4.00	6.59	0.18	W
		46.03	wsnp_Ex_c40247_47349166	QNoDevSplt.Spk.aww-7A2	Secondary	3 and 9	Control	4.33	7.00	0.22	W
		54.67	wsnp_Ku_c4591_8286583	QNoDevSplt.Bot.aww-7A2	Secondary	3	Heat	4.59	7.92	0.11	W
				QNoDevSplt.Mid.aww-7A2	Secondary	3	Heat	4.56	9.80	0.14	W
				QNoDevSplt.Spk.aww-7A2	Secondary	3	Heat	4.63	10.79	0.39	W
		54.67	wsnp_Ku_c4591_8286583	QNoDevSplt.Spk.aww-7A2	Secondary	9	Heat	5.08	11.09	0.40	W
		56.11	wsnp_Ex_c1482_2834254	QNoDevSplt.Mid.aww-7A2	Secondary	9	Heat	3.02	7.11	0.10	W
				QNoDevSplt.Top.aww-7A2	Secondary	9	Heat	4.60	10.04	0.13	W
		66.97	wsnp_Ex_c12102_19361467	QGrNo.Spk.aww-7A2	Primary	9	Tolerance	3.98	5.36	1.64	W
					Secondary	3	Heat	4.61	6.05	1.62	W
					Primary	9	Heat	4.70	6.67	2.01	W
					Secondary	3 and 9	Control	3.48	4.50	1.09	W
				QGrNoSplt.1&2.Mid.aww-7A2	Primary	9	Heat	3.88	7.06	0.32	W
							Tolerance	3.47	6.63	0.24	W
				QGrNoSplt.1&2.Top.aww-7A2	Primary	9	Heat	3.62	6.19	0.18	W
				QGrNoSplt.Spk.aww-7A2	Primary	9	Heat	4.65	6.73	0.13	W
							Tolerance	4.05	5.62	0.11	W
				QNoDevSplt.Bot.aww-7A2	Secondary	3 and 9	Control	4.58	7.63	0.08	W
				QNoDevSplt.Mid.aww-7A2	Secondary	3 and 9	Control	3.41	5.39	0.06	W
				QNoDevSplt.Top.aww-7A2	Secondary	3	Heat	4.60	8.32	0.11	W
						3 and 9	Control	6.77	11.64	0.09	W
7B	QTL27	34.87	#N/A	QAwnL.Mat.aww-7B	Secondary	3 and 9	Control	4.89	18.91	0.25	D
		44.32	wsnp_Ex_c24376_33619527		Primary	3	Heat	4.91	10.25	0.15	D
						9	Heat	5.04	10.51	0.17	D
						3 and 9	Control	4.99	11.39	0.20	D

Appendix table 4.3. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
7B	QTL27	45.06	wsnp_Ra_c1654_3265291	QAwnL.Mat.aww-7B	Secondary	3	Heat	7.03	17.64	0.20	D
		45.06	wsnp_Ra_c1654_3265291			9	Heat	7.87	18.61	0.21	D
		45.80	wsnp_Ex_rep_c68815_67687712				Tolerance	3.66	9.45	0.05	D
		56.94	wsnp_CAP7_c90_52035	QDay.AILtoAnth.aww-7B	Primary	3	Heat	5.89	15.75	0.26	D

Appendix table 4. 4 Summary of QTLs detected in the Drysdale × Waagan DH population sorted by Linkage group, assigned QTL number, positon of each QTL, closest marker(s), traits/QTL name, tiller, stage (AIL in cm), treatment (in control, heat-treated or tolerance, their LOD score, percentage of explained variation (R^2), additive effect, and high value allele (Drysdale, D; Waagan, W) are presented. Yellow highlights indicate QTL co-localized with QTL for heat response for fertility.

Linkage group	Assigned QTL number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
1A1	QTL1	59.67	wsnp_Ex_c1997_3756118	QPlHt.GroPostH.aww-1A1	Secondary	9	Control	3.84	4.65	1.02	W
		66.15	wsnp_BE517729A_Ta_2_1	QHt.Mat.aww-1A1	Primary	3 and 9	Control	2.25	0.32	1.30	W
1B	QTL2	26.24	wsnp_Ex_c14273_22230844	QGrNoSplt.>2.Bot.aww-1B	Primary	3	Heat	2.44	3.46	0.03	D
						9	Heat	5.37	11.35	0.05	D
		53.21	wsnp_Ku_c34980_44256215	QGrNo.Spk.aww-1B	Primary	3	Tolerance	3.58	8.05	1.15	D
		62.46	wsnp_Ex_c22377_31571527	QGrNoSplt.Spk.aww-1B	Secondary	3	Tolerance	3.74	8.33	0.12	D
		69.00	wsnp_Ex_c23235_32471358		Primary	3	Heat	4.02	7.80	0.12	D
2A	QTL3	0.00	wsnp_Ex_c2772_5130007	QNoDevSplt.Spk.aww-2A	Secondary	9	Heat	3.73	8.02	0.34	W
				QNoDevSplt.Top.aww-2A			Heat	2.95	6.24	0.10	W
	QTL4	70.54	wsnp_CAP12_c1269_649827	QSpkL.Mat.aww-2A	Primary	9	Heat	5.23	10.77	0.16	D
						3 and 9	Control	5.07	10.39	0.16	D
		74.99	wsnp_Ex_c42720_49228237	QGrNoSplt.>2.Bot.aww-2A	Primary	3	Heat	4.20	6.86	0.04	W
					Secondary	3	Heat	4.77	10.12	0.04	W
						9	Heat	4.68	10.25	0.04	W
					Primary	3 and 9	Control	4.26	7.17	0.05	W
		77.95	wsnp_Ex_rep_c102538_87682273	QGrNoSplt.>2.Mid.aww-2A	Primary	3	Heat	3.64	8.52	0.06	W
						3 and 9	Control	3.94	7.91	0.05	W
		93.58	wsnp_Ex_rep_c105158_89662129	QUndvSplt.Spk.aww-2A	Secondary	3	Tolerance	3.71	9.56	0.09	D
						9	Tolerance	3.52	9.03	0.14	D
		96.55	wsnp_Ku_c8927_15048149	QProUndsplt.aww-2A	Secondary	3	Heat	3.476	6.146	0.017	D
				QNoDevSplt.Spk.aww-2A			Tolerance	4.12	10.65	0.22	W
		97.29	wsnp_Ku_c4271_7774388	QNoDevSplt.Bot.aww-2A	Secondary	3	Tolerance	3.66	9.35	0.07	W
	QTL5	101.00	wsnp_JD_c15127_14676522	QAIL.GroPostH.aww-2A	Primary	3	Heat	4.16	2.90	0.53	D
		108.44	wsnp_Ex_c3808_6924802	QAIL.Mat.aww-2A	Primary	3	Heat	4.57	2.27	0.46	D

Appendix table 4.4. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
2A	QTL5	108.44	wsnp_Ex_c3808_6924802	QPlHt.GroPostH.aww-2A	Primary	3	Heat	4.70	1.59	1.16	D
							Control	2.64	1.08	1.35	D
						9	Control	4.22	1.24	1.26	D
							Heat	3.77	1.62	0.97	D
		110.66	wsnp_RFL_Contig4779_5764326	QHt.Mat.aww-2A	Primary	9	Heat	6.43	0.91	1.78	D
		146.98	wsnp_CAP11_c360_282889		Secondary	9	Tolerance	3.91	10.18	0.20	D
2B1	QTL6	26.05	wsnp_Ra_rep_c106119_89961852	QDay.Anth.aww-2B1	Primary	3	Heat	3.89	8.67	1.46	D
						3 and 9	Control	3.67	7.92	1.34	D
		41.41	wsnp_Ra_rep_c106119_89961852	QDay.AwnEm.aww-2B1	Primary	9	Heat	3.62	7.71	1.34	D
						3	Heat	3.62	7.78	1.29	D
						3 and 9	Control	3.60	7.67	1.28	D
	QTL7	49.10	wsnp_Ra_rep_c106119_89961852/wsnp_Ex_c10441_17078853	QGrNoSplt.1&2.Bot.aww-2B1	Primary	3	Heat	7.64	20.16	0.49	W
				QAwnL.Mat.aww-2B1	Secondary	9	Heat	3.51	10.32	0.16	D
				QGrNoSplt.>2.Bot.aww-2B1	Primary	9	Heat	5.50	18.01	0.06	W
		56.78	wsnp_Ex_c10441_17078853	QGrNoSplt.1&2.Mid.aww-2B1	Primary	3 and 9	Control	6.17	19.24	0.17	W
				QGrNoSplt.>2.Bot.aww-2B1	Primary	3	Tolerance	5.15	18.03	0.01	W
						9	Tolerance	5.17	18.11	0.05	W
		74.25	Ppd-B1	QAwnL.Mat.aww-2B1	Primary	3	Heat	4.68	9.50	0.15	D
						9	Heat	4.76	9.63	0.16	D
						3 and 9	Control	4.37	9.65	0.18	D
		79.52	wsnp_JD_c3732_4781170	QGrNoSplt.1&2.Top.aww-2B1	Primary	3	Tolerance	9.96	26.00	0.33	W
				QGrNoSplt.1&2.Mid.aww-2B1	Primary	3	Heat	12.61	32.15	0.63	W
				QGrNo.Spk.aww-2B1	Primary	9	Tolerance	22.25	48.75	4.96	W
				QGrNoSplt.1&2.Bot.aww-2B1	Primary	9	Heat	19.99	44.53	0.86	W
							Tolerance	17.74	42.74	0.74	W
				QGrNoSplt.1&2.Mid.aww-2B1	Primary	3	Tolerance	12.19	31.21	0.44	W
						9	Heat	12.22	29.51	0.65	W
							Tolerance	10.08	24.88	0.47	W
				QGrNoSplt.1&2.Top.aww-2B1	Primary	3	Heat	9.21	24.17	0.33	W
						9	Heat	11.54	26.29	0.37	W
							Tolerance	11.66	25.11	0.35	W
				QGrNo.Spk.aww-2B1	Secondary	3	Heat	3.81	4.98	1.47	W
					Primary	3	Tolerance	15.94	38.53	2.51	W

Appendix table 4.4. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
2B1	QTL7	79.52	wsnp_JD_c3732_4781170	QGrNo.Spk.aww-2B1	Secondary	3	Tolerance	10.10	26.34	0.99	W
					Primary	9	Heat	21.35	46.70	5.32	W
					Secondary	9	Heat	10.51	23.90	3.07	W
							Tolerance	10.98	28.42	2.69	W
				QGrNoSplt.1&2.Bot.aww-2B1	Secondary	3	Heat	6.26	9.80	0.31	W
						9	Heat	9.81	20.22	0.47	W
							Tolerance	8.95	20.97	0.29	W
				QGrNoSplt.1&2.Top.aww-2B1	Secondary	9	Heat	7.07	18.70	0.38	W
							Tolerance	7.04	18.62	0.38	W
				QGrNoSplt.>2.Mid.aww-2B1	Primary	9	Heat	6.62	17.53	0.08	W
							Tolerance	8.88	23.34	0.08	W
					Secondary	9	Tolerance	4.01	10.36	0.04	W
				QGrNoSplt.Spk.aww-2B1	Secondary	3	Heat	6.04	15.07	0.16	W
					Primary	9	Heat	20.68	45.57	0.33	W
					Secondary	9	Heat	9.23	21.36	0.43	W
					Primary	9	Tolerance	21.39	47.23	0.32	W
					Secondary	9	Tolerance	11.89	30.54	0.40	W
		84.80	wsnp_RFL_Contig4483_5312236	QGrNoSplt.1&2.Bot.aww-2B1	Primary	3	Tolerance	9.52	24.77	0.22	W
				QGrNo.Spk.aww-2B1	Primary	3	Heat	7.53	15.04	3.06	W
				QGrNoSplt.1&2.Top.aww-2B1	Secondary	3	Tolerance	3.58	9.10	0.07	W
				QGrNoSplt.Spk.aww-2B1	Primary	3	Heat	9.17	21.72	0.20	W
							Tolerance	12.80	32.38	0.17	W
		93.92	wsnp_Ex_c13351_21042379	QGrNoSplt.1&2.Bot.aww-2B1	Primary	3 and 9	Control	3.68	5.91	0.15	W
2D3	QTL8	3.60	wsnp_JD_c5919_7081809	QHt.Mat.aww-2D3	Primary	9	Heat	5.33	0.76	1.62	D
				QHt.PreH.aww-2D3	Primary	9	Heat	4.18	0.91	1.06	D
		30.80	wsnp_Ex_c456_896962	QNoSplt.Spk.aww-2D3	Secondary	9	Control	3.554	6.476	0.556	W
2D4	QTL9	7.58	wsnp_Ra_c4712_8489753	QSpkL.Mat.aww-2D4	Secondary	3	Heat	5.34	14.03	0.16	D
						3	Tolerance	3.61	9.24	0.01	D
						9	Heat	4.89	12.81	0.16	D
						3 and 9	Control	4.89	12.81	0.14	D
		13.58	wsnp_RFL_Contig3286_3338919/wsnp_Ku_c30494_40319867	QSpkL.Mat.aww-2D4	Primary	9	Heat	5.35	12.34	0.17	D
						3 and 9	Control	5.29	12.21	0.18	D

Appendix table 4.4. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
2D4	QTL9	18.84	wsnp_Ku_c30494_40319867	QSpkL.Mat.aww-2D4	Primary	3	Heat	3.19	7.11	0.14	D
3A2	QTL10	0.00	wsnp_Ex_c15674_24004810	QNoDevSplt.Spk.aww-3A2	Secondary	3	Heat	4.08	9.21	0.36	D
						9	Heat	4.30	9.01	0.36	D
						3 and 9	Control	4.40	7.37	0.08	D
				QHt.PreH.aww-3A2	Secondary	3	Control	6.14	2.22	1.33	D
							Heat	6.14	2.22	1.28	D
						9	Control	6.14	2.22	1.58	D
							Heat	5.59	1.98	1.51	D
		15.26	wsnp_Ex_c4069_7354375	QHt.Mat.aww-3A2	Primary	3 and 9	Control	5.84	1.03	2.32	D
						3	Heat	5.75	1.05	1.94	D
		22.51	wsnp_Ra_c16278_24893033	QHt.PreH.aww-3A2	Primary	9	Heat	11.79	3.19	1.98	D
		23.23	wsnp_Ex_c24432_33676448	QHt.PreH.aww-3A2	Primary	9	Control	7.48	2.42	1.75	D
							Heat	8.12	1.18	2.03	D
				QNoDevSplt.Spk.aww-3A2	Secondary	3 and 9	Control	6.24	10.73	0.27	D
				QNoDevSplt.Mid.aww-3A2	Secondary	3 and 9	Control	5.87	10.44	0.08	D
				QHt.PreH.aww-3A2	Primary	3	Control	8.25	2.53	1.60	D
							Heat	7.48	2.42	1.58	D
		30.42	wsnp_Ex_c25668_34932304	QNoDevSplt.Mid.aww-3A2	Secondary	3	Heat	5.06	11.17	0.14	D
				QNoDevSplt.Top.aww-3A2	Secondary	3	Heat	3.59	6.30	0.09	D
		31.14	wsnp_Ex_c29742_38738725	QNoSplt.Spk.aww-3A2	Secondary	3	Control	4.362	7.919	0.615	D
3B1	QTL11	18.88	wsnp_BE497169B_Ta_2_1/wsnp_Ku_c3817_7009093	QSpkL.Mat.aww-3B1	Primary	3	Heat	3.55	11.68	0.18	W
						9	Heat	3.47	9.56	0.15	W
						3 and 9	Control	3.70	10.38	0.16	W
3B2	QTL12	80.92	wsnp_Ex_c9594_15882022	QGrNoSplt.1&2.Bot.aww-3B2	Secondary	3	Tolerance	4.07	10.65	0.12	W
		96.32	wsnp_Ex_rep_c101457_86818160	QHt.Mat.aww-3B2	Primary	9	Heat	4.34	0.59	1.43	W
						3 and 9	Control	3.07	0.51	1.63	W
						3	Heat	3.31	0.57	1.44	W
				QHt.PreH.aww-3B2	Primary	3	Control	2.41	0.58	0.76	W
		105.86	wsnp_Ex_rep_c101457_86818160	QGrNoSplt.1&2.Bot.aww-3B2	Secondary	9	Tolerance	5.03	14.46	0.24	W
4A2	QTL13	18.55	wsnp_Ex_c24474_33721784/wsnp_Ku_c1205_2398925	QGrNo.Spk.aww-4A2	Secondary	3	Heat	3.94	6.76	1.71	W

Appendix table 4.4. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
4A2	QTL13	18.55	wsnp_Ex_c24474_33721784/wsnp_Ku_c1205_2398925	QGrNo.Spk.aww-4A2	Secondary	3 and 9	Control	4.16	7.53	1.41	W
		31.43	wsnp_Ex_c4068_7351806	QAIL.PreH.aww-4A2	Primary	9	Heat	2.48	3.55	0.95	W
				QNoDevSplt.Spk.aww-4A2	Primary	3 and 9	Control	3.60	7.10	0.37	W
					Secondary	3 and 9	Control	5.17	8.64	0.25	W
				QNoDevSplt.Bot.aww-4A2	Secondary	3	Heat	4.58	7.83	0.11	W
				QNoDevSplt.Mid.aww-4A2	Secondary	3	Heat	3.96	8.21	0.12	W
						3 and 9	Control	3.86	6.32	0.07	W
				QNoDevSplt.Top.aww-4A2	Secondary	3	Heat	4.21	7.44	0.10	W
						3 and 9	Control	3.90	5.96	0.07	W
				QNoDevSplt.Spk.aww-4A2	Primary	3	Heat	3.58	6.68	0.45	W
		38.68	wsnp_BE442666A_Ta_2_1	QHt.PreH.aww-4A2	Secondary	9	Heat	7.18	2.67	1.75	W
		39.39	wsnp_Ex_c829_1621908	QPlHt.GroPostH.aww-4A2	Primary	3	Heat	3.63	1.17	1.00	W
				QAIL.Mat.aww-4A2	Primary	3	Heat	4.12	2.02	0.44	W
		41.55	wsnp_Ex_rep_c68569_67411985	QHt.Mat.aww-4A2	Primary	9	Heat	7.73	1.15	2.00	W
						3 and 9	Control	5.86	1.05	2.35	W
				QAIL.GroPostH.aww-4A2	Primary	3	Heat	3.91	2.70	0.51	W
				QAIL.Mat.aww-4A2	Primary	9	Heat	4.27	1.52	0.34	W
							Tolerance	4.27	1.52	0.00	D
				QAIL.Mat.aww-4A2	Primary	3 and 9	Control	4.27	1.52	0.58	W
					Secondary	3	Heat	4.09	2.09	2.81	W
							Tolerance	4.09	2.09	0.00	D
						9	Heat	4.09	2.07	2.64	W
						3 and 9	Control	4.09	2.09	3.33	W
					Primary	3	Heat	5.83	1.08	1.98	W
		42.27	wsnp_RFL_Contig25_2082245	QHt.PreH.aww-4A2	Primary	3	Control	5.52	1.56	1.25	W
							Heat	5.33	1.62	1.29	W
						9	Control	5.33	1.62	1.43	W
							Heat	6.33	1.48	1.35	W
		42.99	wsnp_Ex_c1373_2628597	QHt.PreH.aww-4A2	Secondary	3	Control	6.60	2.39	1.39	W
							Heat	6.60	2.39	1.33	W
						9	Control	6.60	2.39	1.64	W
		45.87	wsnp_BE403900A_Ta_2_1	QProUndsplt.aww-4A2	Secondary	3	Heat	4.083	7.327	0.019	D
		69.99	wsnp_RFL_Contig2771_2524880	QPlHt.GroPostH.aww-4A2	Primary	9	Control	3.72	1.34	1.31	W

