PHENOTYPIC AND MOLECULAR GENETIC ANALYSIS OF REPRODUCTIVE STAGE HEAT TOLERANCE IN WHEAT (*TRITICUM AESTIVUM)*

A Dissertation

by

RICHARD ESTEN MASON

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2010

Major Subject: Molecular and Environmental Plant Sciences

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Approved by:

Chair of Committee,	Dirk B. Hays
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	Stephen King
	William Rooney
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ABSTRACT

Phenotypic and Molecular Genetic Analysis of Reproductive Stage Heat Tolerance in Wheat *(Triticum aestivum)*. (May 2010)

Richard Esten Mason, B.A., Texas A&M University

Chair of Advisory Committee: Dr. Dirk B. Hays

Heat stress adversely affects wheat production in many regions of the world and is particularly detrimental during reproductive development. The objective of this study was to identify quantitative trait loci (QTL) associated with improved heat tolerance in hexaploid bread wheat (*Triticum aestivum)*. To accomplish this objective, an analysis of both the phenotypic and genetic responses of two recombinant inbred line (RIL) populations was conducted. RIL populations Halberd x Cutter and Halberd x Karl 92 (H/K) both derive heat tolerance from Halberd and segregate in their response to heat stress. A heat susceptibility index (HSI) was calculated from the reduction of three yield components; kernel number, kernel weight, and single kernel weight, following a three-day 38°C heat stress treatment during early grain-filling. The HSI, as well as temperature depression of the main spike and flag leaf were used as measurements of heat tolerance. Genetic linkage maps were constructed for both populations and were used in combination with phenotypic data and statistical software to detect QTL for heat tolerance.

In a comparison across the two across populations, seven common QTL regions were identified for HSI, located on chromosomes 1B, 3B, 4A, 5A, 5B, and 6D. Subsequent analysis of temperature depression in the H/K population identified seven QTL that co-localized for both cooler organ temperature and improved HSI. Four of the beneficial alleles at these loci were contributed Halberd. The genetic effect of combining QTL, including *QHkw.tam-1B, QHkwm.tam-5A.1*, and *QHskm.tam-6D* showed the potential benefit of selection for multiple heat tolerant alleles simultaneously. Analysis of the H/K population in the field under abiotic stress detected QTL on chromosome 3B and 5A, which were in agreement with results from the greenhouse study. The locus *QYld.tam-3B* was pleiotropic for both temperature depression and HSI in both experiments and was associated with higher biomass and yield under field conditions.

The results presented here represent a comprehensive analysis of both the phenotypic response of wheat to high temperature stress and the genetic loci associated with improved heat tolerance and will be valuable for future understanding and improvement of heat stress tolerance in wheat.

DEDICATION

To my wife and son

ACKNOWLEDGEMENTS

I would like to thank my major advisor, Dr. Hays, for his support and advice the last five years. I thank my committee members, Dr. Finlayson, Dr. King, and Dr. Rooney, for advice and conversation. I thank Dr. Ibrahim for teaching me how to become a better plant breeder, and I thank the late, great, Dr. Norman Borlaug, whose unselfish passion saved billions from hunger and inspired many young scientists, including myself.

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NOMENCLATURE

QTL	Quantitative trait loci
HSI	Heat susceptibility index
Td	Temperature depression

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CHAPTER I

INTRODUCTION: PHYSIOLOGICAL AND MOLECULAR GENETIC BREEDING STRATEGIES FOR UNDERSTANDING AND IMPROVING HEAT STRESS TOLERANCE IN WHEAT

Introduction

Heat stress is a primary constraint to wheat production both globally and in the U.S. Great Plains region. In the Southern Great Plains, temperatures exceeding 30°C during reproductive development occur annually, reducing yield and end-use quality by shortening the duration of grain filling, inducing early developmental seed abortion, and reducing test weights. Heat stress disrupts source to sink relationships and inhibits photosynthesis and carbon fixation, resulting in early senescence of vegetative and reproductive organs. Although periods of high temperature stress are commonly associated with periods of drought, recent evidence points to the yield limitations of current wheat cultivars, particularly hard red winter wheat (HRWW), under heat stress alone (Yang et al. 2002a). With increasing cultivation of wheat occurring on marginal land in sub-optimal environments, it is vital that progress is made toward introgression of reproductive stage heat tolerance into current elite cultivars (Lantican et al. 2003; Trethowan et al. 2002).

This dissertation follows the style of Theoretical and Applied Genetics.

While much research has been devoted to understanding the basic physiology of the heat stress response in plants, traditional breeding approaches have been limited in their ability to incorporate heat tolerance traits into breeding programs and germplasm. Much of this lack of progress can be attributed to the quantitative nature of heat tolerance, for which a combination of both traditional breeding and molecular and physiological genetics is necessary. Sources of heat tolerant germplasm from CIMMYT, Australia, ICARDA, and the Middle East, exhibit superior levels of heat tolerance compared to HRWW cultivars currently grown in the Great Plains region (Hede et al. 1999; Yang et al. 2002a). These lines maintain yield and/or quality under heat stress and may be utilized for understanding reproductive stage heat tolerance and for introgression of heat tolerance traits into current HRWW cultivars.

Wheat breeding and genetics

Importance and progress of wheat breeding

Wheat is one of the most widely grown cereal crops, with over 620 million tons produced worldwide in 2004 (Dubcovsky and Dvorak 2007; FAO 2006). This accounts for nearly one-fifth of global caloric intake. Through the adoption of green-revolution cultivars, use of synthetic fertilizers, and improved farming practices wheat production has increased at the rate of nearly 1% per annum over the last 50 years (Trethowan et al. 2002). Even larger advances have been made in marginal environments, where yields

have increase by as much as 2 to 3% per annum (Lantican et al. 2003; Trethowan et al. 2002). Based on wheat yields from 1950, approximately 1.2 billion hectares of arable land were spared between 1950 and 1999, simultaneously lessening the impact of wheat production on the environment (Borlaug 2004). Even with these advances, nearly 2 billion people remain malnourished and continued progress will be necessary in order to meet the demand spurred by population growth and malnutrition in areas such as sub-Saharan Africa. In addition, environmental instability created by the changing global climate will force breeders to develop cultivars with higher tolerance to intermittent periods of severe and less predictable weather. With exports of wheat to developing countries predicted to double by 2025, it is vital that the major wheat producing countries work to meet these challenges (Rosegrant and Cline 2003).

The wheat genome

Common bread wheat, *Triticum aestivum* (AABBDD), evolved from the hybridization of three diploid ancestors, *Triticum monococcum* (AA), a close relative of *Aegilops speltoides* (BB), and *Aegilops tauschii* (DD), with sympatry between domesticated emmer (genomes AABB) and *Aegilops tauschii* (genomes DD) resulting in common hexaploid bread wheat (genomes AABBDD) (reviewed by (Dubcovsky and Dvorak 2007). The haploid genome of *T. aestivum* consists of 21 chromosomes of varying sizes comprising seven homeologous groups (2n=6x=42). The presence of the *Ph1* gene on the long arm of Chromosome 5B prevents pairing of homeologous chromosomes,

making the genetics of hexaploid wheat comparable to that of a diploid species (Akhunov et al. 2003). The genome size of hexaploid wheat is estimated at 17,000 Mb, roughly 40 times the size of rice, resulting mostly from extensive duplication of gene and intergenic sequences, many of which are now known due to the improving resources available to study polyploidy genetics (Akhunov et al. 2003) . Despite its large genome size, gene order and content on each of the seven homeologous chromosomes is quite conserved, with "diploidization" resulting in the silencing of many duplicated genes (Singh et al. 2007). Like any large genome, as much as 80% of the DNA sequence is comprised of non-coding, repetitive elements. While useful for the development of DNA based markers for mapping of quantitative trait loci (QTL), these long stretches of repetitive sequences make genome sequencing extremely difficult. The recent complete ordered physical map of the 3B chromosome is a step forward for wheat genetics, but the complete sequencing and ordering of the wheat genome remains unlikely in the near future (Paux et al. 2008).

Heat stress

Heat stress is a primary constraint to wheat yield and quality

Despite its high level of adaptive plasticity, extreme environmental stress can still compromise wheat yield and end-use quality on an economic level. Assad and Paulsen in 2002 identified improvement of high temperature tolerance as the most important

factor leading to increased yield from 1874-1994 in the Great Plains region. Periods of high temperature stress in this region generally occur during early reproductive development and grain filling, when wheat is most susceptible. Wheat has as optimal daytime growing temperature of 15°C and for every 1°C above this optimum a reduction in yield of 3-4% has been observed (Wardlaw and Wrigley 1994). With an average daytime temperature of 28°C in the Southern Great Plains region during reproductive development (March-June), yield losses can range from 30-50% due to heat stress alone. Many of the current HRWW varieties grown in this region show susceptibility to heat stress in terms of their inability to maintain kernel number, kernel weight, and duration of grain filling under high temperature stress (Hays et al. 2007b; Yang et al. 2002c). Because moderate heat stress occurs on a yearly basis, and extreme heat stress on a periodic basis the total yield potential for cultivars in this region may never be realized due to a lack of emphasis on heat tolerance by breeders.

Wheat growth and development is divided into three phases: vegetative, reproductive, and grain filling. Heat stress during the vegetative stage is not of major concern due to the sowing of wheat during the winter or spring months. Immediately prior to anthesis, the number of grains are determined and subsequently filled following anthesis. The number of grains and individual kernel weight make up the two major yield components in wheat (Satorre and Slafer 1999). Grain filling in wheat depends on three main sources: current assimilates from photosynthetic leaves and stems, mobilization of stored carbohydrates and N containing compounds in the same organs, and assimilates

produced by tissues in the ear itself. Depending on the timing, intensity, and duration of heat stress, grain set and grain filling may be disrupted, compromising yield. The response of wheat to chronic heat stress (Tashiro and Wardlaw 1989; Wardlaw et al. 1989; Yang et al. 2002a, b, c) and short-term heat shock (Hays et al. 2007a; Plaut et al. 2004; Tashiro and Wardlaw 1990) has been studied in controlled environments where specific heat treatments may be applied. In addition, cultivars and germplasm have been evaluated in the field under late season or off-season heat stress, or using glass or plastic houses to create heat stress during anthesis (Ferris et al. 1998; Khanna-Chopra and Viswanathan 1999). Prior to anthesis, heat stress results in seed sterility, due to the sensitivity of microspore and megaspore development (Tashiro and Wardlaw 1990). The main effect attributed to post-anthesis heat stress is a reduction in individual kernel weight, due in part to both a reduction in the endosperm and embryo maturation and modification of their composition (Randall and Moss 1990; Wardlaw and Wrigley 1994). Others have also reported a significant reduction in kernel number post-anthesis, with the greatest reduction seen shortly after pollination, resulting from short-term high intensity heat shock (Hays et al. 2007a; Plaut et al. 2004; Tashiro and Wardlaw 1990). Heat tolerant varieties that maintain yield components under both chronic and short-term heat shock have been identified and emphasis is starting to be placed on incorporating these heat tolerant sources into current breeding programs (Hays et al. 2007a; Hays et al. 2007b; Yang et al. 2002c).

Heat tolerance is a quantitatively inherited trait

Heat tolerance is quantitatively inherited and is strongly dependent on the timing, intensity, and duration of stress during sensitive plant developmental stages. Cellular membrane stability (CMS) has been used as an indirect measure for both heat and drought tolerance at the seedling stage (Ibrahim and Quick 2001a, b; Ottaviano et al. 1991; Tripathy et al. 2000). A study in maize identified a minimum of six QTL explaining 53% of the variation for CMS in a set of maize recombinant inbred lines (Ottaviano et al. 1991). A subsequent study in the same population found 5 QTL and 6 QTL for pollen germination and pollen tube growth under high temperature stress, respectively (Frova and Sarigorla 1994). Estimation of heritability for membrane stability in wheat was found to be moderately low (.32-.38) but was highly correlated to a triphenyl tetrazolium chloride assay for heat injury (Ibrahim and Quick 2001b). Maternal effects were found to account for 67% of the variation for CMS within a diallele analysis of 6 wheat varieties (Ibrahim and Quick 2001a). This finding is significant and consistent with evidence showing the importance of cytosolic and mitochondrial specific heat shock proteins in the heat stress response (Miroshnichenko et al. 2005; Rhoads et al. 2005). A significant correlation has been observed between measurement of CMS and yield under chronic heat stress in the field, although this finding is inconsistent within the literature (Blum et al. 2001). A minimum of 1.4 genes were estimated to be controlling heat tolerance in an F_2 population based on shortening of grain filling duration (GFD) (Yang et al. 2002c). In the same study, broad sense

heritability for GFD was high (80%) and two marker loci were linked to the trait, explaining 23% of the total variation. Screening of *Arabidopsis thaliana* mutants for inability to acquire thermotolerance identified four genetic loci controlling the trait, with one loci, *Hot1*, determined to encode a HSP101 (Hong and Vierling 2000). A mutation in the *Hot1* gene that caused the conversion of a glutamine to a lysine residue within an ATP binding domain was thought to compromise the ATPase activity of *HSP101*.