Appendix table 4.4. Continued…

4B	QTL14	33.30	wsnp_Ex_rep_c73742_71687509	QNoSplt.Spk.aww-4B	Primary	3	Control	3.939	8.885	0.708	W
		38.53	wsnp_Ex_rep_c73742_71687509/wsnp_Ex_c30695_39579408	QNoSplt.Spk.aww-4B	Primary	9	Heat	3.985	7.977	0.619	W
		49.04	wsnp_Ex_c30695_39579408/wsnp_CAP7_c1723_854530	QNoSplt.Spk.aww-4B	Primary	3	Heat	2.974	6.703	0.614	W
	QTL15	62.94	wsnp_CAP7_c1723_854530/wsnp_Ex_c18433_27269748	QAIL.PreH.aww-4B	Primary	3	Heat	3.92	8.15	1.41	W
		71.55	wsnp_Ex_c18433_27269748	QNoDevSplt.Top.aww-4B	Secondary	3	Heat	6.23	13.37	0.13	D
				QGrNo.Spk.aww-4B	Secondary	9	Heat	6.68	14.43	2.39	D
				QGrNoSplt.1&2.Bot.aww-4B	Secondary	9	Heat	8.31	17.35	0.43	D
				QGrNoSplt.1&2.Top.aww-4B	Secondary	3	Heat	4.44	12.22	0.13	D
						3 and 9	Control	3.97	9.42	0.13	D
				QGrNoSplt.>2.Mid.aww-4B	Primary	3	Heat	3.62	8.93	0.06	D
						3 and 9	Control	1.94	3.88	0.03	D
				QGrNoSplt.Spk.aww-4B	Secondary	9	Heat	6.12	13.65	0.34	D
		77.72	wsnp_Ex_c18433_27269748/Rht-B1	QGrNoSplt.1&2.Bot.aww-4B	Primary	3	Heat	10.99	24.09	0.53	D
				QAIL.PreH.aww-4B	Primary	3	Control	4.24	7.90	1.36	W
				QNoDevSplt.Spk.aww-4B	Secondary	3 and 9	Control	5.67	11.31	0.28	D
				QNoDevSplt.Bot.aww-4B	Secondary	3	Heat	4.64	9.86	0.12	D
						3 and 9	Control	6.29	13.74	0.11	D
				QNoDevSplt.Mid.aww-4B	Secondary	9	Heat	2.08	5.22	0.09	D
						3 and 9	Control	5.07	10.30	0.08	D
				QProUndsplt.aww-4B	Secondary	3	Heat	10.387	26.439	0.036	W
								9.844	26.053	0.04	W
				QGrNoSplt.1&2.Bot.aww-4B	Primary	3 and 9	Control	11.77	26.67	0.32	D
				QGrNo.Spk.aww-4B	Primary	3	Heat	6.27	13.60	2.91	D
					Secondary	3	Heat	13.08	26.09	3.37	D
					Primary	3 and 9	Control	8.25	18.40	2.56	D
					Secondary	3 and 9	Control	11.97	24.29	2.53	D
				QGrNoSplt.1&2.Bot.aww-4B	Secondary	3	Heat	12.92	27.24	0.52	D
						3 and 9	Control	12.06	29.89	0.32	D
				QGrNoSplt.>2.Bot.aww-4B	Primary	3	Heat	1.93	3.59	0.03	D
						3 and 9	Control	2.77	5.58	0.04	D
				QGrNoSplt.Spk.aww-4B	Secondary	3	Heat	8.90	22.81	0.45	D
					Primary	3 and 9	Control	8.06	20.25	0.12	D
					Secondary	3 and 9	Control	7.69	18.15	0.43	D
				QPlHt.GroPostH.aww-4B	Secondary	3	Control	11.80	29.13	2.04	D
		83.90	Rht-B1	QAIL.GroPostH.aww-4B	Primary	9	Control	43.23	47.52	2.86	D
				QHt.PreH.aww-4B	Primary	9	Control	45.14	32.84	6.46	D
				QPlHt.GroPostH.aww-4B	Primary	3	Heat	50.69	47.47	6.35	D
				QPedL.Mat.aww-4B	Primary	3	Heat	33.30	44.16	4.89	D

Appendix table 4.4. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
4B	QTL15	77.72	wsnp_Ex_c18433_27269748/Rht-B1	QHt.Mat.aww-4B	Primary	9	Heat	70.84	42.26	12.12	D
						3 and 9	Control	64.95	42.31	14.90	D
					Secondary	3	Heat	27.36	40.65	2.25	D
						9	Heat	27.76	40.39	1.78	D
						3 and 9	Control	31.30	42.99	3.05	D
				QAIL.GroPostH.aww-4B	Secondary	3	Control	38.42	49.32	3.22	D
					Primary	3	Heat	31.13	40.51	1.99	D
					Secondary	3	Heat	37.21	49.47	2.41	D
						9	Control	38.42	49.32	3.16	D
					Primary	9	Heat	45.05	48.37	1.76	D
					Secondary	9	Heat	15.64	28.26	1.49	D
				QAIL.Mat.aww-4B	Primary	3	Heat	38.05	40.67	1.96	D
						9	Heat	47.02	44.51	1.82	D
							Tolerance	47.01	44.51	0.00	W
						3 and 9	Control	47.02	44.51	3.13	D
				QDay.AILtoAnth.aww-4B	Primary	9	Control	10.76	23.48	0.51	D
							Heat	10.62	23.27	0.51	D
				QHt.PreH.aww-4B	Primary	3	Control	46.97	33.05	5.77	D
					Secondary	3	Control	42.27	33.20	5.16	D
					Primary	3	Heat	45.14	32.84	5.81	D
					Secondary	3	Heat	42.27	33.20	4.97	D
						9	Control	42.27	33.20	6.11	D
					Primary	9	Heat	49.37	45.32	7.47	D
					Secondary	9	Heat	43.25	34.95	6.33	D
				QPlHt.GroPostH.aww-4B	Primary	3	Control	44.62	49.90	9.17	D
						9	Control	53.08	45.64	7.66	D
							Heat	43.07	45.03	5.13	D
				QPedL.Mat.aww-4B	Secondary	3	Heat	34.49	45.63	5.46	D
					Primary	9	Heat	26.27	39.21	4.25	D
					Secondary	9	Heat	32.67	44.91	4.89	D

Appendix table 4.4. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
4B	QTL15	83.90	Rht-B1	QPedL.Mat.aww-4B	Primary	3 and 9	Control	33.27	44.24	5.81	D
					Secondary	3 and 9	Control	34.19	45.35	5.99	D
				QHt.Mat.aww-4B	Secondary	3	Heat	37.45	41.17	12.47	D
							Tolerance	37.45	41.17	0.00	W
						9	Heat	37.57	40.94	11.74	D
						3 and 9	Control	37.45	41.17	14.80	D
					Primary	3	Heat	64.07	41.93	12.30	D
				QUndvSplt.Spk.aww-4B	Primary	3	Heat	16.89	26.34	0.51	W
							Tolerance	16.89	26.34	0.00	D
						9	Heat	16.89	26.34	0.84	W
							Tolerance	16.88	26.33	0.00	D
						3 and 9	Control	16.89	26.34	0.92	W
					Secondary	3	Heat	14.18	25.30	0.55	W
						9	Heat	13.23	23.74	0.66	W
						3 and 9	Control	10.67	19.13	0.68	W
				QProUndsplt.aww-4B	Primary	3	Heat	11.283	26.327	0.033	W
		84.69	wsnp_Ex_c14026_21924297	QNoDevSplt.Top.aww-4B	Secondary	3 and 9	Control	6.98	13.02	0.10	D
				QPlHt.GroPostH.aww-4B	Secondary	9	Control	17.56	31.36	2.66	D
				QProUndsplt.aww-4B	Primary	3	Control	11.721	22.865	0.041	W
						9	Heat	12.274	25.904	0.04	W
					Secondary	9	Control	13.973	31.272	0.045	W
		86.26	wsnp_RFL_Contig4151_4728831	QProUndsplt.aww-4B	Primary	9	Control	6.919	16.017	0.034	W
		108.33	wsnp_CAP12_rep_c4278_1949864		Secondary	3	Control	6.18	22.203	0.038	W
4B	QTL16	117.19	wsnp_Ex_c39876_47057394	QHt.PreH.aww-4B	Primary	9	Heat	5.80	2.83	1.87	W
		127.49	wsnp_Ku_c11570_18860306	QGrNoSplt.1&2.Mid.aww-4B	Primary	3 and 9	Control	6.00	13.59	0.14	D
					Secondary	3	Heat	4.29	9.32	0.04	D
							Tolerance	4.28	9.35	0.00	D
						3 and 9	Control	4.29	9.32	0.03	D
				QGrNoSplt.>2.Bot.aww-4B	Primary	3	Heat	8.25	17.36	0.06	D
					Secondary	3	Heat	9.13	21.77	0.06	D
						9	Heat	7.92	18.94	0.05	D
					Primary	3 and 9	Control	8.01	17.29	0.07	D
					Secondary	3 and 9	Control	7.48	19.26	0.07	D
				QGrNoSplt.>2.Mid.aww-4B	Primary	3 and 9	Control	3.94	8.59	0.05	D

Appendix table 4.4. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
4B	QTL16	127.49	wsnp_Ku_c11570_18860306	QPlHt.GroPostH.aww-4B	Primary	9	Tolerance	4.68	11.94	0.55	D
				QUndvSplt.Spk.aww-4B	Secondary	3 and 9	Control	6.95	11.52	0.53	W
				QProUndsplt.aww-4B	Primary	9	Control	3.649	7.176	0.022	W
				QNoSplt.Spk.aww-4B	Primary	3	Heat	8.993	21.879	1.11	W
					Secondary	3	Control	12.64	28.866	1.174	W
						9	Control	12.581	29.591	1.189	W
		135.47	wsnp_Ex_c4148_7495656	QAIL.PreH.aww-4B	Primary	9	Control	17.44	41.14	3.13	W
							Heat	17.10	38.69	3.14	W
				QDay.AwnEm.aww-4B	Primary	9	Heat	17.43	39.64	3.03	W
				QDay.Anth.aww-4B	Primary	3	Heat	14.66	33.47	2.86	W
						3 and 9	Control	15.54	35.34	2.83	W
				QAIL.PreH.aww-4B	Primary	3	Control	15.43	33.43	2.79	W
							Heat	14.77	32.54	2.82	W
				QDay.AwnEm.aww-4B	Primary	3	Heat	17.10	39.02	2.88	W
						3 and 9	Control	17.33	39.46	2.90	W
				QDay.Anth.aww-4B	Primary	9	Heat	14.86	36.11	2.86	W
						3 and 9	Control	15.54	35.34	2.83	W
				QNoDevSplt.Bot.aww-4B	Secondary	3	Heat	5.70	10.76	0.13	W
						3 and 9	Control	5.04	9.10	0.09	W
				QNoDevSplt.Top.aww-4B	Secondary	3	Heat	5.17	10.06	0.12	W
						3 and 9	Control	5.48	9.70	0.09	W
				QNoDevSplt.Spk.aww-4B	Primary	3	Heat	3.60	6.71	0.45	W
						9	Heat	3.49	6.76	0.35	W
				QPedL.Mat.aww-4B	Secondary	3	Tolerance	5.87	15.14	0.07	D
				QUndvSplt.Spk.aww-4B	Primary	3	Heat	8.71	11.58	0.34	W
							Tolerance	8.71	11.57	0.00	D
						9	Heat	8.71	11.58	0.56	W
							Tolerance	8.71	11.58	0.00	D
						3 and 9	Control	8.71	11.58	0.61	W
					Secondary	3	Heat	9.03	14.12	0.41	W
						9	Heat	8.99	14.41	0.51	W
				QNoSplt.Spk.aww-4B	Primary	3	Control	11.504	26.192	1.216	W
						9	Control	13.46	33.196	1.343	W
							Heat	16.392	35.555	1.308	W

Appendix table 4.4. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
4B	QTL16	135.47	wsnp_Ex_c4148_7495656	QNoSplt.Spk.aww-4B	Secondary	3	Heat	8.989	23.039	1.235	W
						9	Heat	12.859	31.91	1.286	W
				QProUndsplt.aww-4B	Primary	3	Control	3.342	5.07	0.019	W
		141.25	wsnp_BE403378B_Ta_2_1	QAIL.Mat.aww-4B	Primary	9	Heat	4.85	1.82	0.37	D
							Tolerance	4.85	1.82	0.00	W
						3 and 9	Control	4.85	1.82	0.63	D
				QGrNoSplt.>2.Bot.aww-4B	Primary	9	Heat	5.32	11.33	0.05	D
4D	QTL17	0.00	Rht-D1	QAIL.GroPostH.aww-4D	Primary	9	Control	40.44	40.40	2.64	W
				QNoDevSplt.Bot.aww-4D	Primary	3	Heat	5.10	13.11	0.19	W
				QGrNoSplt.1&2.Bot.aww-4D	Primary	3	Heat	8.57	15.15	0.42	W
				QHt.PreH.aww-4D	Primary	9	Control	59.97	59.96	8.73	W
				QPlHt.GroPostH.aww-4D	Primary	3	Heat	46.31	39.05	5.76	W
				QNoDevSplt.Spk.aww-4D	Primary	3 and 9	Control	8.82	21.03	0.63	W
				QPedL.Mat.aww-4D	Primary	3	Heat	29.46	36.00	4.42	W
				QHt.Mat.aww-4D	Primary	9	Heat	76.90	51.70	13.40	W
						3 and 9	Control	70.00	51.31	16.41	W
				QAIL.Mat.aww-4D	Secondary	3	Heat	25.53	36.44	2.13	W
						9	Heat	26.58	37.62	1.71	W
						3 and 9	Control	29.44	38.80	2.90	W
				QAIL.GroPostH.aww-4D	Secondary	3	Control	32.93	37.69	2.82	W
					Primary	3	Heat	26.99	32.48	1.78	W
					Secondary	3	Heat	30.94	36.13	2.06	W
					Secondary	9	Control	32.93	37.69	2.76	W
					Primary	9	Heat	41.46	39.74	1.59	W
					Secondary	9	Heat	16.96	31.35	1.57	W
				QAIL.Mat.aww-4D	Primary	3	Heat	36.14	36.64	1.86	W
						9	Heat	46.05	41.95	1.76	W
							Tolerance	46.04	41.94	0.00	D
						3 and 9	Control	46.05	41.95	3.04	W
				QNoDevSplt.Spk.aww-4D	Secondary	3 and 9	Control	6.82	11.99	0.29	W
						3 and 9	Control	6.82	11.99	0.29	W
				QNoDevSplt.Bot.aww-4D	Primary	9	Heat	5.87	15.17	0.18	W
						3 and 9	Control	5.88	15.20	0.17	W

Appendix table 4.4. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
4D	QTL17	0.00	Rht-D1	QNoDevSplt.Bot.aww-4D	Secondary	3 and 9	Control	6.65	12.07	0.11	W
				QNoDevSplt.Mid.aww-4D	Primary	3	Heat	5.72	14.78	0.22	W
						9	Heat	6.74	17.44	0.19	W
						3 and 9	Control	6.78	17.54	0.19	W
					Secondary	3 and 9	Control	7.40	13.88	0.10	W
				QNoDevSplt.Top.aww-4D	Primary	3	Heat	5.42	13.98	0.22	W
						9	Heat	5.77	14.91	0.16	W
					Secondary	9	Heat	4.01	8.56	0.12	W
					Primary	3 and 9	Control	5.64	14.55	0.18	W
					Secondary	3 and 9	Control	8.63	15.64	0.11	W
				QGrNoSplt.1&2.Bot.aww-4D	Primary	3 and 9	Control	8.99	16.51	0.25	W
				QGrNoSplt.1&2.Top.aww-4D	Primary	9	Tolerance	6.81	12.43	0.25	D
				QGrNo.Spk.aww-4D	Primary	3	Heat	6.30	11.94	2.73	W
					Secondary	3	Heat	12.29	20.53	2.99	W
					Primary	3 and 9	Control	13.08	28.03	3.16	W
					Secondary	3 and 9	Control	15.09	27.64	2.70	W
				QGrNoSplt.1&2.Bot.aww-4D	Secondary	3	Heat	6.81	10.67	0.33	W
						3 and 9	Control	8.91	17.51	0.25	W
					Primary	3	Heat	4.74	7.67	0.04	W
						3 and 9	Control	5.07	8.59	0.05	W
				QGrNoSplt.>2.Mid.aww-4D	Secondary	3	Tolerance	5.90	15.24	0.00	W
					Primary	3 and 9	Control	3.85	7.45	0.05	W
					Secondary	3 and 9	Control	6.10	15.78	0.04	W
				QGrNoSplt.Spk.aww-4D	Secondary	3	Heat	7.79	16.57	0.39	W
					Primary	3 and 9	Control	8.55	18.62	0.11	W
					Secondary	3 and 9	Control	10.89	23.85	0.50	W
				QHt.PreH.aww-4D	Primary	3	Control	61.54	59.33	7.73	W
					Secondary	3	Control	54.21	55.28	6.66	W
					Primary	3	Heat	59.97	59.96	7.85	W
					Secondary	3	Heat	54.21	55.28	6.41	W
						9	Control	54.21	55.28	7.89	W
					Primary	9	Heat	66.95	60.78	8.65	W
					Secondary	9	Heat	52.27	51.90	7.71	W
				QPlHt.GroPostH.aww-4D	Primary	3	Control	38.95	38.44	8.05	W

Appendix table 4.4. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
4D	QTL17	0.00	Rht-D1	QPlHt.GroPostH.aww-4D	Primary	9	Control	51.44	41.96	7.34	W
					Secondary	9	Control	15.60	26.56	2.45	W
					Primary	9	Heat	39.73	38.32	4.74	W
				QNoDevSplt.Spk.aww-4D	Primary	3	Heat	7.97	17.54	0.72	W
						9	Heat	9.09	21.68	0.63	W
				QPedL.Mat.aww-4D	Secondary	3	Heat	31.36	38.81	5.04	W
					Primary	9	Heat	25.35	37.00	4.13	W
					Secondary	9	Heat	29.34	37.59	4.47	W
					Primary	3 and 9	Control	29.35	35.90	5.24	W
					Secondary	3 and 9	Control	31.23	38.88	5.55	W
				QHt.Mat.aww-4D	Secondary	3	Heat	39.29	44.85	13.01	W
							Tolerance	39.29	44.85	0.00	D
						9	Heat	39.76	45.31	12.35	W
						3 and 9	Control	39.29	44.85	15.45	W
					Primary	3	Heat	69.24	51.06	13.58	W
				QUndvSplt.Spk.aww-4D	Primary	3	Heat	10.55	13.43	0.36	D
							Tolerance	10.54	13.42	0.00	W
						9	Heat	10.55	13.43	0.60	D
							Tolerance	10.54	13.42	0.00	W
						3 and 9	Control	10.55	13.43	0.66	D
					Secondary	3	Heat	8.31	12.11	0.38	D
						9	Heat	8.28	12.38	0.47	D
						3 and 9	Control	7.07	9.64	0.48	D
				QProUndsplt.aww-4D	Primary	3	Control	8.363	13.924	0.032	D
							Heat	6.01	12.092	0.022	D
						9	Control	6.698	12.409	0.03	D
							Heat	10.273	20.507	0.035	D
					Secondary	9	Control	7.582	14.408	0.03	D
							Heat	6.423	13.219	0.028	D
		2.90	wsnp_CAP11_c356_280910	QGrNoSplt.1&2.Top.aww-4D	Primary	3 and 9	Control	3.77	9.46	0.14	W
				QGrNoSplt.1&2.Mid.aww-4D	Secondary	3	Heat	6.70	15.95	0.06	W
							Tolerance	6.44	15.33	0.00	W
						3 and 9	Control	6.70	15.95	0.04	W
				QGrNoSplt.1&2.Top.aww-4D	Secondary	3 and 9	Control	5.73	13.59	0.15	W

Appendix table 4.4. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
4D	QTL17	2.90	wsnp_CAP11_c356_280910	QGrNoSplt.>2.Mid.aww-4D	Secondary	3	Heat	5.96	15.35	0.06	W
				QPlHt.GroPostH.aww-4D	Secondary	3	Control	9.72	19.48	1.67	W
		7.95	wsnp_Ex_rep_c107564_91144523	QDay.AILtoAnth.aww-4D	Primary	9	Control	9.26	19.32	0.47	W
							Heat	9.09	19.03	0.46	W
				QGrNoSplt.1&2.Top.aww-4D	Primary	9	Heat	4.93	9.05	0.22	D
5A2	QTL18	143.13	wsnp_Ex_rep_c67394_65974276	QAwnL.Mat.aww-5A2	Primary	3	Heat	3.70	7.38	0.13	D
						9	Heat	3.84	7.67	0.14	D
5B2	QTL19	15.65	wsnp_Ra_c30792_40014791	QHt.PreH.aww-5B2	Primary	9	Heat	3.49	0.78	0.98	W
				QSpkL.Mat.aww-5B2	Primary	3	Heat	2.91	6.18	0.13	D
						9	Heat	5.22	10.76	0.16	D
						3 and 9	Control	5.08	10.42	0.16	D
5D2	QTL20	40.23	wsnp_JD_c12424_12667585	QAwnL.Mat.aww-5D2	Primary	3	Tolerance	3.51	11.32	0.01	W
6A	QTL21	66.03	wsnp_Ex_c1104_2118684	QAwnL.Mat.aww-6A	Primary	3	Heat	4.42	8.71	0.14	D
					Secondary		Heat	3.80	8.12	0.14	D
					Primary	9	Heat	4.33	8.46	0.15	D
					Secondary		Heat	3.90	7.69	0.13	D
					Primary	3 and 9	Control	3.76	7.85	0.16	D
		69.95	wsnp_JD_c10969_11530496	QPedL.Mat.aww-6A	Primary	3	Heat	3.99	2.69	1.21	D
						3 and 9	Control	3.94	2.66	1.42	D
				QHt.Mat.aww-6A	Primary	3 and 9	Control	4.42	0.77	2.01	D
						3	Heat	4.80	0.88	1.78	D
		70.74	wsnp_JD_rep_c62949_40140212	QHt.Mat.aww-6A	Primary	9	Heat	5.66	0.81	1.68	D
				QProUndsplt.aww-6A	Primary	3	Control	2.621	3.608	0.016	W
		74.67	wsnp_CAP8_c5350_2554478	QAIL.GroPostH.aww-6A	Primary	9	Control	6.32	3.07	0.73	D
						3	Heat	3.34	2.26	0.47	D
						9	Heat	6.05	2.73	0.42	D
				QAIL.Mat.aww-6A	Primary	3	Heat	3.89	1.90	0.42	D
				QPlHt.GroPostH.aww-6A	Primary	3	Heat	6.17	2.24	1.38	D
						9	Control	6.92	2.28	1.71	D
							Heat	6.05	2.84	1.29	D

Appendix table 4.4. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
6A	QTL21	74.67	wsnp_CAP8_c5350_2554478	QPlHt.GroPostH.aww-6A	Primary	9	Heat	6.05	2.84	1.29	D
				QUndvSplt.Spk.aww-6A	Primary	3	Heat	4.14	4.51	0.21	W
							Tolerance	4.14	4.52	0.00	D
						9	Heat	4.14	4.51	0.35	W
							Tolerance	4.14	4.51	0.00	D
						3 and 9	Control	4.14	4.51	0.38	W
		87.71	wsnp_Ex_c6870_11844501	QUndvSplt.Spk.aww-6A	Secondary	3 and 9	Control	3.75	6.29	0.39	W
	QTL22	109.09	wsnp_Ex_rep_c67436_66026057	QHt.PreH.aww-6A	Secondary	9	Heat	3.57	1.14	1.15	D
		132.96	wsnp_Ex_c21688_30847181	QGrNoSplt.Spk.aww-6A	Primary	3	Heat	3.99	7.82	0.12	W
6B2	QTL23	83.23	wsnp_CAP11_c2485_1280612	QAIL.GroPostH.aww-6B2	Primary	9	Control	3.20	1.59	0.52	D
							Heat	2.49	1.12	0.27	D
6D2	QTL24	0.00	wsnp_BQ161779D_Ta_2_1	QAIL.GroPostH.aww-6D2	Primary	3	Heat	3.56	2.33	0.48	D
		0.00				9	Heat	3.62	1.49	0.31	D
		0.00		QAIL.Mat.aww-6D2	Primary	3	Heat	3.86	1.86	0.42	D
7A2	QTL25	24.33	wsnp_Ku_c139_279238	QAIL.Mat.aww-7A2	Primary	3	Heat	5.31	3.85	0.60	D
				QHt.Mat.aww-7A2	Primary	9	Heat	6.85	1.43	2.23	D
				QPlHt.GroPostH.aww-7A2	Primary	3	Heat	4.08	1.84	1.25	D
							Control	3.80	2.32	1.98	D
		33.74	wsnp_Ex_c2268_4251636	QAIL.GroPostH.aww-7A2	Primary	9	Control	4.32	2.61	0.67	D
							Heat	5.68	3.47	0.47	D
				QAIL.Mat.aww-7A2	Primary	9	Heat	5.80	3.04	0.47	D
							Tolerance	5.80	3.04	0.00	W
						3 and 9	Control	5.80	3.04	0.82	D
						3 and 9	Control	7.36	2.01	3.24	D
				QPlHt.GroPostH.aww-7A2	Primary	9	Control	5.40	2.25	1.70	D
							Heat	3.67	2.14	1.12	D
	QTL26	43.15	wsnp_Ex_c2268_4251636	QGrNoSplt.1&2.Top.aww-7A2	Primary	9	Tolerance	4.00	6.59	0.18	W
		46.03	wsnp_Ex_c40247_47349166	QNoDevSplt.Spk.aww-7A2	Secondary	3 and 9	Control	4.33	7.00	0.22	W
		54.67	wsnp_Ku_c4591_8286583	QNoDevSplt.Bot.aww-7A2	Secondary	3	Heat	4.59	7.92	0.11	W
				QNoDevSplt.Mid.aww-7A2	Secondary	3	Heat	4.56	9.80	0.14	W
				QNoDevSplt.Spk.aww-7A2	Secondary	3	Heat	4.63	10.79	0.39	W

Appendix table 4.4. Continued…

Linkage group	QTL Assigned number	Position	Closest marker(s)	Trait/QTL	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
7A 2	QTL26	54.67	wsnp_Ku_c4591_8286583	QNoDevSplt.Spk.aww-7A2	Secondary	9	Heat	5.08	11.09	0.40	W
		56.11	wsnp_Ex_c1482_2834254	QNoDevSplt.Mid.aww-7A2	Secondary	9	Heat	3.02	7.11	0.10	W
				QNoDevSplt.Top.aww-7A2	Secondary	9	Heat	4.60	10.04	0.13	W
		66.97	wsnp_Ex_c12102_19361467	QGrNo.Spk.aww-7A2	Primary	9	Tolerance	3.98	5.36	1.64	W
					Secondary	3	Heat	4.61	6.05	1.62	W
					Primary	9	Heat	4.70	6.67	2.01	W
					Secondary	3 and 9	Control	3.48	4.50	1.09	W
				QGrNoSplt.1&2.Mid.aww-7A2	Primary	9	Heat	3.88	7.06	0.32	W
							Tolerance	3.47	6.63	0.24	W
				QGrNoSplt.1&2.Top.aww-7A2	Primary	9	Heat	3.62	6.19	0.18	W
				QGrNoSplt.Spk.aww-7A2	Primary	9	Heat	4.65	6.73	0.13	W
							Tolerance	4.05	5.62	0.11	W
				QNoDevSplt.Bot.aww-7A2	Secondary	3 and 9	Control	4.58	7.63	0.08	W
				QNoDevSplt.Mid.aww-7A2	Secondary	3 and 9	Control	3.41	5.39	0.06	W
				QNoDevSplt.Top.aww-7A2	Secondary	3	Heat	4.60	8.32	0.11	W
						3 and 9	Control	6.77	11.64	0.09	W
7B	QTL27	34.87	wsnp_Ex_c24376_33619527	QAwnL.Mat.aww-7B	Secondary	3 and 9	Control	4.89	18.91	0.25	D
		44.32	wsnp_Ex_c24376_33619527		Primary	3	Heat	4.91	10.25	0.15	D
						9	Heat	5.04	10.51	0.17	D
						3 and 9	Control	4.99	11.39	0.20	D
		45.06	wsnp_Ra_c1654_3265291		Secondary	3	Heat	7.03	17.64	0.20	D
		45.06	wsnp_Ra_c1654_3265291			9	Heat	7.87	18.61	0.21	D
		45.80	wsnp_Ex_rep_c68815_67687712				Tolerance	3.66	9.45	0.05	D
		56.94	wsnp_CAP7_c90_52035	QDay.AILtoAnth.aww-7B	Primary	3	Heat	5.89	15.75	0.26	D
	QTL28	79.85	wsnp_JD_c2701_3626787	QGrNoSplt.1&2.Bot.aww-7B	Primary	9	Heat	3.24	4.63	0.28	W
	QTL29	155.60	wsnp_Ex_c10014_16477392	QGrNoSplt.1&2.Bot.aww-7B	Secondary	3	Heat	4.44	6.40	0.25	D
						9	Heat	4.02	6.78	0.27	D

Appendix table 4. 5 Summary of QTLs detected in the Drysdale × Waagan DH population sorted by traits/QTL name, linkage group, assigned QTL number, positon of each QTL, closest marker(s), tiller, stage (AIL in cm), treatment (in control, heat-treated or tolerance, their LOD score, percentage of explained variation (R^2), additive effect, and high value allele (Drysdale, D; Waagan, W) are presented. Yellow highlights indicate QTL co-localized with QTL for heat response for fertility.

Trait/QTL	Linkage group	QTL Assigned number	Position (cM)	Closest marker(s)	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
Anthesis											
QDay.Anth.aww	2B1	QTL6	26.05	wsnp_Ra_rep_c106119_89961852	Primary	3	Heat	3.89	8.67	1.46	D
						3 and 9	Control	3.67	7.92	1.34	D
	4B	QTL16	135.47	wsnp_Ex_c4148_7495656	Primary	3	Heat	14.66	33.47	2.86	W
						3 and 9	Control	15.54	35.34	2.83	W
						9	Heat	14.86	36.11	2.86	W
QDay.AILtoAnth.aww	4B	QTL15	83.9	Rht-B1	Primary	9	Control	10.76	23.48	0.51	D
							Heat	10.62	23.27	0.51	D
	4D	QTL17	7.95	wsnp_Ex_rep_c107564_91144523	Primary	9	Control	9.26	19.32	0.47	W
							Heat	9.09	19.03	0.46	W
	7B	QTL27	56.94	wsnp_CAP7_c90_52035	Primary	3	Heat	5.89	15.75	0.26	D
Awn											
QAwnL.Mat.aww	2B1	QTL7	49.1	wsnp_Ra_rep_c106119_89961852/wsnp_Ex_c10441_17078853	Secondary	9	Heat	3.51	10.32	0.16	D
					Primary	3	Heat	4.68	9.50	0.15	D
						9	Heat	4.76	9.63	0.16	D
						3 and 9	Control	4.37	9.65	0.18	D
	5A2	QTL118	143.13	wsnp_Ex_rep_c67394_65974276	Primary	3	Heat	3.70	7.38	0.13	D
						9	Heat	3.84	7.67	0.14	D
	5D2	QTL20	40.23	wsnp_JD_c12424_12667585	Primary	3	Tolerance	3.51	11.32	0.01	W
	6A	QTL21	66.03	wsnp_Ex_c1104_2118684	Primary	3	Heat	4.42	8.71	0.14	D
						9	Heat	4.33	8.46	0.15	D
						3 and 9	Control	3.76	7.85	0.16	D
					Secondary	3	Heat	3.80	8.12	0.14	D
						9	Heat	3.90	7.69	0.13	D
	7B	QTL27	34.87	wsnp_Ex_c24376_33619527	Secondary	3 and 9	Control	4.89	18.91	0.25	D
			44.32	wsnp_Ex_c24376_33619527	Primary	3	Heat	4.91	10.25	0.15	D
						9	Heat	5.04	10.51	0.17	D

Appendix table 4.5. Continued…

Trait/QTL	Linkage group	QTL Assigned number	Position (cM)	Closest marker(s)	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
QAwnL.Mat.aww	7B	QTL27	44.32	wsnp_Ex_c24376_33619527	Primary	3 and 9	Control	4.99	11.39	0.20	D
					Secondary	3	Heat	7.03	17.64	0.20	D
						9	Heat	7.87	18.61	0.21	D
			45.8	wsnp_Ex_rep_c68815_67687712	Secondary	9	Tolerance	3.66	9.45	0.05	D
QDay.AwnEm.aww	2B1	QTL6	41.41	wsnp_Ra_rep_c106119_89961852	Primary	3	Heat	3.62	7.78	1.29	D
						9	Heat	3.62	7.71	1.34	D
						3 and 9	Control	3.60	7.67	1.28	D
	4B	QTL16	135.47	wsnp_Ex_c4148_7495656	Primary	3	Heat	17.10	39.02	2.88	W
						9	Heat	17.43	39.64	3.03	W
						3 and 9	Control	17.33	39.46	2.90	W
Spike											
QSpkL.Mat.aww	2A	QTL4	70.54	wsnp_CAP12_c1269_649827	Primary	9	Heat	5.23	10.77	0.16	D
						3 and 9	Control	5.07	10.39	0.16	D
	2D4	QTL9	7.58	wsnp_Ra_c4712_8489753	Secondary	3	Heat	5.34	14.03	0.16	D
							Tolerance	3.61	9.24	0.01	D
						9	Heat	4.89	12.81	0.16	D
						3 and 9	Control	4.89	12.81	0.14	D
			13.58	wsnp_RFL_Contig3286_3338919/wsnp_Ku_c30494_40319867	Primary	9	Heat	5.35	12.34	0.17	D
						3 and 9	Control	5.29	12.21	0.18	D
			18.84	wsnp_Ku_c30494_40319867(C)	Primary	3	Heat	3.19	7.11	0.14	D
	3B1	QTL11	18.88	wsnp_BE497169B_Ta_2_1/wsnp_Ku_c3817_7009093	Primary	3	Heat	3.55	11.68	0.18	W
						9	Heat	3.47	9.56	0.15	W
						3 and 9	Control	3.70	10.38	0.16	W
	5B2	QTL19	15.65	wsnp_Ra_c30792_40014791	Primary	3	Heat	2.91	6.18	0.13	D
						9	Heat	5.22	10.76	0.16	D
						3 and 9	Control	5.08	10.42	0.16	D

Appendix table 4.5. Continued…

Trait/QTL	Linkage group	QTL Assigned number	Position (cM)	Closest marker(s)	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
AIL											
QAIL.PreH.aww	4A2	QTL13	31.43	wsnp_Ex_c4068_7351806	Primary	9	Heat	2.48	3.55	0.95	W
	4B	QTL15	62.94	wsnp_CAP7_c1723_854530/wsnp_Ex_c18433_27269748	Primary	3	Heat	3.92	8.15	1.41	W
			77.72	wsnp_Ex_c18433_27269748/Rht-B1	Primary	3	Control	4.24	7.90	1.36	W
		QTL16	135.47	wsnp_Ex_c4148_7495656	Primary	3	Control	15.43	33.43	2.79	W
							Heat	14.77	32.54	2.82	W
						9	Control	17.44	41.14	3.13	W
							Heat	17.10	38.69	3.14	W
QAIL.Mat.aww	2A	QTL5	108.44	wsnp_Ex_c3808_6924802	Primary	3	Heat	4.57	2.27	0.46	D
	4A2	QTL13	39.39	wsnp_Ex_c829_1621908	Primary	3	Heat	4.12	2.02	0.44	W
			41.55	wsnp_Ex_rep_c68569_67411985	Primary	9	Heat	4.27	1.52	0.34	W
							Tolerance	4.27	1.52	0.00	D
						3 and 9	Control	4.27	1.52	0.58	W
	4B	QTL15	83.9	Rht-B1	Secondary	3	Heat	27.36	40.65	2.25	D
						9	Heat	27.76	40.39	1.78	D
						3 and 9	Control	31.30	42.99	3.05	D
					Primary	3	Heat	38.05	40.67	1.96	D
						9	Heat	47.02	44.51	1.82	D
							Tolerance	47.01	44.51	0.00	W
						3 and 9	Control	47.02	44.51	3.13	D
		QTL16	141.25	wsnp_BE403378B_Ta_2_1	Primary	9	Heat	4.85	1.82	0.37	D
							Tolerance	4.85	1.82	0.00	W
						3 and 9	Control	4.85	1.82	0.63	D
	4D	QTL17	0	Rht-D1	Secondary	3	Heat	25.53	36.44	2.13	W
						9	Heat	26.58	37.62	1.71	W
						3 and 9	Control	29.44	38.80	2.90	W
					Primary	3	Heat	36.14	36.64	1.86	W
						9	Heat	46.05	41.95	1.76	W
							Tolerance	46.04	41.94	0.00	D
						3 and 9	Control	46.05	41.95	3.04	W
	6A	QTL21	74.67	wsnp_CAP8_c5350_2554478	Primary	3	Heat	3.89	1.90	0.42	D
	6D2	QTL24	0	wsnp_BQ161779D_Ta_2_1	Primary	3	Heat	3.86	1.86	0.42	D
	7A2	QTL25	24.33	wsnp_Ku_c139_279238	Primary	3	Heat	5.31	3.85	0.60	D