The heat stress signaling response in plants

Plants are sessile organisms and that must respond rapidly to environmental changes in their surroundings. The optimum temperature for growth and development differs markedly between species as does the temperature above that optimum that elicits a species' heat stress response. As a cool season grass, wheat has a threshold temperature of around 26°C, considerably lower than other crop and vegetable species (Stone and Nicolas 1994; Wahid et al. 2007). Heat shock proteins (HSPs) are ubiquitous to the heat stress response and are one of the earliest proteins induced by heat stress. There are five major classes of HSPs in plants including the HSP70 family, chaperonins (including HSP60), HSP90 family, HSP100 family (Clp), and small heat shock proteins (sHSP) (Maestri et al. 2002; Wahid et al. 2007; Wang et al. 2004). In general, most heat shock proteins have a chaperone function, assisting in protein folding, unfolding, and preventing aggregation in response to heat stress. HSPs have been shown to be expressed in response to heat, drought, and other types of abiotic stresses, as well as

constitutively during growth and development. HSP70 is the best characterized of the HSPs, with specific family members showing tissue specific expression in response to heat stress (Sung et al. 2001). In wheat, a plastid localized sHSP was linked to thermotolerance in a RIL population segregating for cell viability under heat stress (Joshi et al. 1997). Controlling the transcription of heat shock proteins and other heat inducible genes are heat stress transcription factors (HSFs). Much of what is known about HSFs comes from studies in Arabidopsis and tomato. In Arabidopsis, there are 21 known HSFs, assigned to three classes and 14 groups based on sequence conservation (Kotak et al. 2004; Nover et al. 2001). In response to heat stress, HSFs bind to heat stress elements (a palindromic nGAAn motif) conserved in the promoters of heat inducible genes and regulate their expression (Bienz and Pelham 1987; Nover 1987). In tomato, a class A Hsf, *HsfA1*, has been shown to be a master regulator of the heat stress response, with post transcriptional silencing in transgenic tomato plants completely compromising the downstream expression of heat-shock proteins and other HSFs (Mishra et al. 2002). More recently, genomic tools have been used to identify novel genes under the control of HSFs. Micro-array analysis of a *HsfA1a/HsfA1b* double mutant in Arabidopsis identified genes involved in protein biosynthesis and processing, signaling, metabolism, and transport to be under the control of HSFs (Busch et al. 2005). Although a direct sensor for heat stress has not yet been identified, a burst of oxidative stress has been shown to occur immediately following heat stress and has been shown to be necessary for expression of some heat shock proteins (Larkindale and Knight 2002; Volkov et al. 2006; Wang et al. 2006). In addition, ascorbate peroxidase 1 (*Apx1)*, the

main hydrogen peroxide scavenging enzyme in plants, contains a functional Hsf binding motif within its promoter and other Apx genes have been shown to be induced by short term heat-shock (Panchuk et al. 2002). It has since been hypothesized that HSFs may serve as a sensor for heat stress via detection and subsequent regulation of the oxidative stress response (Reviewed by (Miller and Mittler 2006). Crosstalks with other signaling pathways including calcium/calmodulin, ABA, and ethylene have also been shown to be involved in heat stress signaling (Hays et al. 2007a; Larkindale and Knight 2002).

Heat stress impairs photosynthesis, carbon fixation, and disrupts source/sink relationships during reproductive development

During grain filling under optimal temperature conditions, active photosynthesis provides nearly all the carbohydrates for developing kernels. The majority of these carbohydrates come from the flag leaf and photosynthesis in the stems and glumes. High temperature stress has been shown to impair active photosynthesis and carbon assimilation, leading to mobilization of carbohydrate reserves from the leaves and stems stored during vegetative development (Blum et al. 1994; Paulsen 1994). Impairment of photosynthesis and depletion of reserves can lead to a reduction in the duration of grain filling and early flag leaf senescence which in combination negatively impact yield (Yang et al. 2002b). Moderate heat stress above the optimum inhibits photosynthesis through dephosphorylation of the water splitting D-1 protein of Photosystem II (PSII) and disruption of electron transport (Havaux 1993; Rokka et al. 2000). The activation

state of the carbon fixation enzyme, ribulose-1,5-bisphosphate (rubisco), has also been shown to limit photosynthetic capacity under high temperature stress. Additionally, the solubility of carbon dioxide decreases under higher temperatures and low relative oxygen leading to increased rubisco oxygenase activity and higher photorespiration (Laing et al. 1974; Monson et al. 1982). Rubisco is regulated by rubisco activase, an ATPase, which under non-stressed conditions keeps rubisco in the active state by removing tightly bound sugars from its active site (reviewed by (Salvucci and Crafts-Brandner 2004). Under heat stress, rubisco activase becomes impaired leaving rubisco in the de-activated state (Crafts-Brandner and Salvucci 2000). In *Arabidopsis*, creation of rubisco activase variants with enhanced thermostability resulted in increased levels of photosynthesis and increased growth rates under heat stress (Kurek et al. 2007). Levels of 3-phosphoglycerate, the product of carboxylation of ribulose-1, 5-bisphosphate by rubisco have been shown to decrease to undetectable levels in response to heat stress above 35°C, while levels of the precursor, ribulose-1, 5-bisphospate, remained unaffected (Law and Crafts-Brandner 1999).

Adaptation to high temperature stress

Plants utilized both heat avoidance and heat resistance/tolerance mechanisms in order to minimize the damage caused by high temperature stress. Heat avoidance mechanisms include adaptations such as reflective leaf hairs and leaf waxes, leaf rolling and vertical leaf orientation, and growth of small highly dissected leaves that maximize convective

and conductive heat loss. Heat resistance/tolerance mechanisms include remobilization of resources when photosynthesis is impaired (Yang et al. 2002a), induction of heat stress responsive proteins such as HSPs (discussed above), and reducing canopy temperature under high temperature stress through increased transpirational cooling (Ayeneh et al. 2002; Reynolds et al. 2000). Strong correlations between canopy temperature depression (CTD), leaf temperature depression, spike temperature depression and yield have been observed in the field (Ayeneh et al. 2002; Reynolds et al. 2000). The use of CTD has the potential to be a promising tool for selection of drought tolerant and heat tolerant lines due to both its robustness, as it incorporates the collective response of stomatal conductance, reflective waxes, and other cooling mechanisms, and its ease of measurement. A positive correlation between photosynthetic activity and stomatal conductance with crop yield and biomass has also been shown in the field under arid, irrigated conditions (Gutierrez-Rodriguez et al. 2000; Reynolds et al. 2000). Reflective waxes also show promise as an adaptive mechanism that could be exploited in wheat to improve abiotic stress tolerance. Sorghum bicolor has been shown to have epicuticular wax levels five times greater than that of rice, contributing to its high level of drought tolerance (Otoole and Cruz 1983) and high levels of epicuticular wax have also been correlated with yield and spectral reflectance in wheat under heat stress (Hays et al. unpublished).

Molecular genetic tools for wheat improvement

Genetic resources for wheat

As an alternative to direct genome sequencing, a number of tools have been developed to facilitate wheat breeding and genetics and facilitate a better understanding of the wheat genome. In 2000, GenBank contained only nine expressed sequence tags (EST)entries for wheat, and less than 100 for all of the *Triticeae* tribe (wheat, barley, and rye), leading to an international effort to develop EST and other genetics based resources (Lazo et al. 2004). As of today, GenBank contains close to 900,000 wheat EST sequences, while the number of annotated gene sequences is close to 600. Bacterial artificial chromosome libraries for hexaploid wheat as well as its wild progenitors, *Triticum monococcum* (AA) and *A. Tauschii* (DD) have been developed and are being utilized for direct sequencing of complete genes. As part of this same project, unique EST contigs were further characterized by mapping their location to DNA restriction fragments using a set of wheat deletion lines, allowing for the assignment of EST probe sets to chromosomal locations. The use of deletions to map the function of chromosomal segments and/or the genes underlying a deficiency or trait can be traced to classical genetic studies in *Drosophila* and corn. Wheat deletion lines were first characterized by Sears et al. in the 1960's, with more in depth characterization occurring recently (Endo and Gill 1996; Qi et al. 2003). These deletion stocks have since been used for molecular genetic studies, mapping of ESTs to chromosomal segments, mapping of simple sequence repeat

markers (SSRs) to chromosome bins, and more recently for targeted map-based cloning of candidate genes. For mapping of EST to chromosomal segments, a subset of deletion lines were used to define 159 'deletion bins' dispersed throughout the 21 wheat chromosomes, an average of 7.6 deletion bins per chromosome. These bins are annotated based on the size of the deletion relative to the chromosomal arm in which they are located and may range from ~0.03 to 0.50, as a fraction of the arm. On average, the physical size of each deletion bin would be ~100Mb, or the equivalent to the physical distance of three rice chromosomes. If it is taken into consideration that gene synteny and colinearity are conserved between the three wheat genomes, ESTs may be mapped to chromosomal bins at a resolution of 28Mb (Qi et al. 2003). These numbers can be deceiving given the size of the deletion bins may range from as small as 11Mb towards the telomeres where bin size is smaller and genetic density is greater, to as much as 200Mb toward the centromeres. In 2004, Lazo et al. published the results of the construction of a 16,000 locus bin-delineated EST map, mapping the location of 7,637 unigene ESTs, representing nearly 16,000 loci (genes which had multiple locations), around half of the projects goal of 13,635 ESTs. A subsequent study delineated 213 additional ESTs to chromosome bins (Singh et al. 2007). Estimated gene density varies within the literature, but most recent estimates are around 80kb/gene in *T. aestivum (Reviewed by (Stein 2007).* Assuming gene content is conserved between the three genomes, there is an estimated 70,000 unique genes within one of the diploid genomes. Since this EST-bin map used only unigene ESTs, paralogs would have been eliminated, (although mapped as loci) and this subsequently represents around 11% of total

estimated gene number. With 7,637 unique genes representing 16,000 loci, it could be estimated that a total of 147,000 gene loci would be located within the hexaploid genome. This equates to very roughly 924 gene loci per deletion bin, although gene rich telomeric regions would have a disproportionately larger number of loci. The current EST-Bin map averages roughly 100 loci per bin(Peng et al. 2004).

Genetic linkage mapping for loci discovery in wheat

While wheat geneticists do not have the benefit of a genome sequence, high-density genetic maps utilizing polymorphic molecular markers have been developed and can be utilized for QTL mapping of important loci (Quarrie et al. 2005; Somers et al. 2004; Xue et al. 2008). Although the basic concepts of detecting QTL were developed nearly a century ago (Sax 1923), recent advances in marker development and QTL detection techniques that are freely available to researchers have accelerated the field and allowed broad access to the technology (Asins 2002; Dwivedi et al. 2007; Price 2006). The most widely used molecular marker over the last decade has been the simple sequence repeat or micro-satellite marker (SSR) (Hearne et al. 1992) due to its high level of polymorphism and its transferability across genetic backgrounds both within and between species. In addition to genetic linkage maps, a bin-delineated SSR map was constructed to localize SSR markers to deletion bins where ESTs have been mapped, allowing the comparison of physical versus genetic distances in wheat (Sourdille et al. 2004). In total, 725 microsatellite markers were assigned to deletion bins spanning all

21 chromosomes, for an average of 4.6 markers/bin, although disproportionately distributed. As expected telomeric regions represented a disproportionate fraction of the total genetic distance (40% of the genetic map or 1722/3876 cM in the distal 20% of the physical map) due to higher recombination rates in these gene rich regions. On average, it could be estimated that each deletion bin spans 25cM, with telomeric regions disproportionately larger in genetic distance due to increase recombination and centromeric regions much smaller. Based on genetic: physical ratios from Sourdille et al. 2004, telomeric (genetic: physical =2.2) bins would on average span 55 cM compared to 8.25 cM for the most centromeric regions (genetic:physical=.33). The physical mapping of SSRs to chromosome locations and the utilization of common SSRs between studies has allowed wheat researchers to detect QTL for specific chromosomes, not just linkage groups, which has allowed for comparison of QTL locations across studies. In total, over 90,000 molecular markers are currently available in wheat (http://wheat.pw.usda.gov/GG2/index.shtml) providing a powerful tool that wheat researchers can use to dissect complex traits and identify important genetic loci regulating these traits.

Quantitative trait loci regulating heat stress tolerance, drought stress tolerance, and yield in wheat

Despite the detrimental effects of heat stress on yield and quality in wheat, little work has been done to identify loci regulating heat tolerance in terms of yield and quality

stability. As mentioned previously, two marker loci were linked to the genetic control of grain-filling duration under heat stress, explaining 23% of the total phenotypic variation for the trait (Yang et al. 2002c). These two SSR markers, *gwm11* and *gwm293*, are located on chromosomes 1B and 5A, respectively. The same marker on chromosome 1B, *gwm11*, was associated with a QTL for yield that was present in environments with maximum number of days above 30°C across 18 site-years (Kuchel et al. 2007b). In this same study, number of days above 30°C was found to be the environmental factor most closely associated to decreases in yield, pointing to the importance of breeding for tolerance to high temperature stress. Quarrie et al. 2005 characterized a number of QTL present across a range of environments including salinity, nutrient, and drought stress, but could not confirm the expression of a major QTL on the long arm of 7A to be expressed under drought stress in a set of near-isogenic lines (Quarrie et al. 2006). A QTL on chromosome 4A was associated with increased yield, biomass, weight per spike, earliness per se, and other agronomic traits under drought stressed conditions (Kirigwi et al. 2007). Recently, a number of QTL studies have been undertaken to identify loci that contribute to yield, yield components and other agronomic traits across a range of environments in hexaploid wheat (Cuthbert et al. 2008; Groos et al. 2003; Hai et al. 2008; Huang et al. 2006; Kuchel et al. 2007a; McCartney et al. 2005; Quarrie et al. 2005; Wang et al. 2009) and in durum wheat (Maccaferri et al. 2008; Peleg et al. 2009). Although most of these studies do not provide insight into the specific environmental conditions that influenced the presence of these QTL, such as specific abiotic and biotic

stresses, they are useful for comparative analysis to identify common QTL which are having a significant effect across genetic backgrounds and environments.

Approach of the current study

Although the detrimental effects of reproductive stage heat tolerance on wheat yield and quality have been well documented, less is known about the loci controlling the variation seen in the trait at the phenotypic level. This project aims to link variation in heat tolerance/susceptibility to the quantitative trait loci that regulate it.

The *objective of this study* is to define the phenotypic expression and heritability of reproductive stage heat tolerance in terms of its individual yield components and to identify and validate the quantitative trait loci (QTL) regulating these components. In this study, we have defined heat tolerance as the maintenance of individual yield components under early reproductive stage heat stress versus control conditions. The *central hypothesis* is that the quantitative inheritance of reproductive stage heat tolerance can be defined more simply by its individual components and that these components are regulated by a discrete set of QTL. Through hybridization of wheat cultivars genetically diverse in their response to heat stress, it is possible to detect these QTL and validate their significance. The following specific objectives have been used to test this hypothesis:

Objective 1: ***Determine the phenotypic expression and heritability of reproductive stage heat tolerance.*** The working hypothesis is that reproductive stage heat tolerance, while quantitatively inherited, is controlled by more simply inherited components. The Australian heat tolerant cultivar 'Halberd', two susceptible HRWW cultivars, 'Cutter' and 'Karl 92', and recombinant inbred line (RIL) populations in both susceptible backgrounds were characterized for early reproductive stage heat tolerance. Heat tolerance was estimated based on stability of yield components calculated by a heat susceptibility index (HS) under heat stress versus non-stressed conditions in both controlled greenhouse/growth chamber and field conditions.

Objective 2: ***Identify QTL regulating reproductive stage heat tolerance.*** The working hypothesis is that individual heat tolerant components are regulated by a discrete set of QTL, which collectively regulate reproductive stage heat tolerance. Genetic linkage maps were developed for both RIL populations and used for identification of QTL associated with HSI, canopy temperature depression, as well as other adaptive traits.