Appendix table 4.5. Continued…

Trait/QTL	Linkage group	QTL Assigned number	Position (cM)	Closest marker(s)	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
QAIL.Mat.aww	7A2	QTL25	33.74	wsnp_Ex_c2268_4251636	Primary	9	Heat	5.80	3.04	0.47	D
			33.74	wsnp_Ex_c2268_4251636		9	Tolerance	5.80	3.04	0.00	W
						3 and 9	Control	5.80	3.04	0.82	D
QAIL.GroPostH.aww	2A	QTL5	101	wsnp_JD_c15127_14676522	Primary	3	Heat	4.16	2.90	0.53	D
	4A2	QTL13	41.55	wsnp_Ex_rep_c68569_67411985	Primary	3	Heat	3.91	2.70	0.51	W
	4B	QTL15	83.9	Rht-B1	Primary	3	Heat	31.13	40.51	1.99	D
						9	Heat	45.05	48.37	1.76	D
							Control	43.23	47.52	2.86	D
					Secondary	3	Control	38.42	49.32	3.22	D
							Heat	37.21	49.47	2.41	D
						9	Control	38.42	49.32	3.16	D
							Heat	15.64	28.26	1.49	D
	4D	QTL17	0	Rht-D1	Primary	3	Heat	26.99	32.48	1.78	W
						9	Control	40.44	40.40	2.64	W
							Heat	41.46	39.74	1.59	W
					Secondary	3	Control	32.93	37.69	2.82	W
							Heat	30.94	36.13	2.06	W
						9	Control	32.93	37.69	2.76	W
							Heat	16.96	31.35	1.57	W
	6A	QTL21	74.67	wsnp_CAP8_c5350_2554478	Primary	3	Heat	3.34	2.26	0.47	D
						9	Control	6.32	3.07	0.73	D
							Heat	6.05	2.73	0.42	D
	6B2	QTL23	83.23	wsnp_CAP11_c2485_1280612	Primary	9	Control	3.20	1.59	0.52	D
							Heat	2.49	1.12	0.27	D
	6D2	QTL24	0	wsnp_BQ161779D_Ta_2_1	Primary	3	Heat	3.56	2.33	0.48	D
						9	Heat	3.62	1.49	0.31	D
	7A2	QTL25	33.74	wsnp_Ex_c2268_4251636	Primary	9	Control	4.32	2.61	0.67	D
							Heat	5.68	3.47	0.47	D
Peduncle											
QPedL.Mat.aww	4B	QTL15	83.9	Rht-B1	Primary	3	Heat	33.30	44.16	4.89	D
							Control	33.27	44.24	5.81	D
						9	Heat	26.27	39.21	4.25	D
					Secondary	3	Heat	34.49	45.63	5.46	D

Appendix table 4.5. Continued…

Trait/QTL	Linkage group	QTL Assigned number	Position (cM)	Closest marker(s)	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
QPedL.Mat.aww	4B	QTL15	83.9	Rht-B1	Secondary	3	Control	34.19	45.35	5.99	D
						9	Heat	32.67	44.91	4.89	D
		QTL16	135.47	wsnp_Ex_c4148_7495656	Secondary	3	Tolerance	5.87	15.14	0.07	D
	4D	QTL17	0	Rht-D1	Primary	3	Heat	29.46	36.00	4.42	W
						9	Heat	25.35	37.00	4.13	W
						3 and 9	Control	29.35	35.90	5.24	W
					Secondary	3	Heat	31.36	38.81	5.04	W
						9	Heat	29.34	37.59	4.47	W
						3 and 9	Control	31.23	38.88	5.55	W
	6A	QTL21	69.95	wsnp_JD_c10969_11530496	Primary	3	Heat	3.99	2.69	1.21	D
						3 and 9	Control	3.94	2.66	1.42	D
Plant height											
QHt.PreH.aww	2D3	QTL8	3.6	wsnp_JD_c5919_7081809	Primary	9	Heat	4.18	0.91	1.06	D
	3A2	QTL10	0	wsnp_Ex_c15674_24004810	Secondary	3	Control	6.14	2.22	1.33	D
							Heat	6.14	2.22	1.28	D
						9	Control	6.14	2.22	1.58	D
							Heat	5.59	1.98	1.51	D
			22.51	wsnp_Ra_c16278_24893033	Primary	9	Heat	11.79	3.19	1.98	D
			23.23	wsnp_Ex_c24432_33676448	Primary	9	Control	7.48	2.42	1.75	D
						3	Control	8.25	2.53	1.60	D
							Heat	7.48	2.42	1.58	D
	3B2	QTL12	96.32	wsnp_Ex_rep_c101457_86818160	Primary	3	Control	2.41	0.58	0.76	W
	4A2	QTL13	38.68	wsnp_BE442666A_Ta_2_1	Secondary	9	Heat	7.18	2.67	1.75	W
	4A2	QTL15	42.27	wsnp_RFL_Contig25_2082245	Primary	3	Control	5.52	1.56	1.25	W
							Heat	5.33	1.62	1.29	W
						9	Control	5.33	1.62	1.43	W
							Heat	6.33	1.48	1.35	W
					Secondary	3	Control	6.60	2.39	1.39	W
							Heat	6.60	2.39	1.33	W
						9	Control	6.60	2.39	1.64	W
	4B	QTL15	83.9	Rht-B1	Primary	3	Control	46.97	33.05	5.77	D
							Heat	45.14	32.84	5.81	D
						9	Control	45.14	32.84	6.46	D
							Heat	49.37	45.32	7.47	D

Appendix table 4.5. Continued…

Trait/QTL	Linkage group	QTL Assigned number	Position (cM)	Closest marker(s)	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
QHt.PreH.aww	4B	QTL15	83.9	Rht-B1	Secondary	3	Control	42.27	33.20	5.16	D
							Heat	42.27	33.20	4.97	D
						9	Control	42.27	33.20	6.11	D
							Heat	43.25	34.95	6.33	D
		QTL16	117.19	wsnp_Ex_c39876_47057394	Primary	9	Heat	5.80	2.83	1.87	W
	4D	QTL17	0	Rht-D1	Primary	3	Control	61.54	59.33	7.73	W
							Heat	59.97	59.96	7.85	W
						9	Control	59.97	59.96	8.73	W
							Heat	66.95	60.78	8.65	W
					Secondary	3	Control	54.21	55.28	6.66	W
							Heat	54.21	55.28	6.41	W
						9	Control	54.21	55.28	7.89	W
							Heat	52.27	51.90	7.71	W
	5B2	QTL19	15.65	wsnp_Ra_c30792_40014791	Primary	9	Heat	3.49	0.78	0.98	W
	6A	QTL22	109.09	wsnp_Ex_rep_c67436_66026057	Secondary	9	Heat	3.57	1.14	1.15	D
QHt.Mat.aww	1A1	QTL1	66.15	wsnp_BE517729A_Ta_2_1	Primary	3 and 9	Control	2.25	0.32	1.30	W
	2A	QTL5	110.66	wsnp_RFL_Contig4779_5764326	Primary	9	Heat	6.43	0.91	1.78	D
			146.98	wsnp_CAP11_c360_282889	Secondary	9	Tolerance	3.91	10.18	0.20	D
	2D3	QTL8	3.6	wsnp_JD_c5919_7081809	Primary	9	Heat	5.33	0.76	1.62	D
	3A2	QTL10	15.26	wsnp_Ex_c4069_7354375	Primary	3	Heat	5.75	1.05	1.94	D
						3 and 9	Control	5.84	1.03	2.32	D
			23.23	wsnp_Ex_c24432_33676448	Primary	9	Heat	8.12	1.18	2.03	D
	3B2	QTL12	96.32	wsnp_Ex_rep_c101457_86818160	Primary	3	Heat	3.31	0.57	1.44	W
						9	Heat	4.34	0.59	1.43	W
						3 and 9	Control	3.07	0.51	1.63	W
	4A2	QTL13	41.55	wsnp_Ex_rep_c68569_67411985	Primary	3	Heat	5.83	1.08	1.98	W
						9	Heat	7.73	1.15	2.00	W
						3 and 9	Control	5.86	1.05	2.35	W
					Secondary	3	Heat	4.09	2.09	2.81	W
							Tolerance	4.09	2.09	0.00	D
						9	Heat	4.09	2.07	2.64	W
						3 and 9	Control	4.09	2.09	3.33	W
	4B	QTL15	83.9	Rht-B1	Primary	3	Heat	64.07	41.93	12.30	D

Appendix table 4.5. Continued…

Trait/QTL	Linkage group	QTL Assigned number	Position (cM)	Closest marker(s)	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
QHt.Mat.aww	4B	QTL15	83.9	Rht-B1		9	Heat	70.84	42.26	12.12	D
						3 and 9	Control	64.95	42.31	14.90	D
					Secondary	3	Heat	37.45	41.17	12.47	D
							Tolerance	37.45	41.17	0.00	W
						9	Heat	37.57	40.94	11.74	D
						3 and 9	Control	37.45	41.17	14.80	D
	4D	QTL17	0	Rht-D1	Primary	3	Control	70.00	51.31	16.41	W
							Heat	69.24	51.06	13.58	W
						9	Heat	76.90	51.70	13.40	W
					Secondary	3	Heat	39.29	44.85	13.01	W
							Tolerance	39.29	44.85	0.00	D
						9	Heat	39.76	45.31	12.35	W
						3 and 9	Control	39.29	44.85	15.45	W
	6A	QTL21	69.95	wsnp_JD_c10969_11530496	Primary	3	Heat	4.80	0.88	1.78	D
						3 and 9	Control	4.42	0.77	2.01	D
			70.74	wsnp_JD_rep_c62949_40140212	Primary	9	Heat	5.66	0.81	1.68	D
	7A2	QTL25	24.33	wsnp_Ku_c139_279238	Primary	9	Heat	6.85	1.43	2.23	D
			33.74	wsnp_Ex_c2268_4251636	Primary	3 and 9	Control	7.36	2.01	3.24	D
QPlHt.GroPostH.aww	1A1	QTL1	59.67	wsnp_Ex_c1997_3756118	Secondary	9	Control	3.84	4.65	1.02	W
	2A	QTL5	108.44	wsnp_Ex_c3808_6924802	Primary	3	Heat	4.70	1.59	1.16	D
							Control	2.64	1.08	1.35	D
						9	Control	4.22	1.24	1.26	D
							Heat	3.77	1.62	0.97	D
	4A2	QTL13	39.39	wsnp_Ex_c829_1621908	Primary	3	Heat	3.63	1.17	1.00	W
			69.99	wsnp_RFL_Contig2771_2524880	Primary	9	Control	3.72	1.34	1.31	W
	4B	QTL15	77.72	wsnp_Ex_c18433_27269748/Rht-B1	Secondary	3	Control	11.80	29.13	2.04	D
			83.9	Rht-B1	Primary	3	Heat	50.69	47.47	6.35	D
							Control	44.62	49.90	9.17	D
						9	Control	53.08	45.64	7.66	D
							Heat	43.07	45.03	5.13	D
			84.69	wsnp_Ex_c14026_21924297	Secondary	9	Control	17.56	31.36	2.66	D
		QTL16	127.49	wsnp_Ku_c11570_18860306	Primary	9	Tolerance	4.68	11.94	0.55	D
	4D	QTL17	0	Rht-D1	Primary	3	Heat	46.31	39.05	5.76	W

Appendix table 4.5. Continued…

Trait/QTL	Linkage group	QTL Assigned number	Position (cM)	Closest marker(s)	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
QPlHt.GroPostH.aww	4D	QTL17	0	Rht-D1	Primary	3	Control	38.95	38.44	8.05	W
						9	Control	51.44	41.96	7.34	W
							Heat	39.73	38.32	4.74	W
					Secondary	9	Control	15.60	26.56	2.45	W
			2.9	wsnp_CAP11_c356_280910	Secondary	3	Control	9.72	19.48	1.67	W
	6A	QTL21	74.67	wsnp_CAP8_c5350_2554478	Primary	3	Heat	6.17	2.24	1.38	D
						9	Control	6.92	2.28	1.71	D
							Heat	6.05	2.84	1.29	D
	7A2	QTL25	24.33	wsnp_Ku_c139_279238	Primary	3	Heat	4.08	1.84	1.25	D
							Control	3.80	2.32	1.98	D
			33.74	wsnp_Ex_c2268_4251636	Primary	9	Control	5.40	2.25	1.70	D
							Heat	3.67	2.14	1.12	D
Spikelet number											
QNoDevSplt.Top.aww	2A	QTL3	0	wsnp_Ex_c2772_5130007	Secondary	9	Heat	2.95	6.24	0.10	W
	3A2	QTL10	30.42	wsnp_Ex_c25668_34932304	Secondary	3	Heat	3.59	6.30	0.09	D
	4A2	QTL13	31.43	wsnp_Ex_c4068_7351806	Secondary	3	Heat	4.21	7.44	0.10	W
						3 and 9	Control	3.90	5.96	0.07	W
	4B	QTL15	71.55	wsnp_Ex_c18433_27269748	Secondary	3	Heat	6.23	13.37	0.13	D
			84.69	wsnp_Ex_c14026_21924297	Secondary	3 and 9	Control	6.98	13.02	0.10	D
		QTL16	135.47	wsnp_Ex_c4148_7495656	Secondary	3	Heat	5.17	10.06	0.12	W
						3 and 9	Control	5.48	9.70	0.09	W
	4D	QTL17	0	Rht-D1	Primary	3	Heat	5.42	13.98	0.22	W
						9	Heat	5.77	14.91	0.16	W
						3 and 9	Control	5.64	14.55	0.18	W
					Secondary	9	Heat	4.01	8.56	0.12	W
						3 and 9	Control	8.63	15.64	0.11	W
	7A2	QTL26	66.97	wsnp_Ex_c19582_28564743	Secondary	3	Heat	4.60	8.32	0.11	W
						9	Heat	4.60	10.04	0.13	W
						3 and 9	Control	6.77	11.64	0.09	W
QNoDevSplt.Mid.aww	3A2	QTL10	23.23	wsnp_Ex_c24432_33676448	Secondary	3 and 9	Control	5.87	10.44	0.08	D
			30.42	wsnp_Ex_c25668_34932304	Secondary	3	Heat	5.06	11.17	0.14	D
	4A2	QTL13	31.43	wsnp_Ex_c4068_7351806	Secondary	3	Heat	3.96	8.21	0.12	W
						3 and 9	Control	3.86	6.32	0.07	W

Appendix table 4.5. Continued…

Trait/QTL	Linkage group	QTL Assigned number	Position (cM)	Closest marker(s)	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
QNoDevSplt.Mid.aww	4B	QTL15	77.72	wsnp_Ex_c18433_27269748/Rht-B1	Secondary	9	Heat	2.08	5.22	0.09	D
						3 and 9	Control	5.07	10.30	0.08	D
	4D	QTL17	0	Rht-D1	Primary	3	Heat	5.72	14.78	0.22	W
						9	Heat	6.74	17.44	0.19	W
						3 and 9	Control	6.78	17.54	0.19	W
					Secondary	3 and 9	Control	7.40	13.88	0.10	W
	7A2	QTL26	54.67	wsnp_Ku_c4591_8286583	Secondary	3	Heat	4.56	9.80	0.14	W
			56.11	wsnp_Ex_c1482_2834254	Secondary	9	Heat	3.02	7.11	0.10	W
			66.97	wsnp_Ex_c12102_19361467	Secondary	3 and 9	Control	3.41	5.39	0.06	W
QNoDevSplt.Bot.aww	2A	QTL4	97.29	wsnp_Ku_c4271_7774388	Secondary	3	Tolerance	3.66	9.35	0.07	W
	3A2	QTL10	0	wsnp_Ex_c15674_24004810	Secondary	3	Heat	5.00	8.71	0.12	D
						3 and 9	Control	4.40	7.37	0.08	D
	4A2	QTL13	31.43	wsnp_Ex_c4068_7351806	Secondary	3	Heat	4.58	7.83	0.11	W
	4B	QTL15	77.72	wsnp_Ex_c18433_27269748/Rht-B1	Secondary	3	Heat	4.64	9.86	0.12	D
						3 and 9	Control	6.29	13.74	0.11	D
		QTL16	135.47	wsnp_Ex_c4148_7495656	Secondary	3	Heat	5.70	10.76	0.13	W
						3 and 9	Control	5.04	9.10	0.09	W
	4D	QTL17	0	Rht-D1	Primary	3	Heat	5.10	13.11	0.19	W
						9	Heat	5.87	15.17	0.18	W
						3 and 9	Control	5.88	15.20	0.17	W
					Secondary	3 and 9	Control	6.65	12.07	0.11	W
	7A2	QTL26	54.67	wsnp_Ku_c4591_8286583	Secondary	3	Heat	4.59	7.92	0.11	W
			66.97	wsnp_Ex_c12102_19361467	Secondary	3 and 9	Control	4.58	7.63	0.08	W
QNoDevSplt.Spk.aww	2A	QTL3	0	wsnp_Ex_c2772_5130007	Secondary	9	Heat	3.73	8.02	0.34	W
		QTL4	96.55	wsnp_Ku_c8927_15048149	Secondary	3	Tolerance	4.12	10.65	0.22	W
	3A2	QTL10	0	wsnp_Ex_c15674_24004810	Secondary	3	Heat	4.08	9.21	0.36	D
						9	Heat	4.30	9.01	0.36	D
			23.23	wsnp_Ex_c24432_33676448	Secondary	3 and 9	Control	6.24	10.73	0.27	D
	4A2	QTL13	31.43	wsnp_Ex_c4068_7351806	Primary	3	Heat	3.58	6.68	0.45	W
						3 and 9	Control	3.60	7.10	0.37	W
					Secondary	3 and 9	Control	5.17	8.64	0.25	W
	4B	QTL15	77.72	wsnp_Ex_c18433_27269748/Rht-B1	Secondary	3 and 9	Control	5.67	11.31	0.28	D

Appendix table 4.5. Continued…

Trait/QTL	Linkage group	QTL Assigned number	Position (cM)	Closest marker(s)	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
QNoDevSplt.Spk.aww		QTL16	135.47	wsnp_Ex_c4148_7495656	Primary	3	Heat	3.60	6.71	0.45	W
						9	Heat	3.49	6.76	0.35	W
	4D	QTL17	0	Rht-D1	Primary	3	Heat	7.97	17.54	0.72	W
						9	Heat	9.09	21.68	0.63	W
						3 and 9	Control	8.82	21.03	0.63	W
					Secondary	3 and 9	Control	6.82	11.99	0.29	W
	7A2	QTL26	46.03	wsnp_Ex_c40247_47349166	Secondary	3 and 9	Control	4.33	7.00	0.22	W
			54.67	wsnp_Ku_c4591_8286583	Secondary	3	Heat	4.63	10.79	0.39	W
						9	Heat	5.08	11.09	0.40	W
QUndvSplt.Spk.aww	2A	QTL4	93.58	wsnp_Ex_rep_c105158_89662129	Secondary	3	Tolerance	3.71	9.56	0.09	D
	4B	QTL15	83.9	Rht-B1	Primary	3	Heat	16.89	26.34	0.51	W
							Tolerance	16.89	26.34	0.00	D
						9	Heat	16.89	26.34	0.84	W
							Tolerance	16.88	26.33	0.00	D
						3 and 9	Control	16.89	26.34	0.92	W
					Secondary	3	Heat	14.18	25.30	0.55	W
						9	Heat	13.23	23.74	0.66	W
						3 and 9	Control	10.67	19.13	0.68	W
		QTL16	127.49	wsnp_Ku_c11570_18860306	Secondary	3 and 9	Control	6.95	11.52	0.53	W
			135.47	wsnp_Ex_c4148_7495656	Primary	3	Heat	8.71	11.58	0.34	W
							Tolerance	8.71	11.57	0.00	D
						9	Heat	8.71	11.58	0.56	W
							Tolerance	8.71	11.58	0.00	D
						3 and 9	Control	8.71	11.58	0.61	W
					Secondary	3	Heat	9.03	14.12	0.41	W
						9	Heat	8.99	14.41	0.51	W
	4D	QTL17	0	Rht-D1	Primary	3	Heat	10.55	13.43	0.36	D
							Tolerance	10.54	13.42	0.00	W
						9	Heat	10.55	13.43	0.60	D
							Tolerance	10.54	13.42	0.00	W
						3 and 9	Control	10.55	13.43	0.66	D
					Secondary	3	Heat	8.31	12.11	0.38	D
						9	Heat	8.28	12.38	0.47	D

Appendix table 4.5. Continued…

Trait/QTL	Linkage group	QTL Assigned number	Position (cM)	Closest marker(s)	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
QUndvSplt.Spk.aww	4D	QTL17	0	Rht-D1	Secondary	3 and 9	Control	7.07	9.64	0.48	D
	6A	QTL21	74.67	wsnp_CAP8_c5350_2554478	Primary	3	Heat	4.14	4.51	0.21	W
							Tolerance	4.14	4.52	0.00	D
						9	Heat	4.14	4.51	0.35	W
							Tolerance	4.14	4.51	0.00	D
						3 and 9	Control	4.14	4.51	0.38	W
			87.71	wsnp_Ex_c6870_11844501	Secondary	3 and 9	Control	3.75	6.29	0.39	W
QNoSplt.Spk.aww	2D3	QTL8	30.8	wsnp_Ex_c456_896962	Secondary	9	Control	3.554	6.476	0.556	W
	3A2	QTL10	31.14	wsnp_Ex_c29742_38738725	Secondary	3	Control	4.362	7.919	0.615	D
	4B	QTL14	33.3	wsnp_Ex_rep_c73742_71687509	Primary	3	Control	3.939	8.885	0.708	W
			38.53	wsnp_Ex_rep_c73742_71687509/wsnp_Ex_c30695_39579408	Primary	9	Heat	3.985	7.977	0.619	W
			49.04	wsnp_Ex_c30695_39579408/wsnp_CAP7_c1723_854530	Primary	3	Heat	2.974	6.703	0.614	W
		QTL16	127.49	wsnp_Ku_c11570_18860306	Primary	3	Heat	8.993	21.879	1.11	W
					Secondary	3	Control	12.64	28.866	1.174	W
						9	Control	12.581	29.591	1.189	W
			135.47	wsnp_Ex_c4148_7495656	Primary	3	Control	11.504	26.192	1.216	W
						9	Control	13.46	33.196	1.343	W
							Heat	16.392	35.555	1.308	W
					Secondary	3	Heat	8.989	23.039	1.235	W
						9	Heat	12.859	31.91	1.286	W
QProUndsplt.aww	2A	QTL4	96.55	wsnp_Ku_c8927_15048149	Secondary	3	Heat	3.476	6.146	0.017	D
	4A	QTL13	45.87	wsnp_BE403900A_Ta_2_1	Secondary	3	Heat	4.083	7.327	0.019	D
	4B	QTL15	77.72	wsnp_Ex_c18433_27269748/Rht-B1	Secondary	3	Heat	10.387	26.439	0.036	W
								9.844	26.053	0.04	W
			83.9	Rht	Primary	3	Heat	11.283	26.327	0.033	W
			86.26	wsnp_RFL_Contig4151_4728831	Primary	9	Control	6.919	16.017	0.034	W
			108.33	wsnp_CAP12_rep_c4278_1949864	Secondary	3	Control	6.18	22.203	0.038	W
		QTL16	135.47	wsnp_Ex_c4148_7495656	Primary	3	Control	3.342	5.07	0.019	W
	4D	QTL17	0	Rht-D1	Primary	3	Control	8.363	13.924	0.032	D
							Heat	6.01	12.092	0.022	D
						9	Control	6.698	12.409	0.03	D
							Heat	10.273	20.507	0.035	D
					Secondary	9	Control	7.582	14.408	0.03	D
							Heat	6.423	13.219	0.028	D

Appendix table 4.5. Continued…

Trait/QTL	Linkage group	QTL Assigned number	Position (cM)	Closest marker(s)	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
QProUndsplt.aww	6A	QTL21	70.74	wsnp_JD_rep_c62949_40140212	Primary	3	Control	2.621	3.608	0.016	W
Fertility in Top third spike											
QGrNoSplt.1&2.Top.aww	2B1	QTL7	79.52	wsnp_JD_c3732_4781170(C)	Primary	3	Tolerance	9.96	26.00	0.33	W
							Heat	9.21	24.17	0.33	W
						9	Heat	11.54	26.29	0.37	W
							Tolerance	11.66	25.11	0.35	W
					Secondary	9	Heat	7.07	18.70	0.38	W
							Tolerance	7.04	18.62	0.38	W
			84.8	wsnp_RFL_Contig4483_5312236	Secondary	3	Tolerance	3.58	9.10	0.07	W
	4B	QTL15	71.55	wsnp_Ex_c18433_27269748	Secondary	3	Heat	4.44	12.22	0.13	D
						3 and 9	Control	3.97	9.42	0.13	D
	4D	QTL17	0	Rht-D1	Primary	9	Tolerance	6.81	12.43	0.25	D
			2.9	wsnp_CAP11_c356_280910	Primary	3 and 9	Control	3.77	9.46	0.14	W
					Secondary	3 and 9	Control	5.73	13.59	0.15	W
			7.95	wsnp_Ex_rep_c107564_91144523	Primary	9	Heat	4.93	9.05	0.22	D
	7A2	QTL26	43.15	wsnp_Ex_c2268_4251636	Primary	9	Tolerance	4.00	6.59	0.18	W
			66.97	wsnp_Ex_c12102_19361467	Primary	9	Heat	3.62	6.19	0.18	W
Fertility in middle third spike											
QGrNoSplt.>2.Mid.aww	2A	QTL4	77.95	wsnp_Ex_rep_c102538_87682273	Primary	3	Heat	3.64	8.52	0.06	W
						3 and 9	Control	3.94	7.91	0.05	W
	2B1	QTL7	79.52	wsnp_JD_c3732_4781170	Primary	9	Heat	6.62	17.53	0.08	W
							Tolerance	8.88	23.34	0.08	W
					Secondary	9	Tolerance	4.01	10.36	0.04	W
	4B	QTL15	71.55	wsnp_Ex_c18433_27269748	Primary	3	Heat	3.62	8.93	0.06	D
						3 and 9	Control	1.94	3.88	0.03	D
		QTL16	127.49	wsnp_Ku_c11570_18860306	Primary	3 and 9	Control	3.94	8.59	0.05	D
	4D	QTL17	0	Rht-D1	Primary	3 and 9	Control	3.85	7.45	0.05	W
					Secondary	3	Tolerance	5.90	15.24	0.00	W
						3 and 9	Control	6.10	15.78	0.04	W
			2.9	wsnp_CAP11_c356_280910	Secondary	3	Heat	5.96	15.35	0.06	W
QGrNoSplt.1&2.Mid.aww	2B1	QTL7	56.78	wsnp_Ex_c10441_17078853	Primary	3 and 9	Control	6.17	19.24	0.17	W
			79.52	wsnp_JD_c3732_4781170	Primary	3	Heat	12.61	32.15	0.63	W
							Tolerance	12.19	31.21	0.44	W
						9	Heat	12.22	29.51	0.65	W

Appendix table 4.5. Continued…

Trait/QTL	Linkage group	QTL Assigned number	Position (cM)	Closest marker(s)	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
QGrNoSplt.1&2.Mid.aww	2B1	QTL7	79.52	wsnp_JD_c3732_4781170	Primary	9	Tolerance	10.08	24.88	0.47	W
	4B	QTL16	127.49	wsnp_Ku_c11570_18860306	Primary	3 and 9	Control	6.00	13.59	0.14	D
					Secondary	3	Heat	4.29	9.32	0.04	D
							Tolerance	4.28	9.35	0.00	D
						3 and 9	Control	4.29	9.32	0.03	D
	4D	QTL17	2.9	wsnp_CAP11_c356_280910	Secondary	3	Heat	6.70	15.95	0.06	W
							Tolerance	6.44	15.33	0.00	W
						3 and 9	Control	6.70	15.95	0.04	W
	7A2	QTL26	66.97	wsnp_Ex_c12102_19361467	Primary	9	Heat	3.88	7.06	0.32	W
							Tolerance	3.47	6.63	0.24	W
Fertility in bottom third spike											
QGrNoSplt.>2.Bot.aww	1B	QTL2	26.24	wsnp_Ex_c14273_22230844	Primary	3	Heat	2.44	3.46	0.03	D
						9	Heat	5.37	11.35	0.05	D
	2A	QTL4	74.99	wsnp_Ex_c42720_49228237	Primary	3	Heat	4.20	6.86	0.04	W
						3 and 9	Control	4.26	7.17	0.05	W
					Secondary	3	Heat	4.77	10.12	0.04	W
						9	Heat	4.68	10.25	0.04	W
	2B1	QTL7	49.1	wsnp_Ra_rep_c106119_89961852/wsnp_Ex_c10441_17078853	Primary	9	Heat	5.50	18.01	0.06	W
			56.78	wsnp_Ex_c10441_17078853	Primary	3	Tolerance	5.15	18.03	0.01	W
						9	Tolerance	5.17	18.11	0.05	W
	4B	QTL15	77.72	wsnp_Ex_c18433_27269748/Rht-B1	Primary	3	Heat	1.93	3.59	0.03	D
						3 and 9	Control	2.77	5.58	0.04	D
		QTL16	127.49	wsnp_Ku_c11570_18860306	Primary	3	Heat	8.25	17.36	0.06	D
						3 and 9	Control	8.01	17.29	0.07	D
					Secondary	3	Heat	9.13	21.77	0.06	D
						9	Heat	7.92	18.94	0.05	D
						3 and 9	Control	7.48	19.26	0.07	D
			141.25	wsnp_BE403378B_Ta_2_1	Primary	9	Heat	5.32	11.33	0.05	D
	4D	QTL17	0	Rht-D1	Primary	3	Heat	4.74	7.67	0.04	W
						3 and 9	Control	5.07	8.59	0.05	W
QGrNoSplt.1&2.Bot.aww	2B1	QTL7	49.1	wsnp_Ra_rep_c106119_89961852/wsnp_Ex_c10441_17078853	Primary	3	Heat	7.64	20.16	0.49	W
			79.52	wsnp_JD_c3732_4781170	Primary	9	Heat	19.99	44.53	0.86	W
							Tolerance	17.74	42.74	0.74	W

Appendix table 4.5. Continued…

Trait/QTL	Linkage group	QTL Assigned number	Position (cM)	Closest marker(s)	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
QGrNoSplt.1&2.Bot.aww	2B1	QTL7	79.52	wsnp_JD_c3732_4781170	Secondary	3	Heat	6.26	9.80	0.31	W
						9	Heat	9.81	20.22	0.47	W
							Tolerance	8.95	20.97	0.29	W
			84.8	wsnp_RFL_Contig4483_5312236	Primary	3	Tolerance	9.52	24.77	0.22	W
			93.92	wsnp_Ex_c13351_21042379	Primary	3 and 9	Control	3.68	5.91	0.15	W
	3B2	QTL12	80.92	wsnp_Ex_c9594_15882022	Secondary	3	Tolerance	4.07	10.65	0.12	W
			105.86	wsnp_Ex_rep_c101457_86818160	Secondary	9	Tolerance	5.03	14.46	0.24	W
	4B	QTL15	71.55	wsnp_Ex_c18433_27269748	Secondary	9	Heat	8.31	17.35	0.43	D
			77.72	wsnp_Ex_c18433_27269748/Rht-B1	Primary	3	Heat	10.99	24.09	0.53	D
						3 and 9	Control	11.77	26.67	0.32	D
					Secondary	3	Heat	12.92	27.24	0.52	D
						3 and 9	Control	12.06	29.89	0.32	D
	4D	QTL17	0	Rht-D1	Primary	3	Heat	8.57	15.15	0.42	W
						3 and 9	Control	8.99	16.51	0.25	W
					Secondary	3	Heat	6.81	10.67	0.33	W
						3 and 9	Control	8.91	17.51	0.25	W
	7B	QTL28	79.85	wsnp_JD_c2701_3626787	Primary	9	Heat	3.24	4.63	0.28	W
		QTL29	155.6	wsnp_Ex_c10014_16477392	Secondary	3	Heat	4.44	6.40	0.25	D
						9	Heat	4.02	6.78	0.27	D
Fertility in whole spike											
QGrNoSplt.Spk.aww	1B	QTL2	62.46	wsnp_Ex_c22377_31571527	Secondary	3	Tolerance	3.74	8.33	0.12	D
			69	wsnp_Ex_c23235_32471358	Primary	3	Heat	4.02	7.80	0.12	D
	2B1	QTL7	79.52	wsnp_JD_c3732_4781170	Primary	9	Heat	20.68	45.57	0.33	W
							Tolerance	21.39	47.23	0.32	W
					Secondary	3	Tolerance	6.04	15.07	0.16	W
						9	Heat	9.23	21.36	0.43	W
							Tolerance	11.89	30.54	0.40	W
			84.8	wsnp_RFL_Contig4483_5312236	Primary	3	Heat	9.17	21.72	0.20	W
			84.8	wsnp_RFL_Contig4483_5312236	Primary	3	Tolerance	12.80	32.38	0.17	W
	4B	QTL15	71.55	wsnp_Ex_c18433_27269748	Secondary	9	Heat	6.12	13.65	0.34	D
			77.72	wsnp_Ex_c18433_27269748/Rht-B1	Primary	3 and 9	Control	8.06	20.25	0.12	D
					Secondary	3	Heat	8.90	22.81	0.45	D
						3 and 9	Control	7.69	18.15	0.43	D
	4D	QTL17	0	Rht-D1	Primary	3 and 9	Control	8.55	18.62	0.11	W

Appendix table 4.5. Continued…

Trait/QTL	Linkage group	QTL Assigned number	Position (cM)	Closest marker(s)	Tiller	Stage (AIL in cm)	Treatment	LOD	R^2	Add[b]	Tole allele[c]
QGrNoSplt.Spk.aww	4D	QTL17	0	Rht-D1	Secondary	3	Heat	7.79	16.57	0.39	W
						3 and 9	Control	10.89	23.85	0.50	W
	6A	QTL22	132.96	wsnp_Ex_c21688_30847181	Primary	3	Heat	3.99	7.82	0.12	W
	7A2	QTL26	66.97	wsnp_Ex_c12102_19361467		9	Heat	4.65	6.73	0.13	W
							Tolerance	4.05	5.62	0.11	W
QGrNo.Spk.aww	1B	QTL2	53.21	wsnp_Ku_c34980_44256215	Primary	3	Tolerance	3.58	8.05	1.15	D
	2B1	QTL7	79.52	wsnp_JD_c3732_4781170	Primary	3	Tolerance	15.94	38.53	2.51	W
						9	Tolerance	22.25	48.75	4.96	W
							Heat	21.35	46.70	5.32	W
					Secondary	3	Heat	3.81	4.98	1.47	W
							Tolerance	10.10	26.34	0.99	W
						9	Heat	10.51	23.90	3.07	W
							Tolerance	10.98	28.42	2.69	W
			84.8	wsnp_RFL_Contig4483_5312236	Primary	3	Heat	7.53	15.04	3.06	W
	4A2	QTL13	18.55	wsnp_Ex_c24474_33721784/wsnp_Ku_c1205_2398925	Secondary	3	Heat	3.94	6.76	1.71	W
						3 and 9	Control	4.16	7.53	1.41	W
	4B	QTL15	71.55	wsnp_Ex_c18433_27269748	Secondary	9	Heat	6.68	14.43	2.39	D
			77.72	wsnp_Ex_c18433_27269748/Rht-B1	Primary	3	Heat	6.27	13.60	2.91	D
						3 and 9	Control	8.25	18.40	2.56	D
					Secondary	3	Heat	13.08	26.09	3.37	D
						3 and 9	Control	11.97	24.29	2.53	D
	4D	QTL17	0	Rht-D1	Primary	3	Heat	6.30	11.94	2.73	W
						3 and 9	Control	13.08	28.03	3.16	W
					Secondary	3	Heat	12.29	20.53	2.99	W
						3 and 9	Control	15.09	27.64	2.70	W
	7A2	QTL26	66.97	wsnp_Ex_c12102_19361467	Primary	9	Tolerance	3.98	5.36	1.64	W
							Heat	4.70	6.67	2.01	W
					Secondary	3	Heat	4.61	6.05	1.62	W
						3 and 9	Control	3.48	4.50	1.09	W

Appendix table 5. 1 Measured temperatures (ºC) and humidity in greenhouse during fine mapping of tolerance QTL (Chapter 5) and its mode of expression (sporophytic or gamentophytic) (chapter 7) experimental period. Booting occurred during beginning of January and March for sporophytic or gamentophytic and fine mapping experiment, respectively. Columns indicate daily average (Day), nightly average (Night), average daily maximum (Maximum average), average daily minimum (Minimum average), maximum, minimum and number of days where temperature greater or equal to 30ºC.