Objective 3: ***Validate QTL across environments and susceptible backgrounds.*** The working hypothesis is that major QTL regulating reproductive stage heat tolerance in wheat are stable across genetic backgrounds and in different environments. A comparative analysis combining genetic maps from both RIL populations was carried out and used to analyze the genetic effect and location of the most stable QTL. In addition, field analysis of Halberd x Karl 92 (n=121) RILs was carried out to detect QTL

associated with improved yield potential and co-localization of these loci with those for HSI.

The approach of this study is innovative in that it uses both traditional plant breeding and molecular genetics to define a quantitative trait, reproductive stage heat tolerance, in terms of yield individual components, allowing for the regulatory loci to be identified. A thorough understanding of the importance of both the individual yield components of reproductive stage heat tolerance and the QTL regulating them will assist in the introgression of heat tolerance traits into current elite cultivars within a breeding program and work toward a better biological understanding of the genes regulating reproductive stage heat tolerance.

CHAPTER II

QUANTITATIVE TRAIT LOCI ASSOCIATED WITH HEAT SUSCEPTIBILITY INDEX IN THE HALBERD X CUTTER RECOMBINANT INBRED LINE POPULATION

Introduction

Heat stress is a primary constraint to global wheat (*Triticum aestivum* L.) production. Over the last 50 years, wheat production has increased at the rate of nearly 1% per annum (Trethowan et al. 2002) and tolerance to high temperature stress has been identified as an important factor contributing to these increased yields (Assad and Paulsen 2002). Despite these advances, increasing yield potential and yield stability of wheat in marginal environments remains a priority, particularly in areas with high malnutrition and historically low production, such as Sub-Saharan Africa, and in areas with increasing environmental instability created by the changing global climate (Reynolds et al. 2007a). As a cool season crop, wheat has as an optimal daytime growing temperature of 15°C and for every 1°C above this optimum a reduction in yield of 3-4% has been observed (Wardlaw et al. 1989). Based on a yearly average temperature of 28°C during reproductive development in regions of the Southern Great Plains, it has been estimated that wheat may lose 30-50% of its yield potential due to high temperatures alone (Hays and Ibrahim, unpublished). The response of wheat to

both chronic heat stress (Tashiro and Wardlaw 1989; Wardlaw et al. 1989; Yang et al. 2002a, b, c) and short-term heat shock (Hays et al. 2007a; Plaut et al. 2004; Tashiro and Wardlaw 1990) is well documented and many of the current hard red winter wheat grown in the Southern Great Plains region have shown susceptibility in terms of their inability to maintain yield and quality under high temperatures (Hays et al. 2007b; Yang et al. 2002b). High temperature stress during reproductive development is particularly detrimental, with post-anthesis heat stress resulting in a reduction in both individual kernel weight and kernel number (Hays et al. 2007a; Plaut et al. 2004; Randall and Moss 1990; Tashiro and Wardlaw 1990; Wardlaw and Wrigley 1994). Although varieties that show improved yield stability under heat stress have been identified (Hays et al. 2007a; Hays et al. 2007b; Yang et al. 2002c), the quantitative nature of heat tolerance and unpredictability of heat stress in the field makes it particularly difficult for breeders to effectively select for the trait. Yang et al. 2002c identified two markers associated with grain-filling duration under reproductive stage heat stress, located on chromosomes 1B and 5A. With exception of this study, there are no reports indentifying quantitative trait loci (QTL) regulating heat tolerance in wheat that could be used as molecular markers to expedite the development of heat tolerant germplasm.

Thus, the objective of this study was to carry out a detailed characterization of the effect of heat shock on plant yield and yield components during early grain-filling and to identify molecular markers linked to QTL for heat susceptibility index (HSI) and other potentially beneficial phenotypic traits. HSI was calculated based on difference in yield

and yield components between the heat stressed and control treated RIL replications and used as a phenotypic measure of heat tolerance for QTL mapping.

Materials and methods

Line development

A cross between the heat tolerant spring wheat cultivar 'Halberd' and heat susceptible winter wheat cultivar 'Cutter' was carried out in 2003 in the greenhouse in College Station, TX. Lines were advance by single seed descent in the greenhouse to the F_4 generation and seed from individual F_4 plants was bulked to create $F_{2:4:5}$ lines. Sixty-four $F_{2:4:5}$ lines were selected for evaluation in 2005 and F_6 families were evaluated in 2006. A cross between a heat tolerant spring wheat and a heat susceptible winter wheat was chosen to determine the pleiotropic interaction between heat tolerance/susceptibility and the vernalization requirement.

Phenotypic analysis

Parental cultivars and recombinant inbred lines (RILs) were germinated in petri dishes and vernalized for six weeks at 4°C. Seedlings were transplanted at two plants per pot (1:3 peat: sandy loam soil in 12 x 15 cm pots) and replicated ten times, resulting in ten single plant replications each for control and heat stress treatments. Seedlings were

transplanted on January 2 in 2005 and January 21 in 2006 and arranged in a completely randomized design. Initially, plants were grown under optimal management in air-conditioned greenhouses at ~20^{o}C/18^{o}C day/night cycles with a 14 h photoperiod from 6am to 8pm, under natural sunlight with 600 μmol m^{-2} s^{-1} PAR supplemental light. Inflorescences of the main or first pollinated spike from each plant were scored for day of pollination (DAP) upon emergence of the anther from the pollinated spikelet. At 10 DAP, all replications within a RIL were transferred into two identical growth chambers. Half received a three-day heat stress treatment at 38^{o}C/18^{o}C day/night (with maximum 38^{o}C heat stress from 8am to 5pm), while the second half were maintained at control conditions of 20^{o}C/18^{o}C day/night cycles in the second identical growth chamber. A light intensity of 600 mmol m^{-2} s^{-1} PAR was maintained in both the control and heat stress chambers. Following the three-day transfer to growth chambers, plants were returned to the greenhouse and maintain under optimal conditions, as described above until maturity. Grain filling duration (Gfd) was estimated as the date of pollination until 90% senescence of the main inflorescence and days to flowering (Dtf) was calculated as the time from planting to pollination of the main-spike. At maturity, plants were harvested and hand threshed. Kernel number and kernel weight of the main-spike was measured and used to calculate individual yield components (Table 2.1). Heat tolerance was calculated as a heat susceptibility index (HSI) for each individual RIL using the equation by Fisher and Maurer (Fischer and Maurer 1978): $HSI = (1-Y_h/Y)/(1-X_h/X)$, where Y_h and Y are the phenotypic means for each genotype under control and heat

stressed conditions, respectively, and X_h and X are the phenotypic means for all lines under control and heat stressed conditions, respectively.

Table 2.1 Phenotypic traits evaluated in the greenhouse for the Halberd x Cutter RIL population

Trait	Symbol	Method of Measurement
Kernel number of main-spike	Knm	Number of kernels of main-spike at maturity
Kernel weight of main-spike (g)	Kwm	Yield of main-spike at maturity
Single kernel weight of main-spike (g)	Skm	Kernel weight of main-spike / kernel number of main-spike
HSI	HSI or H for QTL	Heat susceptiblity index calculated for each yield component[1]
Days to flowering	Dtf	Days from planting to flowering of main-spike
Grain-filling duration	Gfd	Days from flowering to 90% senescence of main-spike
Flag leaf length (cm)	Fll	Length of flag leaf from base of leaf to tip
Flag leaf width (cm)	Flw	Width of the widest section of the flag leaf
Visual wax	Wax	Visual score (0-5) of abaxial flag leaf wax

[1]$HSI = (1-X_h/X)/(1-Y_h/Y)$, where X_h and X are the phenotypic means for each genotype under control and heat stressed conditions, respectively, and Y_h and Y are the phenotypic means for all lines under control and heat stressed conditions, respectively.

The HSI for individual main-spike yield components for each RIL were used as phenotypic data for quantitative trail loci (QTL) mapping. In addition, the length, width, and visual wax score of the flag leaf were recorded at 10 DAP in 2006. Width was measured on the widest part of the flag leaf and length was measured from the base of the flag leaf to the tip. Visual wax score was based on a visual rating of abaxial wax

accumulation on a scale of 0 to 5, with a score of 0 showing no wax accumulation and a score of 5 showing wax accumulation on 100% of the flag leaf.

Statistical analysis

Statistical analysis was carried out using the MIXED procedure (SAS v8.2, SAS Institute Inc., Cary, NC, USA). Significant differences between means of treatments were detected by considering treatment as having a fixed effect. The broad sense heritability (H^2) of yield components was estimated from the variance components derived from PROC MIXED:

$H^2 = \sigma_g^2 / \sigma_g^2 + \sigma^2_{ge/e} + \sigma^2_{error/re}$

where σ_g^2 σ_{ge} and σ^2_{error} represent the genotype, genotype x environment, and error variances, respectively.

H^2 was estimated for both control and heat stressed treatments, treating all effects as random (genotype, year, replication (year), genotype x year). Because heterogeneous error and significant genotype x environmental variance was observed between the two treatment years, each year of data was analyzed separately and not combined. Pearson's correlations were done using the statistical software package SPSS for Macintosh (SPSS inc. Chicago, IL). An allele contrast analysis for the phenotypic means of markers most closely associated with select QTL was done using GLM-ANOVA in SPSS, treating each marker as having a fixed effect and individual traits as dependent variables.

Molecular marker analysis

Fifteen F_5 seed of each parent and RIL were germinated on filter paper and coleoptiles were bulked into 1.5ml microtubes. Tissue was ground in liquid nitrogen and DNA was extracted using a maize mini-prep method as described by (Dellaporta et al. 1983). A set of 700 wheat WMC, GWM, and BARC primers were used for genotypic analysis (Roder et al. 1998; Somers et al. 2004) in 10µl PCR reactions containing; 10mM Tris-HCl, pH8.3, 50mM KCl, 1.5mM MgCl2, 0.2mM dNTP, 25 ng of each primer, 24-48 ng genomic DNA and 0.5U *Taq* polymerase. Conditions for PCR were as follows: 94^{o}C for 5min, 40 cycles of 94^{o}C for 1 min, 51/61^{o}C for 1min, and 72^{o}C for 1 min, followed by 10min at 72^{o}C. SSR markers were screened for polymorphisms between the parents and the resulting polymorphic markers were used to screen the RILs. Allele bands were visualized on 3% SFR agarose gels (Amresco) or 6% wide polyacrylamide gels (Wang et al. 2003) depending on required band resolution and were visually scored.

Linkage mapping and QTL analysis

A genetic linkage map was generated from marker data using Mapmaker/Exp v3. For linkage map construction, two or three markers with known chromosomal locations based on a wheat consensus map were anchored and used as an initial framework for all 21 wheat chromosomes (Somers et al. 2004). Markers were added to this framework using the assign and try commands, with confirmation of final marker order using ripple.

The Kosambi mapping function was used for conversion of recombination into genetic distance. QTL controlling HSI and other phenotypic traits were identified using QTL Cartographer version 2.5 (WINQTL) (Wang et al. 2007). Initially, single marker analysis was used to identify genetic markers significantly associated with phenotypic traits. Composite interval mapping (CIM) was then used to determine likely QTL positions and a 1000 permutation test at a significance level of P=0.05 was used to determine the LOD threshold for each trait. For composite interval mapping, up to 10 cofactors were identified using forward and reverse regression at P=0.10. A 10cm window was used for CIM. For discussion purposes, putative QTL below the 1000 permutation threshold that co-localized with other significant QTL are included in the tables and discussion and are noted. QTL were designated based on the nomenclature in the catalog for gene symbols for wheat (http://wheat.pw.usda.gov/ggpages/wgc/98/), consisting of a "Q" followed by the trait name, institution designation, and chromosome assignment. QTL for HSI of multiple traits that co-localized within the same LOD interval were assigned a common QTL name.

Results

Phenotypic analysis of heat tolerance

The heat tolerant cultivar, Halberd, showed minimal and non-significant reduction under heat stress for all main-spike yield components in both years (Table 2.2). All yield

components for Cutter were significantly reduced across both years with the exception of kernel number in 2006. In 2005, percent reduction of yield components ranged from 0.0% to 3.0% for Halberd and from 10.9% to 28.3% for Cutter, with the largest reduction observed for kernel-weight. In 2006, percent reduction of yield components ranged from 5.1% to 7.4% in Halberd and 0.5% to 10.4% in Cutter. For the RIL population, a significant treatment effect was observed for kernel weight and single kernel weight in both years, but not for kernel number. Percent reduction of yield components ranged from 1.3% to 7.9% in 2005 and from 3.1% to 9.9% in 2006. Mean kernel number did show a reduction in both years and this reduction was often large for individual RILs (data not shown). Broad sense heritability estimates (H^2) for yield components were moderate for both the control and heat stress treatments, ranging from 0.44 to 0.75 (Table 2.3). All yield components had higher H^2 under control conditions compared to heat stress, with differences due to greater genotype x environmental variance present under heat tress.