		Day	Night	Maximum average	Minimum average	Maximum	Minimum	Days ≥ 30ºC
Temperature (ºC)								
2015	November	27.0	21.8	31.8	19.8	35.6	17.5	20
	December	24.4	20.8	27.6	19.4	31.9	17.6	5
	January*	24.6	20.6	28.2	19.5	34.7	17.9	7
2016	February	23.9	20.0	26.9	18.7	29.4	17.8	
	March**	24.5	21.2	27.5	19.5	32.5	17.7	9
	April	23.1	19.9	25.4	18.1	26.4	17.1	
	May	22.9	19.6	24.9	18.0	25.7	17.2	
	June	22.6	19.3	24.9	17.8	25.9	16.6	
% relative humidity								
2015	November	43.4	54.7	60.9	29.0	84.8	18.1	
	December	54.1	62.1	70.5	39.3	82.1	16.1	
	January*	60.3	69.0	75.3	43.3	85.2	29.9	
2016	February	62.9	68.7	76.6	49.1	84.3	35.6	
	March**	63.3	69.9	74.5	49.2	85.9	31.9	
	April	53.3	64.0	71.4	40.4	84.9	27.9	
	May	52.3	59.2	68.8	39.9	81.0	27.0	
	June	51.4	60.1	67.4	37.5	85.7	22.0	

Appendix table 5. 2 Mapping the QHFert.aww-2B on 2B by using 144 DH lines, and treating the QTL as a single point locus. The 2B QTL allele call was defined by the frequency distributions in Fig. 5.2 (>1.5 and < 1.0 grains/spikelet in floret positions 1&2, for Waagan and Drysdale allele calls, respectively). The yellow arrows indicates the position of the tolerance locus relative to that of the recombination point, based on the 2B QTL allele call for that line.

		KASParMAS048	wsnp_Ex_c5412_9565527	AHW_DW_001	AHW_DW_010	wsnp_JD_c3732_4781170	0071AHW_DW_031	AHW_DW_037	AHW_DW_032	AHW_DW_054	AHW_DW_053	0076AHW_DW_036	AHW_DW_011	AHW_DW_024	AHW_DW_027	AHW_DW_030	AHW_DW_013	AHW_DW_014	wsnp_Ex_c13351_21042379	AHW_DW_004	AHW_DW_003	AHW_DW_005	wsnp_Ex_c944_1810245	Fertility M&B 1&2 H	Fertility M&B 1&2 H (Re phenotyped	2B QTL allele call
Drysdale x Waagan DH 2B cM		74.3	75.7	79.9	79.9	79.9	80.6	80.6	80.6	84.0	84.0	84.7	85.4	85.4	85.4	85.4	85.4	85.4	94.5	94.5	94.5	96.5	96.5			
NRGene 2B cM		38.7	40.3	42.7	47.2	47.2	50.4	50.5	50.5	50.8	50.8	50.8	51.6	51.6	51.6	54.5	54.5	54.7	55.7	55.7	55.6	56.6	56.6			
Drysdale		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD			
Waagan		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW			
WW28463		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	0.4		
WW28455		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	0.6		
WW28504		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	0.8		
WW28418_WW28427		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	0.9		
WW28373		WW		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	0.9		
WW28393		WW	WW	WW	WW	WW	WW	WW		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.0		
WW28523		WW	WW	WW	WW	WW	WW	WW		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.0		
WW28544		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.0		
WW28519		WW	WW	WW	WW	WW	WW	WW		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.1		
WW28383_WW28385_WW28390		WW	WW	WW	WW	WW	WW		WW		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.1		
WW28454		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.2		
WW28366		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.2		
WW28469		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.3		
WW28419		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.3		
WW28422		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.3		
WW28482		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.3		
WW28363_WW28365		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.4		
WW28535		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.4		
WW28517		WW	WW	WW	WW	WW	WW	WW		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.4		
WW28444_WW28445		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.5		
WW28492		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.5		
WW28485_WW28488		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.5		
WW28428		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.5		
WW28397		WW	WW	WW	WW	WW	WW	WW		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.6		Waagan
WW28500_WW28507		WW	WW	WW	WW	WW	WW	WW		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.6		Waagan
WW28403		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.6		Waagan
WW28543		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.6		Waagan
WW28417		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.6		Waagan
WW28499		WW	WW	WW	WW	WW	WW	WW		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.6		Waagan
WW28410		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.6		Waagan
WW28537		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.6		Waagan
WW28416_WW28430		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.6		Waagan
WW28407		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.6		Waagan
WW28498		WW	WW	WW	WW	WW	WW	WW		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.7		Waagan
WW28449		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.7		Waagan
WW28451		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.7		Waagan
WW28387		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.7		Waagan
WW28395		WW	WW	WW	WW	WW	WW	WW		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.7		Waagan
WW28524		WW	WW	WW	WW	WW	WW	WW		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.8		Waagan
WW28367_WW28368		WW		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.8		Waagan
WW28512		WW	WW	WW	WW	WW	WW	WW		WW	WW	WW		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.8		Waagan
WW28525		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.8		Waagan
WW28478		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.8		Waagan
WW28362		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.8		Waagan
WW28486		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	2.0		Waagan
WW28538		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	2.1		Waagan
WW28531_WW28533		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	2.1		Waagan
WW28404	repeat genotype	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	0.7		Drysdale
WW28404	repeat phenotype	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD		1.60	Waagan
WW28536	repeat genotype	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	1.3		
WW28536	repeat phenotype	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD		0.68	Drysdale
WW28389	repeat genotype	WW	WW	WW	WW	WW	WW	WW		WW	WW	WW		WW	WW	WW	WW	WW	DD		DD	DD	DD	1.4		
WW28389	repeat phenotype	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD		0.87	Drysdale
WW28468		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	1.5		

Appendix table 5.2 continued

		KASParMAS048	wsnp_Ex_c5412_9565527	AHW_DW_001	AHW_DW_010	wsnp_JD_c3732_4781170	0071AHW_DW_031	AHW_DW_037	AHW_DW_032	AHW_DW_054	AHW_DW_053	0076AHW_DW_036	AHW_DW_011	AHW_DW_024	AHW_DW_027	AHW_DW_030	AHW_DW_013	AHW_DW_014	wsnp_Ex_c13351_21042379	AHW_DW_004	AHW_DW_003	AHW_DW_005	wsnp_Ex_c944_1810245	Fertility M&B 1&2 H	Fertility M&B 1&2 H (Re phenotyped	2B QTL allele call
Drysdale x Waagan DH 2B cM		74.3	76.5	80.7	80.7	80.7	81.4	81.4	81.4	85.5	85.5	86.2	86.9	86.9	86.9	86.9	86.9	86.9	95.9	95.9	95.9	98.0	98.0			
NRGene 2B cM		n.a.	40.3	42.7	47.2	47.2	50.4	50.5	50.5	50.8	50.8	50.8	51.6	51.6	51.6	54.5	54.5	54.7	55.7	55.7	55.6	56.6	56.6			
Drysdale		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD			
Waagan		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW			
WW28481	WW28481	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	1.5		Waagan
WW28481	repeat phenotype	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD		1.53	Waagan
WW28501	WW28501	WW	WW	WW	WW	WW	WW	WW		WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	1.7		Waagan
WW28501	repeat phenotype	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD		1.12	Waagan
WW28379	WW28379	WW	WW	WW	WW	WW	WW	WW		WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	1.7		Waagan
WW28379	repeat phenotype	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD		1.57	Waagan
WW28450		WW		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	0.9		Drysdale
WW28477		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW		WW	WW	DD	DD	1.7		Waagan
WW28370		DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	0.5		Drysdale
WW28369		DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	1.1		
WW28493		DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.5		Waagan
WW28527	WW28527	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.6		Waagan
WW28527	repeat phenotype	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW		1.78	Waagan
WW28520	WW28520	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.8		Waagan
WW28520	repeat phenotype	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW		1.58	Waagan
WW28539		WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.7		Waagan
WW28423	WW28423	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.4		
WW28423	repeat phenotype	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		1.69	Waagan
WW28510		DD	WW	WW	WW	WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.1		
WW28360		WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.8		Drysdale
WW28380	WW28380	WW	WW	DD	DD	DD	DD	DD			DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.9		Drysdale
WW28380	repeat phenotype	WW	WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		0.69	Drysdale
WW28547	WW28547	WW	WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.9		Drysdale
WW28547	repeat phenotype	WW	WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		0.23	Drysdale
WW28466	WW28466	WW	DD	DD	WW			DD	DD	DD	WW		WW	WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.7		
WW28466	repeat phenotype	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	WW	WW	WW	WW	WW		0.82	Drysdale
WW28371		DD	WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.9		
WW28372	WW28372	DD	WW	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.6		
WW28372	repeat phenotype	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD		1.72	Waagan
WW28471_WW28473	WW28471_WW28473	DD	DD	DD	DD	DD	DD	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.2		
WW28471	repeat phenotype	DD	DD	DD	DD	DD	DD	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW		1.30	
WW28480	WW28480	DD	DD	DD	DD	DD	DD	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.5		
WW28480	repeat phenotype	DD	DD	DD	DD	DD	DD	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW		1.17	
WW28490		DD	DD	DD	DD	DD	DD	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	0.8		Drysdale
WW28382	WW28382	DD	DD	DD	DD	DD	DD	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	1.3		
WW28382	repeat phenotype	DD	DD	DD	DD	DD	DD	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW		0.28	Drysdale
WW28518		DD	DD	DD	DD	DD	DD	DD		WW	WW	WW	WW	WW	WW	WW	WW	WW	DD	DD	DD	DD	DD	0.6		Drysdale
WW28391_WW28394	WW28391_WW28394	DD	DD	DD	DD	DD	DD	DD		DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	0.7		Drysdale
WW28391	repeat phenotype	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW		0.54	Drysdale
WW28526_WW28528		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	WW	WW	WW	WW	WW	0.9		Drysdale
WW28405		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.2		Drysdale
WW28409		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.2		Drysdale
WW28433_WW28434_WW28436_WW28440		DD	-	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	0.3		Drysdale
WW28359		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.3		Drysdale
WW28476		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.4		Drysdale
WW28441		DD	-	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.4		Drysdale
WW28412_WW28426		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.5		Drysdale
WW28540_WW28542_WW28546		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.5		Drysdale
WW28413		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.5		Drysdale
WW28375		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.5		Drysdale
WW28483_WW28484		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	?	DD	DD	DD	0.5		Drysdale
WW28460_WW28467		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.5		Drysdale
WW28408		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	0.5		Drysdale
WW28505		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	0.6		Drysdale
WW28461_WW28465		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.6		Drysdale
WW28462		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.6		Drysdale
WW28377_WW28378		DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.6		Drysdale
WW28376		DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	0.6		Drysdale
WW28439		DD	-	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.7		Drysdale
WW28534		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.7		Drysdale
WW28532		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.7		Drysdale

Appendix table 5.2 continued

	KASParMA5048	wsnp_Ex_c5412_9565527	AHW_DW_001	AHW_DW_010	wsnp_JD_c3732_4781170	0071AHW_DW_031	AHW_DW_037	AHW_DW_032	AHW_DW_054	AHW_DW_053	0076AHW_DW_036	AHW_DW_011	AHW_DW_024	AHW_DW_027	AHW_DW_030	AHW_DW_013	AHW_DW_014	wsnp_Ex_c13351_21042379	AHW_DW_004	AHW_DW_003	AHW_DW_005	wsnp_Ex_c944_1810245	Fertility M&B 1&2 H	Fertility M&B 1&2 H (Re phenotyped	2B QTL allele call
Drysdale x Waagan DH 2B cM	74.3	76.5	80.7	80.7	80.7	81.4	81.4	81.4	85.5	85.5	86.2	86.9	86.9	86.9	86.9	86.9	86.9	95.9	95.9	95.9	98.0	98.0			
NRGene 2B cM	n.a.	40.3	42.7	47.2	47.2	50.4	50.5	50.5	50.8	50.8	50.8	51.6	51.6	51.6	54.5	54.5	54.7	55.7	55.7	55.6	56.6	56.6			
Drysdale	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD			
Waagan	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW	WW			
WW28432	DD	-	DD	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.8		Drysdale
WW28402	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.8		Drysdale
WW28494_WW28509	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.8		Drysdale
WW28435_WW28437_WW28442	DD	-	DD	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.8		Drysdale
WW28521_WW28522	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.8		Drysdale
WW28502	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.8		Drysdale
WW28386	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.8		Drysdale
WW28541	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	DD	DD	0.9		Drysdale
WW28511	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.9		Drysdale
WW28398	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	0.9		Drysdale
WW28456	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.9		Drysdale
WW28411	DD	DD	DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.9		Drysdale
WW28388	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD		DD	DD	DD		DD	DD	DD	DD	DD	DD	0.9		Drysdale
WW28475	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	0.9		Drysdale
WW28470_WW28472	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	0.9		Drysdale
WW28448_WW28457_WW28506	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.0		Drysdale
WW28514_WW28515	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	1.0		Drysdale
WW28459	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.0		Drysdale
WW28421	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	1.0		Drysdale
WW28474	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.0		
WW28438	DD	-	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.0		
WW28401	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.1		
WW28487_WW28495	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.1		
WW28400	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.1		
WW28496	DD	DD	DD	DD	DD	DD	DD		DD	DD		DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	1.1		
WW28364	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.2		
WW28443_WW28453	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.2		
WW28399	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.3		
WW28392	DD	DD	DD	DD	DD	DD	DD		DD	?	DD	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	1.3		
WW28545_WW28548	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.3		
WW28361	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.3		
WW28446	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	1.3		
WW28381_WW28384	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	1.4		
WW28503_WW28508	DD	DD	DD		DD	DD	DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.4		
WW28447_WW28458	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.4		
WW28420	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	1.4		
WW28497	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	1.4		
WW28452_WW28464	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.4		
WW28414	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.5		
WW28396	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.5		
WW28529	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.5		
WW28513	DD	DD	DD	DD	DD	DD	DD		DD	DD		DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	1.5		
WW28491	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	1.5		
WW28479	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.6		Waagan
WW28415_WW28424_WW28429	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD		DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	1.8		Waagan
WW28374																		DD	DD	DD	DD	DD	1.0		

Appendix table 5. 3 Details of KASP assays designed in the QGrNoSplt.Spk.aww-2B1 region but which failed to identify polymorphism between Drysdale and Waagan or reliably map to the 2B QTL region.

Marker/assay	Scaffold number	Base position of SNP within the scaffold	Primer	Primer sequence (5′ to 3′)
AHW_DW-002	144010	3583167	Allele X	GAAGGTGACCAAGTTCATGCTACAGGACCTAGGGAGCCATGAA
			Allele Y	GAAGGTCGGAGTCAACGGATTACAGGACCTAGGGAGCCATGAT
			Common	GGGTTCAAAAATCCATGCCCACTCAA
AHW_DW-006	116647	312291	Allele X	GAAGGTGACCAAGTTCATGCTCATGGATTTCCCTAGTACTACTCAAC
			Allele Y	GAAGGTCGGAGTCAACGGATTCATGGATTTCCCTAGTACTACTCAAT
			Common	GGGCTTCTACTTCGGTGGGCAA
wsnp_Ex_c5412_9565527	110402	1272933	Allele X	GAAGGTGACCAAGTTCATGCTCAAAAGAACATCAAAGAATCAGTTAGGAAA
			Allele Y	GAAGGTCGGAGTCAACGGATTAAAAGAACATCAAAGAATCAGTTAGGAAG
			Common	GCAGCAGGCCCTGAATAGTGCAT
AHW_DW-007	110402	1272933	Allele X	GAAGGTGACCAAGTTCATGCTGTACCATTTTGAACGTCAACGTTATG
			Allele Y	GAAGGTCGGAGTCAACGGATTGTACCATTTTGAACGTCAACGTTATC
			Common	TTGCCAGGCATGGGTTGACAAGTAA
AHW_DW-012	10981	190410	Allele X	GAAGGTGACCAAGTTCATGCTGGCCTCGAGCGTCCTCAAA
			Allele Y	GAAGGTCGGAGTCAACGGATTGGCCTCGAGCGTCCTCAAG
			Common	GTCGAGCCAGACCTTGCCCTT
wsnp_RFL_Contig4483_512236	10981	1,026,889	Allele X	GAAGGTGACCAAGTTCATGCTGTCCTAGCGGTTGCCCATAGATA
			Allele Y	GAAGGTCGGAGTCAACGGATTCCTAGCGGTTGCCCATAGATG
			Common	GGTCTCATCCGACACGGTCCTA
AHW_DW-029	84097	302657	Allele X	GAAGGTGACCAAGTTCATGCTGAGCTCGGTGCTGGAGTCC
			Allele Y	GAAGGTCGGAGTCAACGGATTGAGCTCGGTGCTGGAGTCG
			Common	GCAGCGGCAGCGCCACCAT
AHW_DW-033	85263	648098	Allele X	GAAGGTGACCAAGTTCATGCTATCAGGTCGACCTAGGGCAGT
			Allele Y	GAAGGTCGGAGTCAACGGATTCAGGTCGACCTAGGGCAGC
			Commn	AGGGCGCGCGGCGGCTAT

Appendix table 5.3 Continued…

Marker/assay	Scaffold number	Base position of SNP within the scaffold	Primer	Primer sequence (5′ to 3′)
AHW_DW-034	108993	4821957	Allele X	GAAGGTGACCAAGTTCATGCTTGCAGTCACATGCGATCCG
			Allele Y	GAAGGTCGGAGTCAACGGATTGCTTGCAGTCACATGCGATCCA
			Common	ACTGATATGACAGGAACTATATAGCGCTT
AHW_DW-035	4059	2274788	Allele X	GAAGGTGACCAAGTTCATGCTCAGGAGTTCTTTGAAACCAGAAATGG
			Allele Y	GAAGGTCGGAGTCAACGGATTACAGGAGTTCTTTGAAACCAGAAATGA
			Common	CTAGCACCGCACACCAAGGCTT
AHW_DW-038	39667	2251472	Allele X	GAAGGTGACCAAGTTCATGCTGAACACGAGCGCGCCGAGAT
			Allele Y	GAAGGTCGGAGTCAACGGATTAACACGAGCGCGCCGAGAC
			Common	ATTGGCTGAGGGCCGCGAGAT
AHW_DW-040	85263	100094	Allele X	GAAGGTGACCAAGTTCATGCTGACAAGCTAAAAGTTCAGTATACATACCT
			Allele Y	GAAGGTCGGAGTCAACGGATTCAAGCTAAAAGTTCAGTATACATACCC
			Common	CCACCCAAATGCCTCCCCTCAA
AHW_DW-041	85263	184340	Allele X	GAAGGTGACCAAGTTCATGCTCCTTCAAGCAAACAACAAAGGTAAAC
			Allele Y	GAAGGTCGGAGTCAACGGATTCCTTCAAGCAAACAACAAAGGTAAAG
			Common	ATTCTTTCCCATTGGACAAGAGAACCATT
AHW_DW-042	85263	1011535	Allele X	GAAGGTGACCAAGTTCATGCTGCCATCGTAAACTGACTATAGCG
			Allele Y	GAAGGTCGGAGTCAACGGATTGGCCATCGTAAACTGACTATAGCA
			Common	GTAAACGTCTTTGACCATTCCAGAAGAAA
AHW_DW-043	85263	1137234	Allele X	GAAGGTGACCAAGTTCATGCTCACAAATCCACTAGACAATCAAACAC
			Allele Y	GAAGGTCGGAGTCAACGGATTCACAAATCCACTAGACAATCAAACAG
			Common	CTTTGTTGTTCATGTACCTTGTGGATCTT
AHW_DW-044	108993	361667	Allele X	GAAGGTGACCAAGTTCATGCTCCATTAAAGGTCCACCATCATCAAGT
			Allele Y	GAAGGTCGGAGTCAACGGATTCATTAAAGGTCCACCATCATCAAGG
			Common	TCCTCGAACAGTGTTATACAGGTTTTGAA

Appendix table 5.3 Continued…

Marker/assay	Scaffold number	Base position of SNP within the scaffold	Primer	Primer sequence (5′ to 3′)
HW_DW-045	108993	365701	Allele X	GAAGGTGACCAAGTTCATGCTCTGGCTCTGGCCACAGCC
			Allele Y	GAAGGTCGGAGTCAACGGATTGTCTGGCTCTGGCCACAGCT
			Common	TTTAGAGTTGAAGATGGACTTTCGACGTA
AHW_DW-046	108993	3589623	Allele X	GAAGGTGACCAAGTTCATGCTGTTCTGGCACCCTGCCG
			Allele Y	GAAGGTCGGAGTCAACGGATTCCTGTTCTGGCACCCTGCCA
			Common	GAAGATGGCCACCGATGAGGGAT
AHW_DW-048	108993	4981643	Allele X	GAAGGTGACCAAGTTCATGCTATAAAAATACAGGAATACTCCCTCCG
			Allele Y	GAAGGTCGGAGTCAACGGATTAATTATAAAAATACAGGAATACTCCCTCCA
			Common	GCATCCATTTTTATCCATTTCTCCGACAA
AHW_DW-049	4059	858670	Allele X	GAAGGTGACCAAGTTCATGCTCCAACAGCTTGTCTATATGGTCGTT
			Allele Y	GAAGGTCGGAGTCAACGGATTCAACAGCTTGTCTATATGGTCGTC
			Common	GTCGAGGTTGATTTAGAGGATGTGTATAT
AHW_DW-050	4059	1190338	Allele X	GAAGGTGACCAAGTTCATGCTAAAAGCATAACGTTAGCCATAGAGC
			Allele Y	GAAGGTCGGAGTCAACGGATTGTAAAAGCATAACGTTAGCCATAGAGA
			Common	TTCGGAATGGTTTGTCTTCACTACAGATT
AHW_DW-051	4059	2629070	Allele X	GAAGGTGACCAAGTTCATGCTGTCGGTTTTTCTGCAAGTTGGTGA
			Allele Y	GAAGGTCGGAGTCAACGGATTGTCGGTTTTTCTGCAAGTTGGTGT
			Common	CGCATGGCGTGGCTTTGACCTT
AHW_DW-052	16147	880109	Allele X	GAAGGTGACCAAGTTCATGCTCGATCAGAAACCCTTCGGGAAAC
			Allele Y	GAAGGTCGGAGTCAACGGATTACGATCAGAAACCCTTCGGGAAAA
			Common	TCTTACACCGTTGTTTGCGATTATTGCAT

Appendix table 5. 4 Summary of 263 predicted genes within the QHFert.aww-2B interval corresponding with homology in Arabidopsis and rice.

Scaffold ID	List of Candidate genes	MIPS annotation description	Rice-drived annotation description
Scaffold110402	ID: Traes_2BS_D0218FFD5.1	protein	expressed protein
	ID: Traes_2BS_52121D3F3.1	protein	expressed protein
	ID: Traes_2BS_43ED9166F.1	protein	expressed protein
	ID: Traes_2BS_F164B8CB9.1	Hypersensitive-induced response protein	hypersensitive-induced response protein, putative, expressed
	ID: Traes_2BS_EE94553EA.1	Importin-11	importin subunit beta, putative, expressed
	ID: Traes_2BS_23E0AC565.1	Bifunctional pinoresinol-lariciresinol reductase	isoflavone reductase, putative, expressed
	ID: Traes_6BS_F9EA1E55D.1	Bifunctional pinoresinol-lariciresinol reductase	isoflavone reductase, putative, expressed
	ID: Traes_2BS_49E1AF0CC.1	Bifunctional pinoresinol-lariciresinol reductase	isoflavone reductase, putative, expressed
	ID: Traes_2BS_3ACCAD67F.1	Bifunctional pinoresinol-lariciresinol reductase	nmrA-like family domain containing protein, expressed
	ID: Traes_2BS_A20CCBEC3.1	Bifunctional pinoresinol-lariciresinol reductase	nmrA-like family domain containing protein, expressed
	ID: Traes_2BS_8F932941C.1	Bifunctional pinoresinol-lariciresinol reductase	nmrA-like family domain containing protein, expressed
	ID: Traes_2BS_3FEDED0E8.1	Bifunctional pinoresinol-lariciresinol reductase	nmrA-like family domain containing protein, expressed
	ID: Traes_2BS_249C5DF99.1	Bifunctional pinoresinol-lariciresinol reductase	soflavone reductase, putative, expressed
	ID: Traes_2BS_3D49D0B77.1	Unknown protein	transposon protein, putative, Pong sub-class, expressed
	ID: Traes_3B_3D49D0B77.1	Unknown protein	transposon protein, putative, Pong sub-class, expressed
	ID: Traes_7BL_21C0362FB.1	Unknown protein	transposon protein, putative, Pong sub-class, expressed
Scaffold88768	ID: Traes_2BS_2FB6DB866.1	26S protease regulatory subunit 4 homolog	26S protease regulatory subunit 4, putative, expressed
	ID: Traes_2BS_CB8DF8A02.1	60S ribosomal protein	60S ribosomal protein L19-3, putative, expressed
	ID: Traes_2BS_B7BAC6B4F1.1	ACT domain repeat	ACT domain containing protein, putative, expressed
	ID: Traes_2BS_B7BAC6B4F.1	ACT domain repeat	ACT domain containing protein, putative, expressed
	ID: Traes_2BS_97934559D.1	receptor lectin kinase	bZIP transcription factor domain containing protein, expressed
	ID: Traes_2BS_DB8EB0632.1	COBRA-like protein	COBRA-like protein 7 precursor, putative, expressed
	ID: Traes_2BS_BD49453B7.1	D-alanine--D-alanine ligase family	D-alanine--D-alanine ligase family, putative, expressed
	ID: Traes_2BS_BA425ADE0.1	Heat shock protein DnaJ with tetratricopeptide repeat	DNAJ heat shock N-terminal domain-containing protein, putative, expressed
	ID: Traes_2BS_928B08FD2.1	cAMP-regulated phosphoprotein 19-related protein	expressed protein
	ID: Traes_2BS_96D64756B.1	Eukaryotic translation initiation factor 2 subunit	expressed protein
Scaffold88768	ID: Traes_2BS_8506C57C5.1	Protein kinase superfamily protein	expressed protein
	ID: Traes_2BS_2BADB9AFD.1	Protein of unknown function	expressed protein
	ID: Traes_2BS_5B27C2264.1	Protein of unknown function	expressed protein
	ID: Traes_2BS_65BC9BB6B.1	xyloglucan endotransglucosylase/hydrolase	glycosyl hydrolases family 16, putative, expressed
	ID: Traes_2BS_BC7099C57.1	galactinol synthase	glycosyl transferase 8 domain containing protein, putative, expressed
	ID: Traes_2BS_E69390845.1	galactinol synthase	glycosyl transferase 8 domain containing protein, putative, expressed
	ID: Traes_2BS_ECF9B4EB4.1	Heat stress transcription factor A-2b	heat stress transcription factor, putative, expressed

Appendix table 5.4 Continued…

Scaffold ID	List of Candidate genes	MIPS annotation description	Rice-drived annotation description
Scaffold88768	ID: Traes_2BS_84F813947.1	DNA repair protein-related	helicase conserved C-terminal domain containing protein, expressed
	ID: Traes_2BS_456235DA9.1	UPI0002336EA8 related cluster	helicase conserved C-terminal domain containing protein, expressed
	ID: Traes_2BS_710A67321.1	receptor lectin kinase	lectin receptor-type protein kinase, putative, expressed
	ID: Traes_2BS_1CEE22532.1	L-type lectin-domain containing receptor kinase	lectin-like receptor kinase, putative, expressed
	ID: Traes_2BS_123DDF962.1	receptor kinase	lectin-like receptor kinase, putative, expressed
	ID: Traes_2BS_C1FFCFF3E.1	CASP-like protein	membrane associated DUF588 domain containing protein, putative, expressed
	ID: Traes_2BS_B88CDE912.1	Protein kinase family protein	MRH1, putative, expressed
	ID: Traes_2BS_AF98B1454.1	Bifunctional pinoresinol-lariciresinol reductase	nmrA-like family domain containing protein, expressed
	ID: Traes_4BS_1AF04720C.1	cytochrome C biogenesis	nothing found in rice
	ID: Traes_2BS_33981FD61.1	O-methyltransferase family protein	O-methyltransferase, putative, expressed
	ID: Traes_2BS_A17045AA9.1	protein	OsFBDUF23 - F-box and DUF domain containing protein, expressed
	ID: Traes_2BS_2FD1D68FB.1	lon protease	OsLonP3 - Putative Lon protease homologue, expressed
Scaffold88768	ID: Traes_2BS_8D25129AA.1	lon protease	OsLonP3 - Putative Lon protease homologue, expressed
	ID: Traes_2BS_CF59DCDF31.1	Short-chain dehydrogenase reductase	oxidoreductase, short chain dehydrogenase/reductase family, putative, expressed
	ID: Traes_2BS_BE7D83F34.1	pectinesterase family protein	pectinesterase, putative, expressed
	ID: Traes_2BS_D7A73A455.1	PHD and RING finger domain-containing protein	PHD-finger family protein, expressed
	ID: Traes_2BS_45DB3F48E.1	receptor-like kinase	protein Kinase-like protein TMKL1 precursor, putative, expressed
	ID: Traes_2BS_4AC449580.1	Protein phosphatase 2C family	protein phosphotase protein, putative, expressed
	ID: Traes_2BS_F9F811144.1	Protein transport protein Sec24-like	protein transport protein Sec24-like, putative, expressed
	ID: Traes_2BS_372400B94.1	pyruvate decarboxylase-2	pyruvate decarboxylase isozyme 2, putative, expressed
	ID: Traes_2BS_228023FCA.1	pyruvate decarboxylase-2	pyruvate decarboxylase isozyme 2, putative, expressed
	ID: Traes_2BS_C5CDBD022.1	Leucine-rich receptor-like protein kinase family protein	receptor-like protein kinase precursor, putative, expressed
	ID: Traes_2BS_3827D0CC3.1	Transposon Ty1-H Gag-Pol polyprotein	retrotransposon protein, putative, unclassified, expressed
	ID: Traes_2BS_2C594923E.1	NADP-binding Rossmann-fold superfamily protein	sex determination protein tasselseed-2, putative, expressed
	ID: Traes_2BS_56373D360.1	Short-chain dehydrogenase reductase 2a	sex determination protein tasselseed-2, putative, expressed
	ID: Traes_2BS_8DBB523E3.1	pyruvate decarboxylase-2	thiamine pyrophosphate enzyme, C-terminal TPP binding domain containing protein, expressed
	ID: Traes_2BS_6DB91427E.1	BRCT domain-containing protein	TOPBP1B - Similar to DNA replication protein TOPBP1 from, expressed
	ID: Traes_2BS_FC3070266.1	polyubiquitin 10	ubiquitin fusion protein, putative, expressed
	ID: Traes_2BS_42897F765.1	ubiquitin-activating enzyme	ubiquitin-activating enzyme, putative, expressed
	ID: Traes_2BS_A22F0E3381.1	Wound-induced protein	Wound-induced protein 1
	ID: Traes_2BS_A22F0E338.1	Wound-induced protein	wound-induced protein WI12, putative, expressed
	ID: Traes_2BS_6428AA6CC.1	Abscisic acid receptor PYR1	yclase/dehydrase family protein, putative, expressed
	ID: Traes_2BS_494F02775.1	zinc-finger protein	ZOS3-11 - C2H2 zinc finger protein, expressed

Appendix table 5.4 Continued…

Scaffold ID	List of Candidate genes	MIPS annotation description	Rice-drived annotation description
Scaffold144010	ID: Traes_2BS_7BB456215.1	60S ribosomal protein	60S ribosomal protein L18a, putative, expressed
	ID: Traes_2BS_0B2869248.1	Aldehyde dehydrogenase	aldehyde dehydrogenase, putative, expressed
	ID: Traes_2BS_E5732CD2C.1	alpha-L-arabinofuranosidase	alpha-N-arabinofuranosidase A, putative, expressed
	ID: Traes_2BS_6CBC3A542.1	protein kinase family protein	AMK_KIN1/SNF1/Nim1_like.32 - CAMK includes calcium/calmodulin depedent protein kinases, expressed
	ID: Traes_2BS_4E07DACE7.1	ASF1 like histone chaperone	anti-silencing protein, ASF1-like domain containing protein, expressed
	ID: Traes_2BS_7BEE046C4.1	CBL-interacting protein kinase	CAMK_KIN1/SNF1/Nim1_like.2 - CAMK includes calcium/calmodulin depedent protein kinases, expressed
	ID: Traes_2BS_2974A0A92.1	Coiled-coil domain-containing protein 111	coiled-coil domain-containing protein 111, putative, expressed
	ID: Traes_2BS_0A58CA8ED.1	Coiled-coil domain-containing protein 111 homolog	coiled-coil domain-containing protein 111, putative, expressed
	ID: Traes_2BS_2A4BEA731.1	DNA primase	coiled-coil domain-containing protein 111, putative, expressed
	ID: Traes_2BS_24E1B5A9B.1	UPI000233F451 related cluster	eucine-rich repeat family protein, putative, expressed
	ID: Traes_2BS_CD0E277C1.1	Expressed protein	expressed protein
	ID: Traes_2BS_894109D01.1	polyA polymerase	expressed protein
	ID: Traes_2BS_7C75EBA4A.1	UPI000234E7C4 related cluster	expressed protein
	ID: Traes_2BS_D32231DB0.1	GRAS family transcription factor family protein	gibberellin response modulator protein, putative, expressed
	ID: Traes_2BS_6C74475F9.1	galactinol synthase	glycosyl transferase 8 domain containing protein, putative, expressed
	ID: Traes_2BS_C65FEAB31.1	galactinol synthase	glycosyl transferase 8 domain containing protein, putative, expressed
	ID: Traes_2BS_486296DE7.1	Guanine nucleotide-binding protein-like 3-like protein	GTPase of unknown function domain containing protein, putative, expressed
	ID: Traes_2BS_B64C334AC.1	Importin subunit alpha-1b	importin subunit alpha-1b, putative, expressed
	ID: Traes_2BS_E8D2BD66E.1	myb domain protein	MYB family transcription factor, putative, expressed
	ID: Traes_2BS_CB79BAFB1.1	Nicotianamine synthase	nicotianamine synthase, putative, expressed
	ID: Traes_2BS_591904A01.1	Unknown protein	nothing found in rice
	ID: Traes_2BS_E52D09CE6.1	beta glucosidase	Os1bglu5 - beta-glucosidase homologue, similar to G. max isohydroxyurate hydrolase, expressed
	ID: Traes_2BS_6AECC4811.1	calmodulin	OsCam1-2 - Calmodulin, expressed
	ID: Traes_2BS_B4F22F1BC.1	F-box domain containing protein	OsFBDUF17 - F-box and DUF domain containing protein, expressed
	ID: Traes_2BS_EA73B7F93.1	F-box family protein	OsFBDUF17 - F-box and DUF domain containing protein, expressed
	ID: Traes_2BS_31C9F9A6F.1	Prolyl oligopeptidase family protein	OsPOP17 - Putative Prolyl Oligopeptidase homologue, expressed
	ID: Traes_2BS_3935EBE62.1	Chaperone SurA	parvulin-type peptidyl prolyl cis/trans isomerase, putative, expressed
	ID: Traes_2BS_7EE368753.1	Pectin lyase-like superfamily protein	pectinesterase, putative, expressed
	ID: Traes_2BS_F68DDC360.1	Pentatricopeptide repeat-containing protein	PPR repeat containing protein, expressed
	ID: Traes_2BS_16EF7B45A.1	UPI000234F058 related cluster	retrotransposon, putative, centromere-specific, expressed

Appendix table 5.4 Continued…

Scaffold ID	List of Candidate genes	MIPS annotation description	Rice-drived annotation description
Scaffold144010	ID: Traes_4BS_7FF921484.1	Involved in 10-formyltetrahydrofolate bi s in 780 species: Archae - 12; Bacteria - 1396; Metazoa - 17338; Fungi - 3422; Plants - 5037; Viruses - 0; Other Eukaryotes - 2996 source: NCBI Blink	serine hydrolase domain containing protein, expressed
	ID: Traes_2BS_75DF965FC.1	5'-AMP-activated protein kinase beta-2 subunit protein	SNF1-related protein kinase regulatory subunit beta-1, putative, expressed
	ID: Traes_2BS_8D66D6B82.1	bZIP transcription factor family protein	transcription factor, putative, expressed
	ID: Traes_2BS_2FA63E59F.1	Disease resistance protein RPM1	zinc knuckle family protein, expressed
	ID: Traes_2BS_88286B258.1	UPI00023B2DC4 related cluster	ZOS10-02 - C2H2 zinc finger protein, expressed
Scaffold57860	ID: Traes_2BS_03B72D2B2.1	2-dehydro-3-deoxyphosphooctonate aldolase	2-dehydro-3-deoxyphosphooctonate aldolase, putative, expressed
	ID: Traes_2BS_84FB90D88.1	ABSCISIC ACID-INSENSITIVE 5-like protein	bZIP transcription factor domain containing protein, expressed
	ID: Traes_2BS_F0B2CD12C.1	Protein of unknown function DUF630 and DUF632	DUF630/DUF632 domains containing protein, putative, expressed
	ID: Traes_2BS_4351F4CF6.1	UPI000234E76B related cluster	expressed protein
Scaffold57860	ID: Traes_2BS_23FC6D52B.1	RAD50-interacting protein	MAG2, putative, expressed
	ID: Traes_2BS_B5D02612D.1	PI000234FC49 related cluster	OsFBX251 - F-box domain containing protein, expressed
	ID: Traes_2BS_ED3652A2E.1	subtilisin-like serine protease	OsSub54 - Putative Subtilisin homologue, expressed
Scaffold57860	ID: Traes_2BS_85FAA954F.1	2-oxoglutarate 2OG and FeII-dependent oxygenase superfamily protein	retrotransposon protein, putative, unclassified, expressed
	ID: Traes_2BS_67E0AD439.1	arbonyl reductase [NADPH]	short-chain dehydrogenase/reductase, putative, expressed
	ID: Traes_2BS_40D276D8D.1	protein kinase 2A	tyrosine protein kinase domain containing protein, putative, expressed
	ID: Traes_2BS_C5ABA1E79.1	protein kinase 2A	tyrosine protein kinase domain containing protein, putative, expressed
	ID: Traes_2BS_E446D97FD.1	Protein kinase superfamily protein	tyrosine protein kinase domain containing protein, putative, expressed
	ID: Traes_2BS_708555CAC.1	rotein kinase superfamily protein	tyrosine protein kinase domain containing protein, putative, expressed
Scaffold39667	ID: Traes_2BS_F7DCC979B.1	30S ribosomal protein S16, chloroplastic	chloroplast 30S ribosomal protein S16, putative, expressed
	ID: Traes_2BS_93D50B0D6.1	UPI000234EBA5 related cluster	EMB1011, putative, expressed
	ID: Traes_2BS_647C82888.1	Ethylene insensitive 3 family protein	ethylene-insensitive 3, putative, expressed
	ID: Traes_2BS_6859C24E5.1	DOF zinc finger protein	expressed protein
	ID: Traes_2BS_D852B503C.1	WUSCHEL related homeobox	homeobox domain containing protein, expressed
	ID: Traes_2BS_8A1FE01EE.1	WUSCHEL related homeobox	homeobox domain containing protein, expressed
	ID: Traes_2BS_AEB23633E.1	Hydrolase, alpha/beta fold family protein, expressed	hydrolase, alpha/beta fold family domain containing protein, expressed
	ID: Traes_2BS_34669CA7B.1	myb-like transcription factor family protein	Myb transcription factor, putative, expressed
	ID: Traes_2BS_B8359978A.1	NAC domain protein,	no apical meristem protein, putative, expressed
	ID: Traes_2BS_C02C5A463.1	pectinesterase	pectinesterase, putative, expressed
	ID: Traes_2BS_4C405B620.1	Carbonyl reductase [NADPH]	short-chain dehydrogenase/reductase, putative, expressed
	ID: Traes_2BS_FBE40AC05.1	thioredoxin F2	thioredoxin, putative, expressed
	ID: Traes_2BS_1B3E61DE0.1	Nuclear pore complex protein	