Table 2.2 Data for yield components in wheat parents (Mean ± SE) and Halberd x Cutter RILs (mean ± SD) for 2005 and 2006

		Halberd		Cutter		Recombinant Inbred Lines		
Trait	Year	Control	Treated	Control	Treated	Control	Treated	HSI
Kernel number of main-spike	2005	49.22 ± 1.39	47.91 ± 1.36^{ns}	58.64 ± 1.34	52.27 ± 2.68*	36.33 ± 8.89	35.84 ± 8.38^{ns}	0.74
	2006	43.64 ± 1.34	44.83 ± 1.61^{ns}	56.40 ± 1.45	56.14 ± 2.50^{ns}	33.29 ± 8.24	32.26 ± 8.49^{ns}	1.29
Kernel weight of main-spike (g)	2005	1.68 ± 0.06	1.63 ± 0.12^{ns}	1.84 ± 0.09	1.32 ± 0.13**	1.32 ± 0.34	1.26 ± 0.34*	0.86
	2006	1.78 ± 0.08	1.69 ± 0.08^{ns}	1.35 ± 0.04	1.21 ± 0.03*	0.81 ± 0.26	0.73 ± 0.26**	1.10
Single kernel weight of main-spike (g)	2005	0.034 ± 0.00	0.034 ± 0.00^{ns}	0.031 ± 0.00	0.026 ± 0.00*	0.038 ± 0.01	0.035 ± 0.01**	0.82
	2006	0.041 ± 0.00	0.038 ± 0.00^{ns}	0.024 ± 0.00	0.022 ± 0.00*	0.025 ± 0.01	0.023 ± 0.01**	0.94
Days to flowering (days)	2005	75.44 ± 0.18	75.45 ± 0.25^{ns}	83.45 ± 0.51	82.73 ± 0.79^{ns}	71.12 ± 4.9	70.98 ± 3.8	
	2006	70.86 ± 0.14	71.22 ± 0.22^{ns}	61.00 ± 0.52	61.07 ± 0.59^{ns}	62.89 ± 5.5	62.69 ± 5.4	
Grain-filling duration (days)	2006	30.54 ± 0.39	28.28 ± 0.30*	31.78 ± 0.36	29.00 ± 0.70**	31.04 ± 2.93	29.47 ± 3.30**	1.05
Flag leaf length (cm)	2006		21.77 ± 0.87		19.94 ± 0.45		24.44 ± 4.35	
Flag leaf width (cm)	2006		1.41 ± 0.02		1.08 ± 0.02^{++}		1.28 ± 0.17	
Wax score (0-5)	2006		2.55 ± 0.18		0.89 ± 0.17^{++}		1.38 ± 1.10	

** Significant at P = .01, * Significant at P = .05 between control and heat-treated plants of parental and recombinant inbred lines
%%Significant at P = .01 between parental lines Halberd and Cutter by F-test
[1] Average HSI calculated across all RILs

Table 2.3 Variance components and broad sense heritability estimates for yield components of Halberd x Cutter RILs (n=64) under control and heat stress treatments in the greenhouse, 2005 and 2006

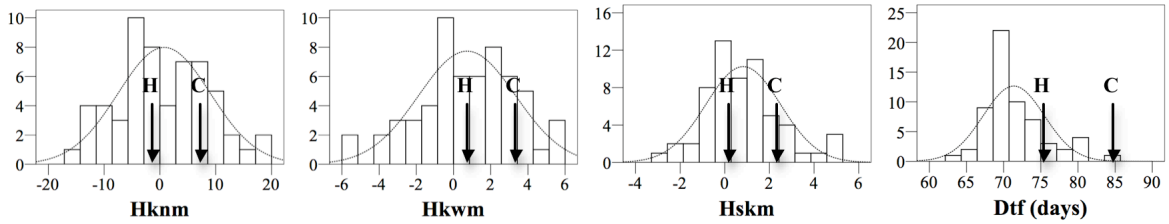
Trait	σ_g^2	σ_e^2	σ_{g*e}^2	H^2 Control	σ_g^2	σ_e^2	σ_{g*e}^2	H^2 Heat
Days to flowering	8.8***	35.7***	4.9***	0.75	8.5***	28.5***	6.7***	0.69
Kernel number	16.2***	6.0**	12.0***	0.67	17.3***	6.1**	16.7***	0.63
Kernel weight	0.017***	0.141***	0.011***	0.68	0.013*	0.129***	0.023***	0.49
Single kernel weight	9.8E-06***	8.7E-05***	9.6E-06***	0.62	5.4E-06*	7.5E-05***	1.1E-05***	0.44

H^2, broad sense heritability estimated from the variance components for the 64 RIL of the Halberd x Cutter mapping population
$H^2 = \sigma_g^2 / \sigma_g^2 + \sigma^2_{ge/e} + \sigma^2_{e/re}$

Replication variance was not significant and is not shown

The significant treatment effect and genetic variation within the population for reduction of yield components allowed for the calculation of a HSI for each yield component and RIL. The average HSI for the population was positive, indicating susceptibility. The RILs showed a normal distribution for HSI of most yield components with a large amount of transgressive segregation (Fig. 2.1). Mean HSI was consistent for most traits across years with some differences in the range of values. In addition to HSI, phenotypic data for days to flowering, grain-filling duration, flag-leaf length and width, and visual wax was also collected. Halberd, Cutter, and the RILs showed wide phenotypic variation for these characters in the 2005 experiment, which made them targets for analysis in the 2006 experiment. In addition, they have the potential for influencing plant productivity

(a) Traits measured in 2005



(b) Traits measured in 2006

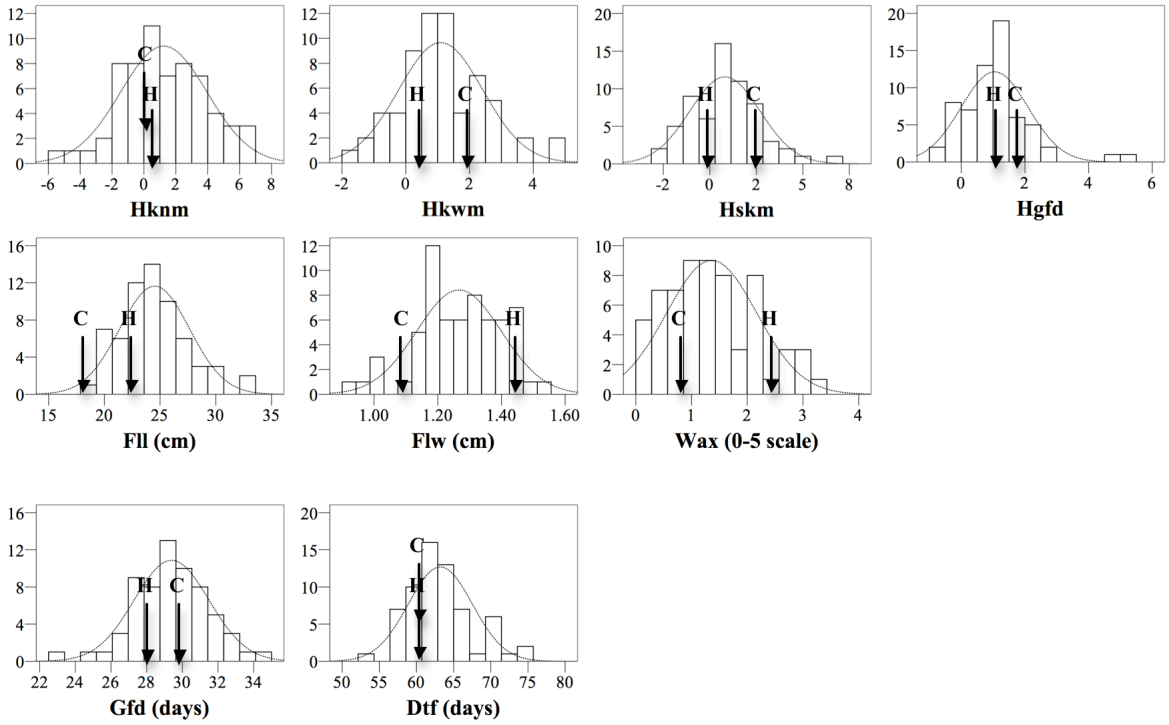


Fig. 2.1 Frequency distribution of heat susceptibility index (HSI) for main spike yield components for Halberd x Cutter recombinant inbred lines in 2005 (a) and 2006 (b). Data is presented for HSI of kernel number (Hknm), kernel weight (Hknw), single kernel weight (Hskm), and grain-filling duration (Hgfd) in addition to phenotypic data for days to flowering (Dtf), grain-filling duration (Gfd), flag leaf length (Fll), flag leaf width (Flw), and visual wax score (Wax). The locations of parental lines are also presented. A positive HSI corresponds to a higher level of heat stress susceptibility.

Correlations

HSI for the three main-spike yield components were moderately to highly correlated and as expected in terms of sign (Table 2.4). HSI of kernel number was positively correlated with HSI of kernel weight in both years (r=0.53 and r=0.45) and negatively correlated with HSI of single kernel weight in 2005 (r=-0.37). HSI of kernel weight was also positively correlated with HSI of single kernel weight (r=0.48) in both years. Flag leaf width showed significant correlation with yield components (not HSI) kernel number (r=0.57), kernel weight (r=0.58), and single kernel weight (r=0.33) under heat stress and with the visual wax score (r=0.48), but not with HSI of these yield components (data no shown in Table 2.4). These results suggest that the phenotypic characters for flag leaf may play a role in plant productivity as has been reported in the literature, but their influence on heat tolerance may be minimal given that they did not correlate with HSI.

Table 2.4 Correlations between heat susceptibility index and phenotypic characters for Halberd x Cutter RILs

Trait	Hknm	Hkwm	Hskm	Hgfd	Gfd	Fll	Flw	Wax
2005								
Days to flowering	0.145	0.35**	0.16					
HSI_kernel number	-	0.53**	-0.37**					
HSI_kernel weight		-	0.48**					
HSI_single kernel weight			-					
2006								
Days to flowering	0.12	-0.03	-0.23	-0.18	-0.27*	0.34**	0.10	0.06
HSI_kernel number	-	0.45**	-0.17	-0.03	0.03	0.16	-0.21	-0.11
HSI_kernel weight		-	0.48**	0.34**	-0.24	0.04	-0.00	-0.07
HSI_single kernel weight			-	0.46**	-0.23	-0.14	0.11	0.03
HSI_grain filling				-	-0.62**	0.07	-0.02	-0.18
Grain-filling duration					-	-0.09	0.13	0.20
Flag leaf length						-	0.38**	-0.06
Flag leaf width							-	0.48**

*Significant at P=.05

**Significant at P=.01

Gfd, Fll, Flw, and Wax were not measured in 2005

Genetic linkage map and QTL mapping

A total of 170 SSR markers were used to construct a genetic linkage map for all 21 wheat chromosomes, with a total map distance of 2256.6 cm and an average distance of 13.3 cm between markers. In general, marker order was conserved with published SSR consensus maps (Roder et al. 1998; Somers et al. 2004). Markers were well distributed across linkage groups, with the exception of 3D, 4D, 6A, and 6B, which had fewer than four makers. Large gaps (>40cm) were observed on 2A, 2B, 3B, 3D, 4A, 5D, 6A, and 7D. Large gaps are often present in wheat genetic maps due to the large chromosome arms, which can result in low marker density on average across the genome. Only

chromosomes that showed significant QTL are presented. Additional data regarding the full Halberd x Cutter genetic map is presented in the Appendix of this dissertation.

Phenotypic data collected from the RILs was converted into a HSI and used as phenotypic data for QTL mapping with the goal of detecting QTL associated with yield stability under heat stress. Data for days to flowering, grain-filling duration, flag-leaf length and width, and visual wax were also collected and used for mapping. These traits were included in order to identify regions of pleiotropy and to elucidate which, if any yield component HSIs they influence. Single marker analysis identified 24 single marker QTL in 2005 and 96 in 2006 (P=0.05), with the greater number mostly a result of more phenotypic traits evaluated in 2006. Composite interval mapping (CIM) detected a total of 17 and 23 QTL in 2005 and 2006, respectively. Both unique QTL present in only one of the two years as well as stable QTL present in both years were detected (Fig. 2.2).

Heat susceptibility index QTL

CIM detected 15 and 12 QTL for HSI in 2005 and 2006, respectively. In 2005, five QTL for HSI were detected for each of the three yield components and were distributed on nine of the 21 wheat chromosomes including 1A, 1B, 2A, 2B, 3B, 4A, 5A, 5B, and 6D (Table 2.5). Both parents contributed favorable alleles to the population, with eight from Halberd and seven from Cutter. Individual QTL explained from 11.3% to 27.4%

of the additive phenotypic variance for HSI of kernel number, from 10.6% to 38.6% of the variance for HSI of kernel weight, and 9.8% to 23.5% of the variance for HSI of single kernel weight (Table 2.5).

In 2006, QTL were detected on eight of the 21 wheat chromosomes including 1A, 1D, 2A, 2B, 3B, 5A, 6D, and 7A (Table 2.6). Five QTL for HSI of kernel number, one for HSI of kernel weight, and three for HSI of single kernel weight were detected. Three QTL each for grain-filling duration under heat stress and HSI of grain filling duration were also detected. Each parent contributed six favorable alleles for HSI in 2006. Individual QTL explained 10.1% to 32.1% of the phenotypic variance for HSI of kernel number and from 16.5% to 31.6% of the variance for HSI of single kernel weight. The only QTL identified for HSI of kernel weight explained 21.2% of the phenotypic variation.

Fig. 2.2 Linkage map and quantitative trait loci (QTL) for heat susceptibility index (HSI) of main spike yield components and phenotypic traits in Halberd x Cutter recombinant inbred lines. QTL detected in both 2005 (dark bars) and 2006 (hatched bars) are presented as 2LOD intervals. Markers positions were calculated using the Kosambi mapping function and are listed in cm position from the top of each linkage group. QTL are annotated based on trait, linkage group and relative position on each chromosome. Details for QTL are presented in Tables 2.5-2.7. Abbreviations: Hkwm (HSI of kernel weight), Knm (HSI of kernel number), Hskm (HSI of single kernel weight), Dtf (days to flowering), grain-filling duration (Gfd), Fll (flag-leaf length), Flw (flag-leaf width), Wax (visual wax score).

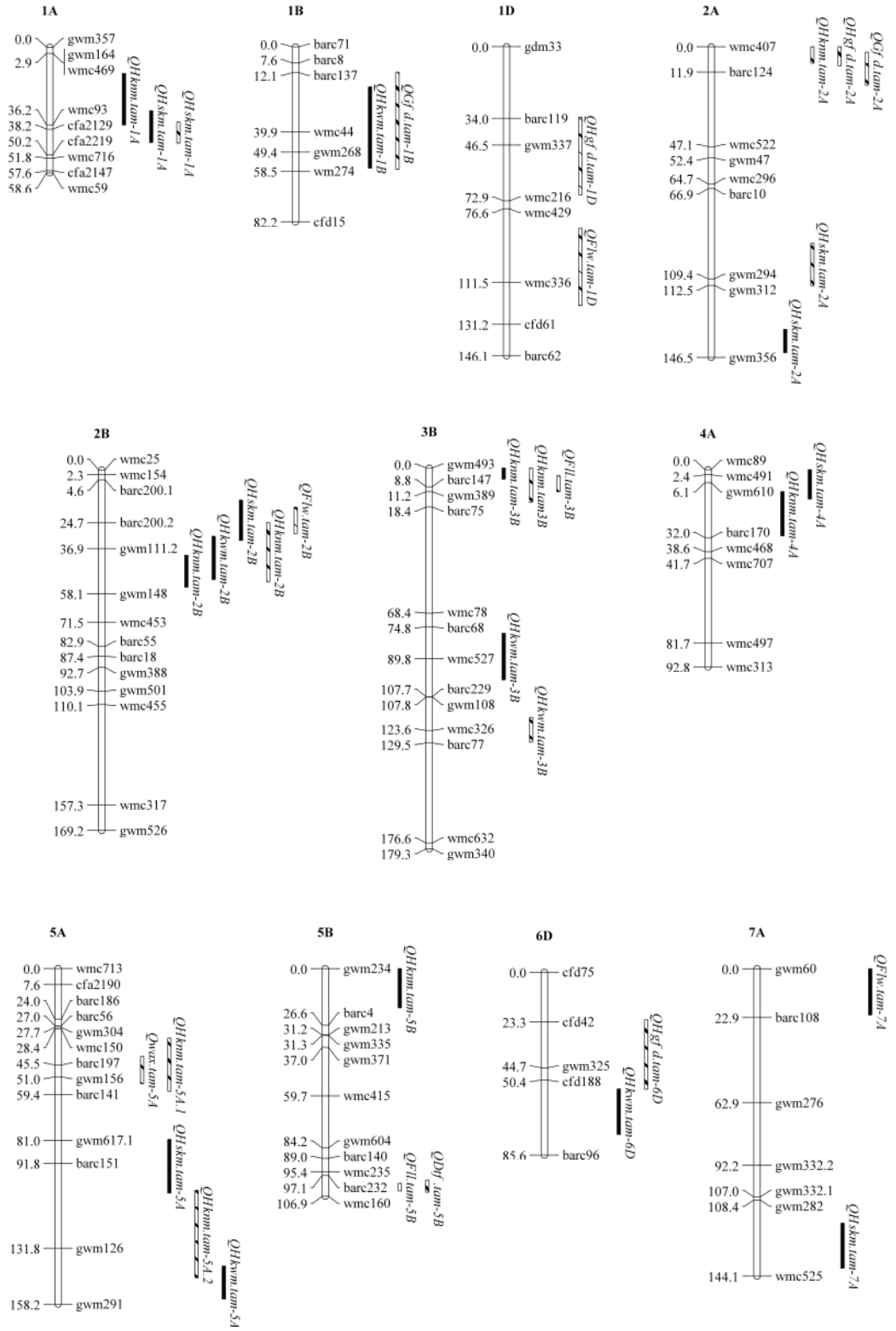


Fig. 2.2 Continued

Table 2.5 QTL detected for heat susceptibility index of main spike yield components and days to flowering in 2005