Appendix table 5.4 Continued…

Scaffold ID	List of Candidate genes	MIPS annotation description	Rice-drived annotation description
Scaffold85263	ID: Traes_2BS_00566A85C.1	NAC domain containing protein 100	no apical meristem protein, putative, expressed
	ID: Traes_2BS_759FEB9DB.1	FIZZY-related	WD repeat-containing protein, putative, expressed
Scaffold108993	ID: Traes_2BS_A0152F1E1.1	Protein kinase superfamily protein	AGC_AGC_other_RS6K_like.2 - ACG kinases include homologs to PKA, PKG and PKC, expressed
	ID: Traes_4BS_13C44FE57.1	Serine/threonine-protein kinase AtPK19	AGC_AGC_other_RS6K_like.2 - ACG kinases include homologs to PKA, PKG and PKC, expressed
	ID: Traes_2BS_5B00A8C50.1	B3 domain-containing transcription factor ABI3	B3 DNA binding domain containing protein, putative, expressed
	ID: Traes_2BS_1B0F27580.1	Basic-leucine zipper bZIP transcription factor family protein	bZIP transcription factor domain containing protein, expressed
	ID: Traes_2BS_064B02A89.1	Cellulose synthase family protein	CESA8 - cellulose synthase, expressed
	ID: Traes_2BS_82FCD24BE.1	Cytochrome P450 superfamily protein	cytochrome P450, putative, expressed
	ID: Traes_2BS_101E8FAC1.1	Translation initiation factor 2 subunit beta	eukaryotic translation initiation factor 2 subunit beta, putative, expressed
	ID: Traes_2BS_18158906C.1	Ribonuclease PH	exosome complex exonuclease, putative, expressed
	ID: Traes_2BS_E5560133F.1	PI000234EBFA related cluster	expressed protein
	ID: Traes_2BS_C527278E9.1	protein	expressed protein
	ID: Traes_2BS_C2250DD00.1	protein	expressed protein
Scaffold108993	ID: Traes_2BS_ADDECA43A.1	UPI000234EBFA related cluster	expressed protein
	ID: Traes_2BS_51E9E0AD1.1	Na+-translocating NADH-quinone reductase subunit F	fruit protein PKIWI502, putative, expressed
	ID: Traes_2BS_DCCA0F4DE.1	galacturonosyltransferase	glycosyl transferase, family 8, putative, expressed
	ID: Traes_2BS_CA0F39B3D.1	Acid phosphatase	HAD superfamily phosphatase, putative, expressed
	ID: Traes_2BS_5A8E027D3.1	Acid phosphatase	HAD superfamily phosphatase, putative, expressed
Scaffold108993	ID: Traes_2BS_F63DCE7C1.1	ATP-dependent DNA helicase RecQ	helicase conserved C-terminal domain containing protein, expressed
Scaffold108993	ID: Traes_2BS_CE9D4EA2B.1	Chromodomain-helicase-DNA-binding protein	helicase conserved C-terminal domain containing protein, expressed
	ID: Traes_2BS_28EB7EB86.1	Protein of unknown function DUF616	hydrolase, acting on carbon-nitrogen, putative, expressed
	ID: Traes_2BS_BDB1BA66F.1	receptor-like kinase	inactive receptor kinase At2g26730 precursor, putative, expressed
	ID: Traes_2BS_0F9BB4981.1	30S ribosomal protein S7	mitochondrial ribosomal protein S7, putative, expressed
	ID: Traes_2DL_1227978B4.1	30S ribosomal protein S7	mitochondrial ribosomal protein S7, putative, expressed
	ID: Traes_2BS_0D569FC2A.1	Mitochondrial transcription termination factor family protein	mTERF family protein, expresse
	ID: Traes_2BS_87944581C.1	Mitochondrial transcription termination factor family protein	mTERF family protein, expressed
	ID: Traes_2BS_E5A5144E0.1	tRNA guanine26-N2-dimethyltransferase	N-dimethylguanosine tRNA methyltransferase, putative, expressed
	ID: Traes_2BS_924D34AA1.1	NAC domain protein	no apical meristem protein, putative, expressed
	ID: Traes_2BS_C116E5668.1	NAC domain protein	no apical meristem protein, putative, expressed
	ID: Traes_2BS_9704A64CB.1	Ectonucleoside triphosphate diphosphohydrolase	nucleoside-triphosphatase, putative, expressed
	ID: Traes_2BS_6EAAACBDB.1	calmodulin like 43	OsCML24 - Calmodulin-related calcium sensor protein, expressed
	ID: Traes_2BS_9F1E76FCA.1	Sorting and assembly machinery component 50 homolog A	outer membrane protein, OMP85 family protein, expressed
	ID: Traes_2BS_3212EB7DF.1	Heavy metal transport/detoxification superfamily protein	proline-rich protein, putative, expressed

Appendix table 5.4 Continued…

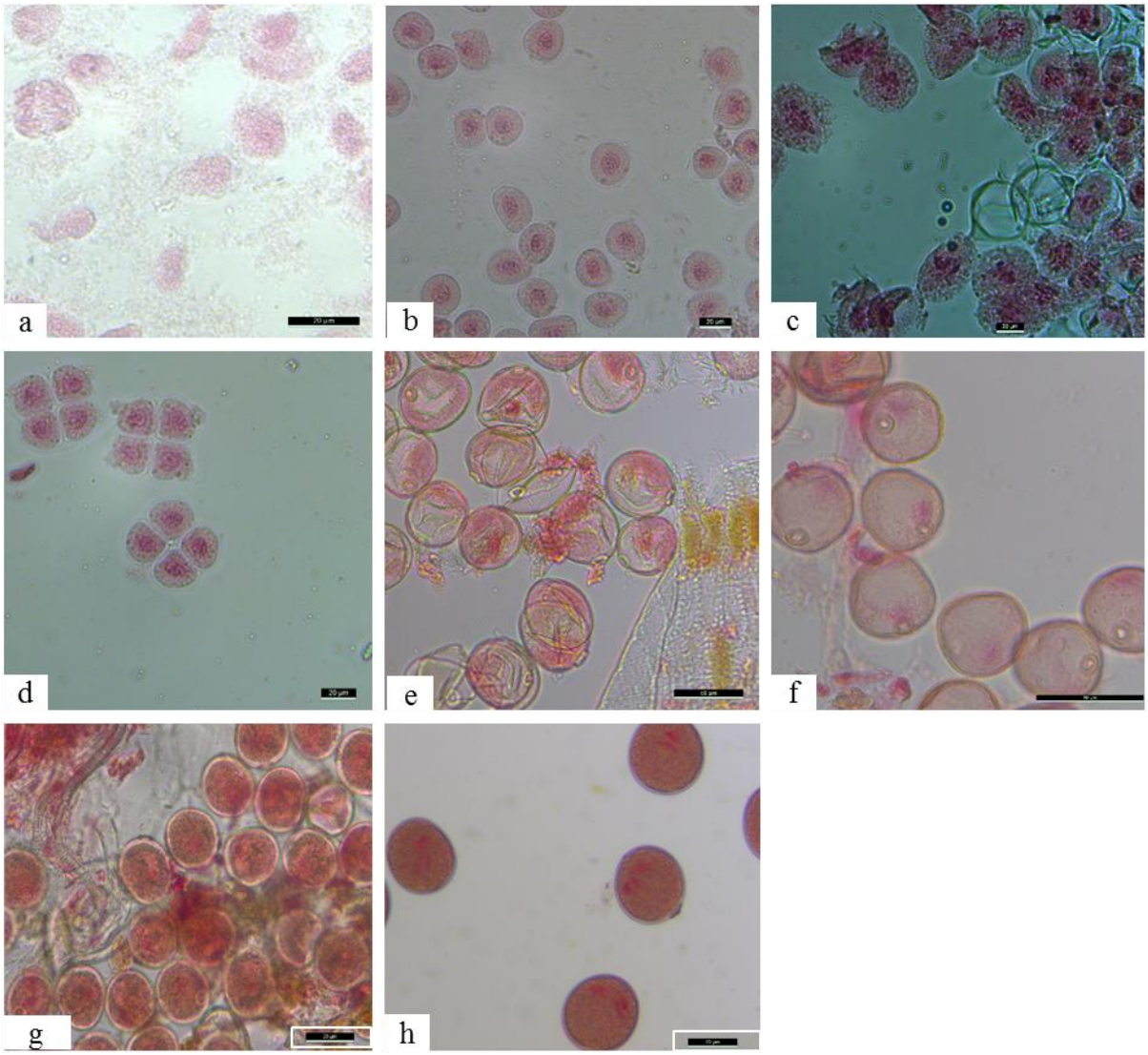
Scaffold ID	List of Candidate genes	MIPS annotation description	Rice-drived annotation description
Scaffold108993	ID: Traes_2BS_90939D3D0.1	Zinc finger CCCH domain-containing protein	RNA-binding zinc finger protein, putative, expressed
	ID: Traes_2BS_8D88641BC.1	Expressed protein	stress responsive protein, putative, expressed
	ID: Traes_2BS_5A45D68C2.1	Expressed protein	stress responsive protein, putative, expressed
	ID: Traes_2BS_762E60A0B.1	Expressed protein	stress responsive protein, putative, expressed
	ID: Traes_2BS_DD098DA8B.1	UPI00032A6D97 related cluster	structural constituent of ribosome, putative, expressed
	ID: Traes_4DL_28812A63E.1	protein	transposon protein, putative, unclassified, expressed
	ID: Traes_2BS_590A24E6A.1	Ubiquinol-cytochrome c reductase complex 6.7 kDa protein	ubiquinol-cytochrome c reductase complex 6.7 kDa protein, putative, expressed
	ID: Traes_2BS_9C5E6C127.1	vacuolar sorting receptor homolog	vacuolar-sorting receptor precursor, putative, expressed
Scaffold4059	ID: Traes_2BS_30F417369.1	Actin-related protein 2/3 complex subunit 5A	actin polymerization factor, putative, expressed
	ID: Traes_2BS_EF1490FF1.1	alpha-galactosidase	alpha-galactosidase precursor, putative, expressed
	ID: Traes_4BS_B03DE0CDA.1	B12D protein	B12D protein, putative, expressed
	ID: Traes_2BS_47F79F636.1	CBL-interacting protein kinase	CAMK_KIN1/SNF1/Nim1_like.30 - CAMK includes calcium/calmodulin depedent protein kinases, expressed
	ID: Traes_2BS_6A7A2DE84.1	protein kinase family protein	CAMK_KIN1/SNF1/Nim1_like.30 - CAMK includes calcium/calmodulin depedent protein kinases, expressed
	ID: Traes_2BS_0ABED462E.1	protein kinase family protein	CAMK_KIN1/SNF1/Nim1_like.31 - CAMK includes calcium/calmodulin depedent protein kinases, expressed
	ID: Traes_2BS_95E669E89.1	LRR receptor-like serine/threonine-protein kinase EFR	expressed protein
	ID: Traes_2BS_246F40694.1	PI0001983BB3 related cluster	expressed protein
	ID: Traes_2BS_323D22B38.1	Peroxidase superfamily protein	peroxidase precursor, putative, expressed
	ID: Traes_2BS_430425C78.1	Peroxidase superfamily protein	peroxidase precursor, putative, expressed
	ID: Traes_2BS_E67905E6E.1	potassium transporter	potassium transporter, putative, expressed
	ID: Traes_2BS_78AFC31F4.1	protein	retrotransposon protein, putative, unclassified
	ID: Traes_2BS_8CFEE207F2.1	Retrotransposon protein, putative, unclassified	retrotransposon protein, putative, unclassified, expressed
	ID: Traes_2BS_8CFEE207F.1	Retrotransposon protein, putative, unclassified	retrotransposon protein, putative, unclassified, expressed
	ID: Traes_2BS_8CFEE207F1.1	Retrotransposon protein, putative, unclassified	retrotransposon protein, putative, unclassified, expressed
	ID: Traes_2BS_89A3A269B.1	thiamin pyrophosphokinase	thiamin pyrophosphokinase 1, putative, expressed
	ID: Traes_2BS_45B041C1F.1	Unknown protein	transposon protein, putative, unclassified, expressed
Scaffold16147	ID: Traes_2BS_5CD0C8850.1	acyl-CoA dehydrogenase-related	acyl-CoA dehydrogenase family member 10, putative, expressed
	ID: Traes_2BS_CE3D56DEF.1	transcription regulators	basic helix-loop-helix domain containing protein, expressed
	ID: Traes_4BS_A37887801.1	Peroxidase superfamily protein	eroxidase precursor, putative, expressed
	ID: Traes_2BS_A6F901E98.1	Unknown protein	nothing found in rice
	ID: Traes_2BS_8FF7B0EA2.1	Peroxidase superfamily protein	peroxidase precursor, putative, expressed
	ID: Traes_2BS_B973866E7.1	Peroxidase superfamily protein	peroxidase precursor, putative, expressed
	ID: Traes_2BS_DFE31095E.1	Peroxidase superfamily protein	peroxidase precursor, putative, expressed

Appendix table 5.4 Continued…

Scaffold ID	List of Candidate genes	MIPS annotation description	Rice-drived annotation description
Scaffold16147	ID: Traes_2BS_4FF4CEA9F.1	Peroxidase superfamily protein	peroxidase precursor, putative, expressed
	ID: Traes_2BS_748791A12.1	Peroxidase superfamily protein	peroxidase precursor, putative, expressed
	ID: Traes_2BS_990895438.1	Peroxidase superfamily protein	peroxidase precursor, putative, expressed
	ID: Traes_2BS_8F766B5E9.1	Peroxidase superfamily protein	peroxidase precursor, putative, expressed
	ID: Traes_2BS_1702DBE8E.1	Peroxidase superfamily protein	peroxidase precursor, putative, expressed
	ID: Traes_2BS_1733E4AAF.1	Peroxidase superfamily protein	peroxidase precursor, putative, expressed
	ID: Traes_2BS_FF5A68083.1	Peroxidase superfamily protein	peroxidase precursor, putative, expressed
	ID: Traes_2BS_40C683B47.1	Peroxidase superfamily protein	peroxidase precursor, putative, expressed
	ID: Traes_2BS_19F05C27A.1	Peroxidase superfamily protein	peroxidase precursor, putative, expressed
	ID: Traes_2BS_B6EBC0962.1	Peroxidase superfamily protein	peroxidase precursor, putative, expressed
	ID: Traes_2BS_EAB2C09D0.1	Peroxidase superfamily protein	peroxidase precursor, putative, expressed

Appendix table 6. 1 Measured temperatures (ºC) and humidity in greenhouse during identifying developmental stages (chapter 6) and exploration of 2B QTL mode of expression (pollen stain and crossing) (Chapter 7) experimental period. Booting occurred during Sptember. Columns indicate daily average (Day), nightly average (Night), average daily maximum (Maximum average), average daily minimum (Minimum average), maximum, minimum and number of days where temperature greater or equal to 30ºC.

		Day	Night	Maximum average	Minimum average	Maximum	Minimum	Days ≥ 30ºC
Temperature (ºC)								
2016	July	20.5	20.5	24.4	17.5	25.0	16.4	-
	August	22.8	18.9	24.7	17.5	25.5	16.5	-
	September*	22.9	19.0	24.7	17.6	26.0	16.8	-
	October	22.9	19.4	24.8	17.6	25.9	16.7	-
	November	22.6	19.8	25.5	17.9	27.8	17.7	-
	December	25.7	22.7	29.8	20.3	36.3	15.6	12
% relative humidity								
2016	July	57.7	57.3	67.3	37.9	79.8	23.4	
	August	46.0	59.9	67.6	33.5	74.6	23.0	
	September*	53.6	65.4	72.6	39.6	79.3	27.8	
	October	49.7	60.5	69.9	36.5	79.3	26.4	
	November	51.6	56.3	67.4	35.1	79.6	26.0	
	December	48.7	53.6	64.2	34.3	80.9	23.3	



Appendix figure 6. 1 Anther tissue sampled at various developmental stages, visualized by squashing the anthers and staining with acetocarmine, illustrating various stages of microsporogenesis: a. early meiosis; b. pre meiosis; c. meiosis; d. post meiosis (tetrad stage); e. Early uni nucleate; f. late uninucleate; g. bi nucleate; and h. tri nucleate (mature pollen).

Appendix table 7. 1 Measured temperatures (ºC) and humidity in greenhouse during the second experiment of manifestation of Waagan vs Drysdale (DAPI and pollen starch) (chapter 7) experimental period. Booting occurred in the month November. Columns indicate daily average (Day), nightly average (Night), average daily maximum (Maximum average), average daily minimum (Minimum average), maximum, minimum and number of days where temperature greater or equal to 30ºC.

		Day	Night	Maximum average	Minimum average	Maximum	Minimum	Days ≥ 30ºC
Temperature (ºC)								
2015	September	21.3	16.9	23.9	15.4	29.4	14.6	-
	October	23.4	19.8	26.2	17.6	33.7	15.8	5
	November*	24.0	20.4	26.2	18.8	31.7	15.7	4
	December	25.3	21.2	27.5	19.5	32.5	15.6	7
2016	January	25.5	21.0	30.1	19.4	48.0	16.1	17
	February	25.4	20.5	29.4	18.8	35.3	16.1	13
% relative humidity								
2015	September	57.3	71.1	79.5	47.0	87.1	35.1	
	October	59.8	60.6	77.1	44.3	90.3	25.9	
	November*	57.3	62.0	74.9	46.3	87.9	27.1	
	December	57.3	64.6	72.3	46.5	83.3	22.9	
2016	January	59.8	69.6	77.2	42.2	87.1	30.8	
	February	61.5	71.7	78.0	50.0	89.4	30.5	