QTL (LOD threshold[a])	Marker	LOD	R^2	Additive[b]	Positive allele
HSI kernel number (2.91)					
QHknm.tam-1A	cfa2129	3.4	0.274	-4.73	Halberd
QHknm.tam-2B	barc200.2	3.4	0.216	-4.66	Halberd
*QHknm.tam-3B**	barc147	2.7	0.113	3.11	Cutter
QHknm.tam-4A	wmc89	4.6	0.155	3.74	Cutter
QHknm.tam-5B	gwm213	5.7	0.246	4.80	Cutter
HSI kernel weight (2.84)					
QHkwm.tam-1B	gwm268	2.9	0.106	-0.95	Halberd
QHkwm.tam-2B	gwm111.2	4.8	0.248	-1.72	Halberd
QHkwm.tam-3B	wmc527	4.8	0.190	1.36	Cutter
QHkwm.tam-5A	gwm291	3.5	0.219	1.26	Cutter
QHkwm.tam-6D	gwm325	6.0	0.386	-1.74	Halberd
HSI single kernel weight (2.91)					
QHskm.tam-1A	cfa2129	7.0	0.226	1.18	Cutter
*QHskm.tam-2A**	gwm356	2.4	0.210	-0.77	Halberd
QHskm.tam-2B	barc200.2	6.4	0.235	-1.08	Halberd
QHskm.tam-4A	barc170	4.6	0.135	-0.66	Halberd
QHskm.tam-5A	barc151	3.0	0.098	0.61	Cutter
Days to flowering					
QDtf.tam-2D	wmc601	6.6	0.216	-2.00	Cutter
QDtf.tam-7D	wmc438	3.3	0.193	-1.78	Cutter

[a]LOD thresholds were estimated in QTL Cartographer v2.0 using 1000 permutation

[b]Additive effect of allele substitution

*Putative QTL

Table 2.6 QTL regulating heat susceptibility index of main spike yield components and other phenotypic traits in 2006

QTL (LOD threshold[a])	Marker	LOD	R^2	Additive[b]	Positive allele
HSI kernel number (2.50)					
QHknm.tam-2A	wmc407	3.2	0.109	1.04	Cutter
QHknm.tam-2B	gwm111.2	3.6	0.127	-1.39	Halberd
*QHknm.tam3B**	barc147	2.6	0.101	0.93	Cutter
QHknm.tam-5A.1	barc197	3.5	0.138	-1.11	Halberd
QHknm.tam-5A.2	gwm126	3.8	0.321	-1.61	Halberd
HSI kernel weight (2.86)					
QHkwm.tam-3B	wmc326	5.4	0.212	0.75	Cutter
HSI single kernel weight (2.89)					
QHskm.tam-1A	cfa2129	4.3	0.165	0.96	Cutter
QHskm.tam-2A	gwm294	3.7	0.178	-0.97	Halberd
QHskm.tam-7A	gwm282	4.3	0.316	-1.22	Halberd
HSI grain filling duration (2.59)					
QHgfd.tam-1D	gwm337	2.9	0.090	-0.41	Halberd
QHgfd.tam-2A	wmc407	4.9	0.150	0.44	Cutter
QHgfd.tam-6D	gwm325	2.9	0.131	0.41	Cutter
Grain-filling duration (2.83)					
*QGfd.tam-1B**	barc137	2.8	0.123	0.77	Halberd
QGfd.tam-2A	wmc407	8.7	0.296	-1.40	Cutter
QGfd.tam-2D	cfd43	4.3	0.146	-0.97	Cutter
Flag leaf length (2.85)					
QFll.tam-3B	barc147	7.9	0.207	1.69	Halberd
QFll.tam-5B	wmc160	5.3	0.130	1.69	Halberd
Flag leaf width (2.50)					
QFlw.tam-1D	wmc336	3.0	0.078	0.04	Halberd
QFlw.tam-2B	barc200.2	8.3	0.259	0.09	Halberd
QFlw.tam-7A	gwm60	4.8	0.190	-0.06	Cutter
Visual wax score (3.30)					
Qwax.tam-5A	wmc150	3.9	0.164	0.40	Halberd
Days to flowering(3.00)					
QDtf.tam-4B	gwm251	3.9	0.122	-1.74	Cutter
QDtf.tam-5B	wmc160	6.1	0.220	2.28	Halberd

[a]LOD thresholds were estimated in QTL Cartographer v2.0 using 1000 permutation

[b]Additive effect of allele substitution

*Putative QTL below 1000 permutation threshold

Five QTL for HSI were detected in both years (Table 2.7). These stable QTL were located on chromosomes 1A, 2A, 2B, and 3B. Favorable alleles at two QTL,

QHskm.tam-2A and QHknm.tam-2B, were contributed by Halberd. *QHskm.tam-2A* explained 21.0% of the phenotypic variance for HSI of single kernel weight in 2005 and 17.0% of the variance in 2006, with an additive effect lowering the HSI by 0.77 and 0.97 for the two years. *QHknm.tam-2B* explained 21.6% and 12.7% of the phenotypic variance for HSI of single kernel weight across the two years, lowering HSI by 4.66 and 1.39. *QHknm.tam-2B* co-localized with a flag leaf width QTL, *QFlw.tam-2B,* which explained 25.9% of the phenotypic variance and increased width by 0.09 cm. This region also co-localized with QTL for HSI of single kernel weight, *QHskm.tam-2B,* and HSI of kernel weight, *QHkwm.tam-2B*.

Cutter contributed favorable alleles at the other three stable QTL, *QHskm.tam-1A, QHknm.tam-3B and QHwm.tam-3B* (Table 2.7). *QHskm.tam-1A* explained 22.6% and 16.5% of the phenotypic variance for HSI of single kernel weight in 2005 and 2006, respectively. *QHknm.tam-3B* and *QHwm.tam-3B* were located on opposite chromosome arms of chromosome 3B. *QHknm.tam*-3B was explained 11.3% and 11.1% of the phenotypic variance for HSI of kernel number in the two years. Co-localization with *QFlw.tam-3B,* a QTL for flag leaf width was present in this region, closely associated with marker *barc147*. The Cutter allele for *QFlw.tam-3B* was associated with a shorter flag leaf with an additive genetic value of -1.69 cm. This is in contrast to the *QHknm.tam-2B* locus where a wider flag leaf was associated with lower HSI for kernel number. The second QTL on 3BL, *QHkwm.tam-3B* explained 19.0% and 21.2% of the phenotypic variance for HSI of kernel weight in 2005 and 2006, respectively.

Table 2.7 QTL regulating heat susceptibility index detected in both 2005 and 2006

QTL (LOD threshold[a])	Year	Marker	LOD	R^2	Additive[b]	Positive allele
HSI_kernel number of main spike						
QHknm.tam-2B	2005	gwm111.2	3.4	0.216	-4.66	Halberd
QHknm.tam-2B	2006	gwm111.2	3.6	0.127	-1.39	Halberd
QHknm.tam-3B	2005	gwm389	2.7	0.113	3.11	Cutter
*QHknm.tam3B**	2006	barc147	2.6	0.101	0.93	Cutter
HSI_kernel weight of main spike						
QHkwm.tam-3B	2005	wmc527	4.8	0.190	1.36	Cutter
QHkwm.tam-3B	2006	wmc326	5.4	0.212	0.75	Cutter
HSI_single kernel weight of main spike						
QHskm.tam-1A	2005	cfa2129	7.0	0.226	1.18	Cutter
QHskm.tam-1A	2006	cfa2129	4.3	0.165	0.96	Cutter
*QHskm.tam-2A**	2005	gwm356	2.4	0.210	-0.77	Halberd
QHskm.tam-2A	2006	gwm294	3.7	0.178	-0.97	Halberd

[a]LOD thresholds were estimated in QTL Cartographer v2.0 using 1000 permutation

[b]Additive effect of allele substitution

*Putative QTL

Discussion

Large phenotypic variation for reduction in yield components as a result of a three day-heat stress treatment allowed for the detection of QTL associated with HSI. Reductions up to 28.3% for individual yield components were observed in the susceptible cultivar, Cutter, with mean HSI for all yield components positive (susceptible) and normally distributed within the RIL population (Table 2.2 and Fig. 2.1). The heat tolerant cultivar, Halberd, showed minimal reduction of yield components in both years. Halberd is an Australian cultivar that has previously been shown to have tolerance to heat stress (Hays et al. 2007a), boron toxicity (Jefferies et al. 2000), sowing depth (Rebetzke et al. 2005) as well as a cooler maximum spike temperature under field

conditions (Panozzo et al. 1999). The results presented here are comparable to previous studies of short-term heat shock in wheat in terms of the susceptibility of yield components to high temperatures (Hays et al. 2007b; Stone and Nicolas 1994; Yang et al. 2002a).

Unlike other cereal crops such as sorghum, millet, and to some extent corn, most wheat genotypes have an indeterminate growth habit making it difficult to eliminate developmental variation caused by non-synchronized flowering of a large number of tillers. Previous studies in wheat have used the main-spike as a target for studying both yield susceptibility and grain-filling duration under heat stress (Hays et al. 2007a; Yang et al. 2002b, c). The experimental design of targeting the stress at 10 DAP appears to have been successful in eliminating most pleiotropic effects associated with differences in treatment and flowering time on estimating HSI, although days to flowering and HSI of kernel weight were moderately correlated in 2005 (r=0.35). However, no QTL for HSI of main spike characters were associated with flowering time QTL or significant single markers. QTL for HSI of whole plant yield components identified in conjunction with this study showed higher co-localization with days to flowering (data not shown) giving additional validity for using only main-spike characters for detecting heat tolerance QTL.

Heritabilities could not be estimated for HSI directly since it is calculated on a mean basis with no replications. Estimates of broad sense heritability were moderate for most

yield components and higher under control conditions (Table 2.3). Lower H^2 under heat stress conditions indicates that genotype x environment (GxE) interaction was higher for heat stress versus control conditions, although GxE was significant for most traits in both treatments.

Composite interval mapping detected a total of 15 and 12 QTL associated with HSI in 2005 and 2006, respectively (Tables 2.5 and 2.6). Most QTL had moderate to high genetic effects, explaining from 10% up to 30% of the phenotypic variance. Due to the small population size used in this study, estimates of variance and QTL effects are most likely inaccurate, as small population sizes can inflate these number due to a lack of degrees of freedom (Asins 2002). However, the results presented here are novel and represent one of the first studies where QTL were identified in response to a targeted heat stress treatment. Previous QTL studies have used a susceptibility index for measuring flooding tolerance in soybean (Githiri et al. 2006) and drought tolerance in both hexaploid wheat (Kirigwi et al. 2007) and durum wheat (Peleg et al. 2009) but the approach of mapping each yield component as a separate HSI is unique and allows for the identification of QTL affecting single traits that would collectively contribute to overall yield stability and heat tolerance. The parental contributions to heat tolerance were nearly even, as both parents contributed multiple favorable alleles each year and favorable alleles for stable QTL. This is a surprising result given the significant decrease in yield components observed in Cutter but not in Halberd, but not completely unexpected given the quantitative nature of heat tolerance. Five QTL from 2005 were

subsequently detected in 2006, which corresponds to 37.0% of the total number of QTL detected for HSI. Lack of QTL detection across years can be attributed to a combination of the small population size used in this study and moderate heritability for the individual yield components influenced by significant GxE variation present between the two years.

Few studies have reported QTL for heat stress tolerance in wheat. Yang et al. 2002c reported two markers, *gwm11* on 1B and *gwm293* on 5A, to be associated with grain-filling duration under long-term heat stress during reproductive development. The 1B marker *gwm11* is in the general region of *QHkwm.tam-1B,* a QTL for HSI of kernel weight, and *QGfd.tam-1B*, a putative QTL for grain-filling duration. *QHkwm.tam-1B* was detected in 2005 but was not detected in 2006. A field study also confirmed *gwm11* and this region on 1B to be associated with QTL x environmental interactions for yield under high temperatures (days with maximum temperature > 30°C) (Kuchel et al. 2007b). Despite the lack of detection of this QTL across both years in this study, the confirmation of the importance of this region for temperature tolerance in the independent studies is meaningful and warrants further examination of this region.

Five QTL for HSI mapped to chromosome 5A, with favorable alleles from both parents, although lack of both marker density on 5AL and lack of QTL detection across years makes it difficult to determine their significance. In addition to the *gwm293* locus on 5A identified by Yang et al. 2002c, the distal region on 5AL is known to contain *VrnA1,* a

vernalization gene regulating ear emergence in response to temperature (Galiba et al. 1995; Law et al. 1976), which could have a pleiotropic effect on heat tolerance by influencing timing of flowering and plant phenology. A cluster of QTL did map to the distal region of 5AL, in the vicinity of *VrnA1*. Surprisingly this region had lower marker polymorphism and as a result, low marker density for resolution of QTL. This region showed no QTL for flowering time or significant single markers associated with days to flowering, in contrast to what would be expected if vernalization was indeed affecting plant development and subsequently influencing heat tolerance.

Chromosome 5AL has also been associated with the effect of high temperature during grain-filling on grain protein content (Groos et al. 2003). In this study, Gross et al. 2003 used factorial regression and environmental covariables to identify genetic regions associated with differential genotypic responses to temperature during grain-filling and interaction with grain-protein content, yield, and thousand-kernel weight. Genomic regions on 1AS, 2BS 5AL, 5BL, and 6DS identified by Groos et al. 2003 are consistent with QTL on these same chromosomes identified for HSI in this study. This is true for both chromosome and chromosome arm, however the lack of common markers used between the two studies does not allow for resolution to the marker level. This includes *QHskm.tam-1A* and *QHknm.tam-2B*, both stable QTL detected in both years.

Pleiotropy and trade-offs between favorable alleles

Multiple QTL regions were determined to be pleiotropic. Regions in which QTL for more than one yield component HSI co-localized (1A, 2A, 2B, and 4A) as well as regions where HSI co-localized with other phenotypic characters (1B, 2A, 2B, 3B, and 5A) were identified.

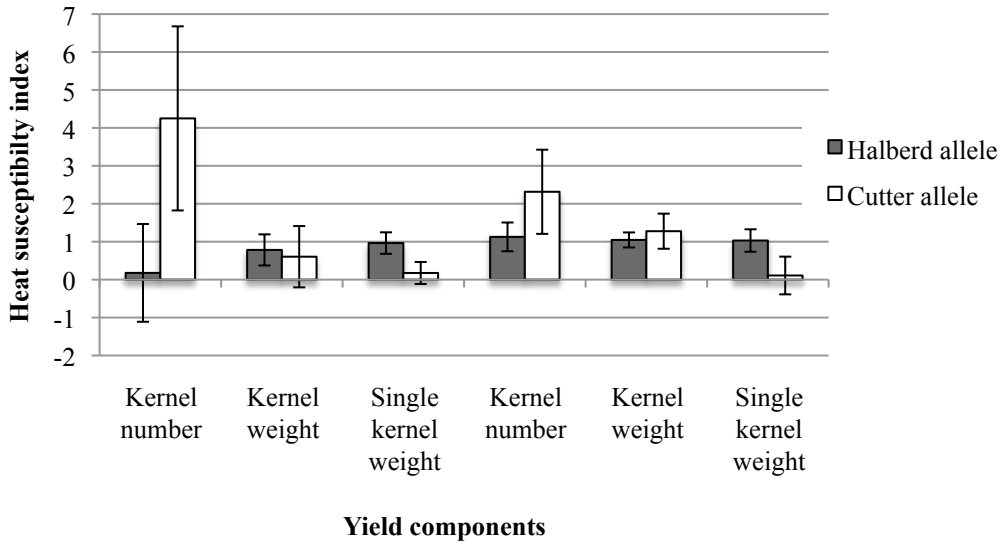


Fig. 2.3 Mean allele contrast analysis of *QHskm.tam-1A* showing the negative pleiotropy detected between heat susceptibility index (HSI) of kernel number and HSI of single kernel weight.

The allele for Halberd was favorable for all QTL on chromosome 2BL, including QTL for HSI of kernel number (both years), kernel weight, single kernel weight and flag leaf

width. This is in contrast to the region on 1AS, where the Cutter allele at *QHskm.tam-1A* was favorable for HSI of single kernel weight (both years), but was unfavorable for HSI of kernel number in 2005 at the *QHknm.tam-1A* locus. There is a negative pleiotropic association between these two traits that results in little net gain in heat tolerance (Fig. 2.3). The potential use of this locus for marker-assisted selection would be negated given this negative trade-off. The possibility of combining QTL that have a pleiotropic trade-off such as *QHskm.tam-1A* with QTL for a larger flag-leaf or higher level of stem carbohydrate reserves could circumvent the trade-off between kernel number and single kernel weight under heat stress.

Conclusions

Several genome regions were determined to be associated with the quantitative regulation of heat tolerance in wheat, as measured by HSI. These include regions previously associated with QTL for grain-filling duration, yield, and QTL x environmental effects related to high temperatures during grain-filling. While some QTL were associated with improvement in HSI of all yield components simultaneously, others, such as *QHskm.tam-1A*, have a pleiotropic trade-off between maintaining kernel number versus maintaining kernel weight. Analysis of these QTL regions in larger mapping populations and genetic backgrounds is under way for validation of their genetic effect, fine mapping of their location, and for the development of molecular markers for selection of heat tolerant germplasm.

CHAPTER III

THE RELATIONSHIP BETWEEN REPRODUCTIVE STAGE HEAT TOLERANCE AND ORGAN TEMPERATURE DEPRESSION IN THE HALBERD X KARL 92 RECOMBINANT INBRED LINE MAPPING POPULATION

Introduction

Wheat is one of the most widely grown cereal crops, with over 620 million tons produced worldwide in 2004 (Dubcovsky and Dvorak 2007; FAO 2006). This accounts for nearly one-fifth of global caloric intake. Despite its high level of adaptive plasticity, extreme heat stress can still compromise wheat yield and end-use quality on an economic level. Through the adoption of green-revolution cultivars, use of synthetic fertilizers, and improved farming practices wheat production has increased at the rate of nearly 1% per annum over the last 50 years (Trethowan et al. 2002). Even larger advances have been made in marginal environments, where yields have increase by as much as 2 to 3% per annum (Lantican et al. 2003; Trethowan et al. 2002). Based on wheat yields from 1950, approximately 1.2 billion hectares of arable land were spared between 1950 and 1999, simultaneously lessening the impact of wheat production on the environment (Borlaug 2004).

Studies have shown high temperature to be the main environmental factor affecting yield across multi-environmental trials (Kuchel et al. 2007b) and improvement of high temperature tolerance has been identified as the most important factor leading to increased yield from 1874-1994 in the Great Plains of the United States (Assad and Paulsen 2002). In the Great Plains region, high temperature stress generally occurs during early reproductive development and grain filling, when wheat is most susceptible (Hays et al. 2007b). Despite improvement in stress tolerance as reported by Assad and Paulsen 2002, many of the current hard red winter wheat varieties grown in the Great Plains region still show susceptibility in terms of their inability to maintain yield and quality under high temperatures (Hays et al. 2007b; Yang et al. 2002b). Because moderate heat stress occurs on a yearly basis, and extreme heat stress on a periodic basis the total yield potential for cultivars in this region may never be realized due to a lack of emphasis on heat tolerance by breeders and the quantitative nature of heat tolerance.

Markers associated with grain-filling duration under reproductive stage heat stress have previously been reported on chromosomes 1B and 5A (Yang et al. 2002c). Other studies have identified regions of the wheat genome associated with QTL x environmental responses to high temperature during grain-filling (Groos et al. 2003; Kuchel et al. 2007a) but did not identify QTL directly associated with heat tolerance. With the exception of the study by Yang et al. 2002, reports associating quantitative trait loci (QTL) directly to improved heat tolerance are lacking.

The benefits of a cooler crop canopy, influenced by a combination of leaf, spike, and peduncle temperature depression have been correlated with yield in the field (Ayeneh et al. 2002; Gutierrez-Rodriguez et al. 2000; Reynolds et al. 2000) and under controlled conditions (Hede et al. 1999) and associations with differences in plant morphology have been documented (Balota et al. 2008). Canopy temperature depression shows promise for use as a physiological marker for genetic gains in yield under conditions of stress given its robustness, its correlation with yield and its ease of measurement (Reynolds et al. 2007b; Richards 2000). However, there are currently no reports of genetic loci associated with temperature depression that would give insight into its genetic regulation, its pleiotropy with loci for phenotypic characters such as leaf morphology, and its influence on heat tolerance.

Given the deficiencies in the current literature relating to detection of genetic loci associated with improved heat tolerance, the approach of the present study was two fold. The first objective was to characterize the phenotypic and physiological response of three wheat cultivars to a two or three day 38°C heat stress treatment during early reproductive development. The second goal was to identify QTL associated with both improved heat tolerance as measured by a heat susceptibility index, confirm QTL previously identified in the Halberd x Cutter population and to associate these loci with those for cooler spike and flag leaf temperature.

Materials and methods

Plant material

Three wheat cultivars, 'Cutter', 'Halberd', and 'Karl 92' and a population of 121 recombinant inbred lines (RILs) derived from a cross between 'Halberd' and 'Karl 92' were characterized for their response to reproductive stage heat stress. For RIL development, a cross between the two parents was carried out in 2003 in the greenhouse in College Station, TX. Lines were advance by single seed descent in the greenhouse to the F_5 generation and seed from individual F_5 plants was bulked to create 121 $_{F2:6}$ lines. F_6 lines were advanced in the field and evaluated in the F_7 generation in the greenhouse.

Plant culture

Cultivars and recombinant inbred lines (RILs) were germinated in petri dishes and vernalized for six weeks at 4°C. Seedlings were transplanted on January 21 at two plants per pot (1:3 peat: sand mixture in 12 x 15 cm pots) and replicated eight times, resulting in eight single plant replications each for both control and heat stress treatments. Plants were initially supplied with 5 grams of Osmocote™ and were supplemented with Peters™ at the recommended rate once every two weeks. The pots were arranged in a completely randomized design. Initially, plants were grown under optimal management in air-conditioned greenhouses at ~20^{o}C/18^{o}C day/night cycles

with a 14 h photoperiod from 6am to 8pm, under natural sunlight with 600 μmol m^{-2} s^{-1} PAR supplemental light. Inflorescences of the main or first pollinated spike from each plant were scored for day of pollination (DAP) upon emergence of the anther from the pollinated spikelet.

Cultivar treatment

At 10 DAP (morning of the 11th day) eight replications of each cultivar were transferred into two identical growth chambers. Half received either a two or three-day heat stress treatment at 38°C/18°C day/night (with maximum 38°C heat stress from 8am to 5pm), while the second half were maintained at control conditions of 20°C/18°C day/night cycles in the second identical growth chamber, resulting in four heat stressed and four control replications of each cultivar. A light intensity of 600 μmol m^{-2} s^{-1} PAR was maintained in both the control and heat stress chambers. Experiments on cultivars were replicated once with a second set of replicated cultivars and data was combined for analysis.

Photosynthesis, conductance, and transpiration measurements

A LI-COR 6400 (LI-COR Biosciences Inc., Lincoln, NE) equipped with an *Arabidopsis* leaf chamber was used to measure rate of photosynthesis, conductance, and transpiration of wheat cultivars during heat stress. Photosynthesis measurements were taken in the

growth chamber under 600 μmol m^{-2} s^{-1} PAR randomly on replications from 1pm to 3pm (5hr to 7hr after initial heat stress) on day-one and day-two of heat stress as well as during recovery on the Day 3, under both control and heat stressed conditions. Organ temperature measurements of both the main spike and flag leaf were taken at 9am, 12pm, and 3pm on each day of heat stress with a handheld thermometer (Model AG-42, Teletemperature Corp, Fullerton, CA), corresponding to 1hr, 4hr, and 7hr after initial heat stress, respectively, as described above. Plants were watered one hour before each measurement to eliminate differences in water deficit. Photosynthesis measurements were only carried out on wheat cultivars and not the RIL populations.

RIL treatment

A greenhouse set at 38°C/18°C day/night temperature was used for heat treatment of RILs. At 10 DAP (morning of 11th day), half of the plants were transferred to the adjacent heat stress greenhouse and received a three-day heat stress treatment at 38^{o}C/18^{o}C day/night (with maximum 38^{o}C heat stress from 8am to 5pm), while the second half were maintained at control conditions of ~20^{o}C/18^{o}C day/night cycles in the control greenhouse. Identical supplemental light of 600 μmol m^{-2} s^{-1} PAR was maintained in both the control and heat stress greenhouses. Following the three-day heat stress treatment, plants were returned to the control greenhouse and maintain under optimal conditions, as described above, until maturity.

Grain filling duration (Gfd) was estimated as the date of pollination until 90% senescence of the main inflorescence, days to flowering (Dtf) as the time from planting to pollination of the main spike, and days to maturity as the time from planting to senescence.

Table 3.1 Phenotypic traits evaluated in greenhouse for Halberd x Karl 92 (n=121) mapping population

Trait	QTL symbol	Method of Measurement
Kernel number of main spike	Knm	Number of kernels of main spike at maturity
Kernel weight of main spike (g)	Kwm	Yield of main spike at maturity
Single kernel weight of main spike (g)	Skm	Kernel weight of main spike / kernel number of main spike
HSI	HSI or H for QTL	Heat susceptibility index calculated for each yield component[1]
Days to flowering (days)	Dtf	Days from planting to flowering of main spike
Days to maturity (days)	Dtm	Days from planting to 90% senescence of the main spike
Grain-filling duration (days)	Gfd	Days from flowering to 90% senescence of main spike
Flag leaf length (cm)	Fll	Length of flag leaf from base of leaf to tip
Flag leaf width (cm)	Flw	Width of the widest section of the flag leaf
Temperature depression (°C)	Td	Air temperature minus organ temperature, measured using a handheld infrared thermometer

[1]$HSI = (1-X_h/X)/(1-Y_h/Y)$, where X_h and X are the phenotypic means for each genotype under control and heat stressed conditions, respectively, and Y_h and Y are the phenotypic means for all lines under control and heat stressed conditions, respectively.

Temperature depression (Td) for both the flag leaf and the main spike were taken on the first and second days of heat stress with a handheld thermometer (Model AG-42,

Teletemperature Corp, Fullerton, CA). Measurements were taken at a 45° angle from the horizon or leaf surface. The Td was calculated as air temperature minus organ temperature. Measurements on Day 1 and Day 2 were taken on RILs in the heated greenhouse.

At maturity, plants were harvested and hand threshed. Kernel number and kernel weight of the main spike were measured and used to calculate single kernel weight. Only the main spike was used for yield analysis to eliminate error in the treatment associated with non-uniform tillering and flowering secondary tillers that would cause unequal application of heat stress. Heat tolerance was calculated as a heat susceptibility index (HSI) for each individual RIL using the equation by Fisher and Maurer (Fischer and Maurer 1978): HSI = $(1-Y_h/Y)/(1-X_h/X)$, where Y_h and Y are the phenotypic means for each genotype under control and heat stressed conditions, respectively, and X_h and X are the phenotypic means for all lines under control and heat stressed conditions, respectively. The HSI for individual yield components and individual RILs was used as phenotypic data for quantitative trail loci (QTL) mapping as opposed to using the yield components directly. The objective of this study was to map QTL regulating yield stability as measured by a HSI not yield per se as would be measured by the individual yield components. In addition, the length and width of the flag leaf were recorded at 10 DAP. Width was measured on the widest part of the flag leaf and length was measured from the base of the flag leaf to the tip. These traits were included, as they have previously co-localized with heat tolerance QTL.

Statistical analysis

Statistical analysis was carried out using the MIXED procedure (SAS v8.2, SAS Institute Inc., Cary, NC, USA). Significant differences between means of treatments were detected by considering treatment as having a fixed effect and genotypes and replications as random. Pearson correlations were done using the statistical software package SPSS for Mac (SPSS inc. Chicago, IL). An allele contrast analysis for the phenotypic means of markers most closely associated with select QTL was done using GLM-ANOVA in SPSS for Mac, treating each marker as having a fixed effect and individual traits as dependent variables.

Molecular marker analysis

Fifteen F_7 seed of each parent and RIL were germinated on filter paper and coleoptiles were bulked into 1.5ml microtubes. Tissue was ground in liquid nitrogen and DNA was extracted using a maize mini-prep method as described by (Dellaporta et al. 1983). A set of 623 wheat WMC, GWM, and BARC primers were used for genotypic analysis (Roder et al. 1998; Somers et al. 2004) in 10µl PCR reactions containing; 10mM Tris-HCl, pH8.3, 50mM KCl, 1.5mM MgCl2, 0.2mM dNTP, 25 ng of each primer, 24-48 ng genomic DNA and 0.5U *Taq* polymerase. Conditions for PCR were as follows: 94^{o}C for 5min, 40 cycles of 94^{o}C for 1 min, 51/61^{o}C for 1min, and 72^{o}C for 1 min, followed by 10min at 72^{o}C. SSR markers were screened for polymorphisms between the parents

and the resulting polymorphic markers were used to screen the RILs. Allele bands were visualized on 4% SFR agarose gels (Amresco) or 6% wide polyacrylamide gels (Wang et al. 2003) depending on required band resolution and were visually scored.

Linkage mapping and QTL analysis

A genetic linkage map was generated from marker data using JoinMap (Kyazma, B.V., Netherlands). Initially, a LOD value of 3.0 was used to establish initial mapping nodes for each linkage group. For linkage map construction, two or three markers with known chromosomal locations based on a wheat consensus map were used as an initial framework for all 21 wheat chromosomes (Somers et al. 2004). Markers were then added to this framework using an initial LOD threshold of 3.0 and a maximum recombination frequency of 0.40. The Kosambi mapping function was used for conversion of recombination into genetic distance. QTL controlling HSI and other phenotypic traits were identified using QTL Cartographer version 2.5 (WINQTL) (Wang et al. 2007). Initially, single marker analysis was used to identify genetic markers significantly associated with phenotypic traits. Composite interval mapping (CIM) was then used to determine likely QTL positions and a 1000 permutation test at a significance level of P=0.05 was used to determine the LOD threshold for each trait. For CIM, forward regression (P=0.05) and backward regression (P=0.05) was used to identify background cofactors with 10cm window. For discussion purposes, putative QTL below the 1000 permutation threshold that co-localized with other significant QTL

may be included in the tables and discussion. QTL were designated based on the nomenclature in the catalog for gene symbols for wheat (http://wheat.pw.usda.gov/ggpages/wgc/98/), consisting of a "Q" followed by the trait name, institution designation, and chromosome assignment.

Results

Flag leaf and main spike temperature depression of wheat cultivars

Temperature of the flag leaf and main spike of three wheat cultivars, Cutter, Halberd, and Karl 92 was measured at 1hr, 4hr, and 7hr after initial heat stress on each day (d1, d2, d3) of a three-day, 38°C heat stress treatment (Fig. 3.1). All Cultivars responded to 38°C heat stress by lowering both main spike and flag leaf temperatures below that of the treatment temperature. Significant differences between cultivars were observed at most time-points. Flag leaf temperatures of both Cutter and Karl 92 increased throughout the day on both d1 and d2, with maximum temperature observed at 7hr after treatment (Fig. 3.la). On each day of heat stress, Halberd showed an increase in flag leaf temperature from 1hr to 4hr, but decreased from 4hr to 7hr, consistently having a cooler flag leaf at 7hr compared to both Cutter and Karl 92.

Karl 92 had the highest spike temperature across all time-points on d1 and d2 and Halberd the lowest, with the exception of 1hr and 4hr on d2, where Halberd was

statistically equal to that of Cutter (Fig. 3.1b). Cutter had the highest main spike temperature across all time-points on d3. The largest difference in main spike temperature was observed at 7hr, with Halberd maintaining a spike temperature 2-3°C cooler than both Cutter and Karl92 on both d1 and d2 of heat stress.

Photosynthetic rate, transpiration, and conductance

Photosynthetic rate, transpiration, and conductance were measured in conjunction with the two-day heat stress experiment. Measurements were taken on d1 and d2 of heat stress and during recovery on d3 with a LI-COR 6400 (LI-COR Inc. Lincoln, NE). Compared to average control, all lines showed nearly complete inhibition of photosynthesis under 38°C heat stress. No significant differences were observed during d1 of heat stress while both Cutter and Halberd had photosynthetic rates that were significantly higher than Karl 92 on d2. (Fig. 3.2a).

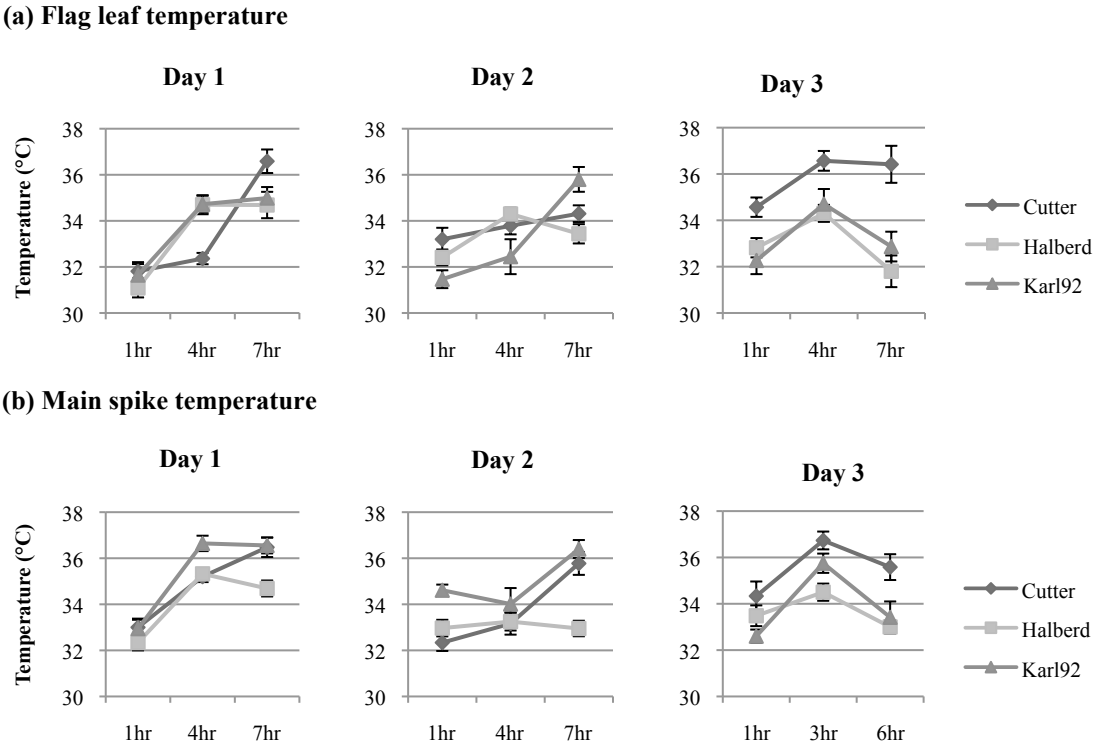


Fig. 3.1 Flag leaf (a) and main spike temperatures (b) of wheat cultivars taken at 1hr, 4hr, and 7hr on each day of a 38°C heat stress treatment at 10 days after anthesis. Measurements were taken with a handheld infrared thermometer. Results are presented as mean ± SE.

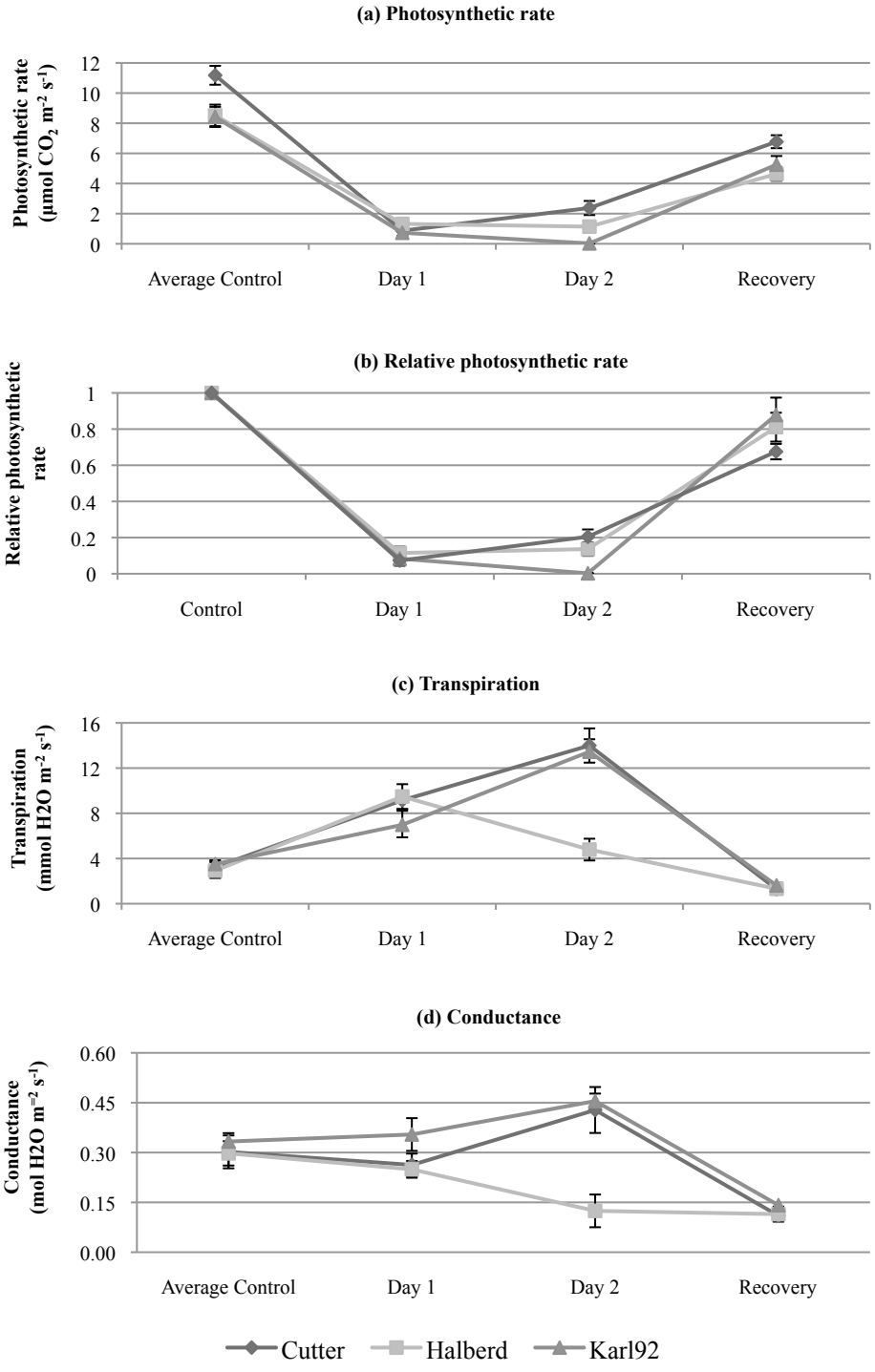


Fig. 3.2 Photosynthetic rate (a), relative photosynthetic rate (b), transpiration (c), and conductance (d) of wheat cultivars under 38°C heat stress at 10 days after pollination. Measurements were taken from 1pm to 3pm (5hr to 7hr) on Day 1 and Day 2 of heat stress as well as during Day 3 recovery.

Cutter had the highest rate of photosynthesis under control, heat stress, and recovery conditions, although relative photosynthesis for Cutter was lower during recovery on d3 (Fig. 3.2b). Similar to photosynthetic rate, there were no significant differences in transpiration (Fig. 3.2c) or conductance (Fig. 3.2d) during d1 of heat stress, with all lines showing large increases in transpiration compared to the control. On d2 of heat stress, both transpiration and conductance in Cutter and Karl 92 increased, while Halberd showed a marked decrease in both of these traits. Recovery of these traits on d3 closely mirrored that of photosynthetic rate, with levels returning to near 50% of the control.

Effect of heat stress on yield components of the main spike

For most traits, both a two-day and three-day heat stress treatment resulted in significant reduction in yield components (Fig. 3.3). Cutter showed a significant reduction of 20% in kernel weight of the main spike following a two-day treatment, with a similar reduction observed when the stress was applied for three-days (Fig. 3.3b). This reduction in kernel weight of the main spike was due to both a 10% reduction in kernel number (Fig. 3.3a) and a 13% reduction in single kernel weight (Fig. 3.3c). Similar to Cutter, Karl 92 had reductions of 38% and 37% for kernel number and kernel weight of the main spike following the three-day treatment, respectively. Karl 92 did not show a decrease in single kernel weight following either of the treatments due to large reduction observed for kernel number and the negative pleiotropic relationship between these two

traits. In contrast to both Cutter and Karl 92, Halberd did not show a significant reduction in any main spike yield component measured.

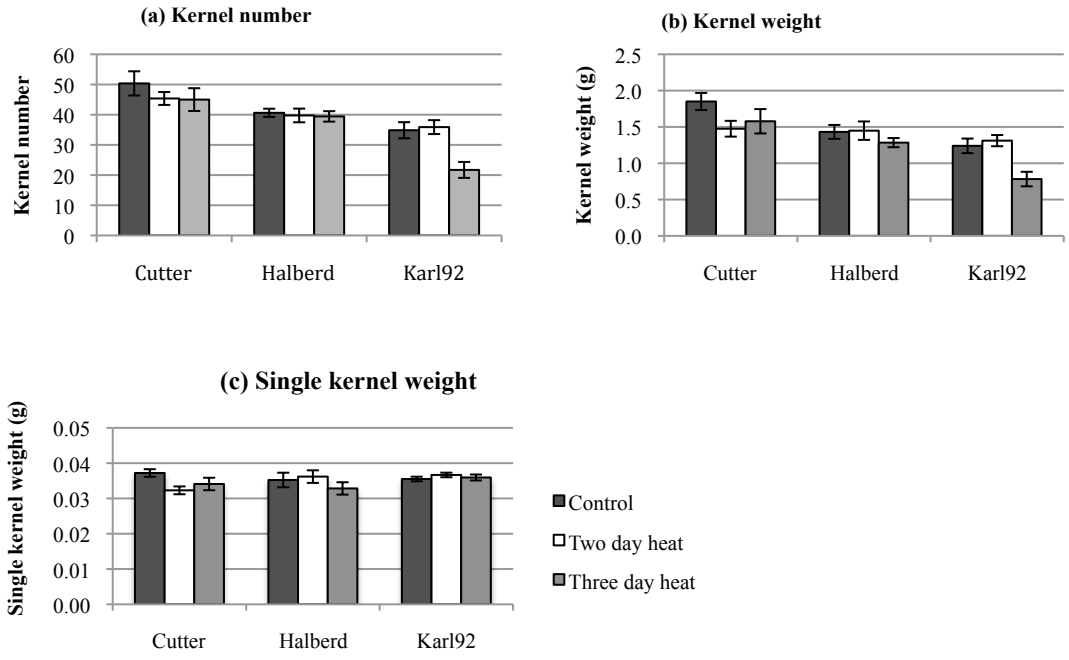


Fig. 3.3 Kernel number (a), kernel weight (b), and single kernel weight (c) of main spike yield components for wheat cultivars Cutter, Halberd, and Karl 92 at maturity following control, two-day heat stress, and three-day heat stress treatments.

Treatment of recombinant inbred lines

Based on results from parental cultivars, a three-day heat stress treatment was imposed on a set of Halberd x Karl 92 recombinant inbred lines using a greenhouse set to a 38°C/20°C day/night cycle. A significant treatment effect was observed for kernel weight and single kernel weight, but not for kernel number (Table 3.2). Individual RILs did show significant reductions in kernel number (data not shown), although the overall treatment means were not significantly different between heat and control groups. No difference was observed for days to flowering or days to maturity. It was expected that no difference would be observed for days to flowering since plants were treated post anthesis. There was a small decrease in days to maturity, due in most part to significant mean reduction of 1.4 days in grain-filling duration between heat-stressed and control treatments.

Significant treatment effect and variation within the Halberd x Karl 92 population for reduction in yield components allowed for the calculation of a heat susceptibility index (HSI). Average HSI for kernel weight and single kernel weight was positive indicating susceptibility (Table 3.2). HSI for most yield components were normally distributed and transgressive segregation observed for most traits (Fig. 3.4). For most yield components, Halberd was the more tolerant parent as calculated by a lower value for the HSI. The exception was HSI of single kernel weight, where very little difference was

observed between the parental cultivars but a large amount of transgressive segregation was present in the RILs.

Flag leaf dimensions and temperature depression

Flag leaf length and width were measured at 10 DAP just prior to heat stress treatment of RILs. The two parents show strong phenotypic differences in the expression of these characters. Mean flag leaf length for Halberd was 5.1 cm longer that Karl 92 and mean flag leaf width for Halberd was 0.12 cm more than Karl 92. Mean data for flag leaf length and width for the RIL population was subsequently used for QTL mapping. Because the parental cultivars show large variation for these traits it is plausible that there could be an association with and possibly co-localization between flag leaf characters and HSI. Co-localization of QTL for HSI and flag leaf dimensions were present in the Halberd x Cutter population, which were useful in dissecting the pleiotropic effects of some heat tolerance QTL (Mason et al. in review).

Table 3.2 Data for yield components in wheat parents (Mean ± SE) and recombinant inbred lines (mean ± SD) in greenhouse 2008 under control and heat treated conditions

	Halberd		Karl 92		Recombinant Inbred Lines		
Trait	Control	Treated	Control	Treated	Control	Treated	HSI[1]
Kernel number of main spike	46.1 ± 1.2	42.4 ± 1.9^{ns}	34.9 ± 2.7	21.7 ± 2.6*	30.8 ± 11.9	31.2 ± 12.6^{ns}	-0.55
Kernel weight of main spike (g)	1.71 ± 0.1	1.73 ± 0.1^{ns}	1.24 ± .10	0.78 ± 0.10*	1.07 ± 0.5	1.02 ± 0.4*	0.38
Single kernel weight of main spike (g)	0.037 ± 0.001	0.041 ± 0.002^{ns}	0.036 ± 0.001	0.036 ± 0.001^{ns}	0.035 ± 0.008	0.033 ± 0.007**	0.90
Grain-filling duration (days)	39.0 ± 1.4	37.7 ± 0.7^{ns}	35.6 ± 0.9	33.6 ± 0.7*	35.9 ± 3.2	34.6 ± 3.4**	1.64
Days to maturity (days)	95.7 ± 0.3	97.0 ± 1.1^{ns}	82.8 ± 1.4	81.0 ± 0.9^{ns}	90.3 ± 9.1	89.7 ± 9.6^{ns}	0.82
Days to flowering (days)	57.0 ± 1.0	59.0 ± 1.5^{ns}	47.1 ± 0.3	47.4 ± 0.3^{ns}	56.4 ± 10.8	56.2 ± 11.3^{ns}	
Temperature depression leaf (°C)		6.8 ± 1.3		6.2 ± 1.3		5.5 ± 1.4	
Temperature depression spike (°C)		4.5 ± 1.0		3.7 ± 1.0		3.8 ± 1.2	
Visual wax score (1-5)		1.33 ± 0.17		2.25 ± 0.31		1.53 ± 1.06	
Flag leaf length (cm)		28.6 ± 1.4		23.5 ± 1.8		23.4 ± 6.9	
Flag leaf width (cm)		1.40 ± 0.02		1.28 ± 0.04		1.24 ± 0.64	

** Significant at P = .01, * Significant at P = .05 between control and heat-treated plants of parental and recombinant inbred lines with treatment as a fixe effect

[1] Average HSI calculated across all RILs

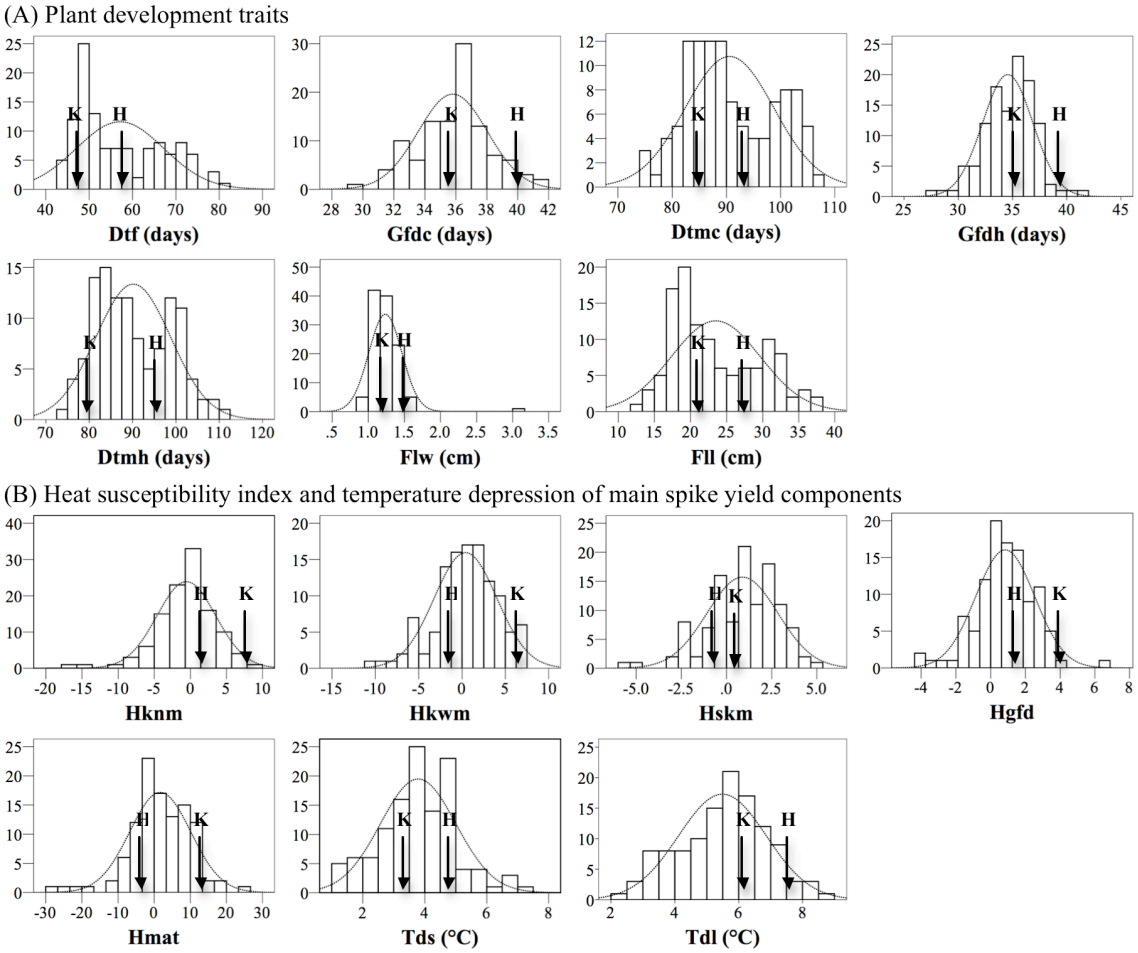


Fig. 3.4. Frequency distribution of phenotypic characters for the Halberd x Karl 92 recombinant inbred lines. **(A)** Plant developmental traits days to flowering (Dtf), grain-filling duration control (Gfdc), days to maturity control (Dtmc), grain-filling duration heat (Gfdh), days to maturity heat (Dtmh), visual wax content (Wax), flag leaf width (Flw), and flag leaf length (Fll); **(B)** Heat susceptibility index of main spike yield components kernel number (Hknm), kernel weight (Hkwm), and single kernel weight (Hskm), grain-filling duration (Hgfd), and maturity (Hmat), and temperature depression of the main spike (Tds) and flag leaf (Tdl). A lower HSI corresponds to higher heat tolerance.

Temperature depression (Td) of the flag leaf and main spike were recorded at 7hr on day 1 and day 2 of heat stress for the RIL population (Table 3.1). These two time-points were selected based on the variation observed between the parental lines. To obtain the measurement for Td, both organ temperature and ambient temperature were measured simultaneously and Td was calculated as the difference between these two measurements. This is in contrast to data presented in Fig. 3.1 in which organ temperatures were presented not temperature depressions. Each organ x day measurement was used as a separate trait for QTL mapping in order to identify QTL specific to the initial cooling response on d1 as well as subsequent response on d2. Halberd had both a cooler mean flag leaf temperature (greater temperature depression) and cooler spike temperature under heat stress than Karl 92 (Table 3.2). Temperature depression of RILs ranged from 2.3°C to 8.55°C for the flag leaf and 1.05°C to 7.10°C for the main spike with a relatively large amount of transgressive segregation (Fig. 3.4). On average, flag leaf temperatures were much cooler than those of the main spike and had a larger range of variability.

Correlations

HSI of main spike yield components were moderately to strongly correlated with each other, with the exception of HSI of kernel number and single kernel weight, which showed no significant correlation (Table 3.3). Organ Td measurements across the different days and organs showed moderate correlation. Correlation between HSI and

Td measurements was only significant during d1 of heat stress with Td negatively (favorably) correlated with HSI of kernel weight and single kernel weight of the flag leaf.

Table 3.3 Correlations among heat susceptibility index, temperature depression, and flag leaf dimensions of the main spike

	Main spike traits								
	Tdl_d1	Tds_d1	Tdl_d2	Tds_d2	Hknm	Hkwm	Hskm	Fll	Flw
Td leaf day 1	-	0.29**	0.37**	0.04	-0.12	-0.25*	-0.28**	0.02	0.05
Td spike day 1		-	0.08	0.42**	-0.02	-0.00	0.00	0.20*	0.09
Td leaf day 2			-	0.16	-0.08	-0.11	-0.14	-0.02	-0.03
Td spike day 2				-	-0.06	-0.08	-0.05	-0.09	-0.08
HSI kernel number					-	0.88**	-0.16	-0.14	0.02
HSI kernel weight						-	0.29**	-0.14	0.05
HSI single kernel weight							-	-0.01	0.06
Flag leaf length								-	0.21*
Flag leaf width									-

Td, temperature depression, HSI, heat susceptibility index

** Correlation is significant at the 0.01 level

* Correlation is significant at the 0.05 level

Genetic linkage map construction

A total of 623 simple-sequence repeat markers were used to screen parental lines, Halberd and Karl 92, for polymorphisms. From these markers, 188 polymorphic primer pairs producing 193 alleles were used for RIL population analysis. Of these, 190 markers, including 189 SSR markers and one phenotypic (*B1/awns*) marker were used to

construct a genetic linkage map for all 21 wheat chromosomes using JoinMap software. Three markers were unlinked and not included in the linkage map. The constructed genetic map spanned a total of 2343.6 cm with an average distance of 12.3 cm maker^{-1}. Markers were unevenly distributed between linkage groups with marker numbers per chromosome ranging from three on chromosome 4D to eighteen on chromosomes 2D and 5B. Notable gaps (>40cm) within linkage groups were present on chromosomes 3D, 4A, 4D, and 6D. In general, marker order was highly conserved with previous wheat genetic maps and consensus SSR maps (Somers et al. 2004).

Single marker QTL analysis

Single marker analysis (SMA) in QTL Cartographer was used to identify single markers significantly associated with HSI, temperature depression, and other phenotypic traits listed in Table 3.1. In total there were 133 significant single marker QTL (P= 0.05) for the 13 traits analyzed (Appendix). The number of markers significant for any one trait ranged from five for HSI of kernel number to twenty for HSI of single kernel weight. SMA detected five, six, and twenty markers significantly associated with HSI of kernel number, kernel weight, and single kernel weight of the main spike at P= 0.05.

Composite interval mapping of main spike QTL

Significant co-factors identified using single-marker analysis were subsequently used as background markers for QTL detection using composite interval mapping (CIM) with QTL Cartographer. A HSI was calculated for five main-spike yield components, including kernel number, kernel weight, single kernel weight, days to maturity, and grain-filling duration, using phenotypic data collected for each of the one-hundred and twenty one RILs. These traits were mapped as a HSI for the purpose of identifying QTL associated with yield stability under heat stress, not yield per se under heat stress. In addition to these HSI traits, days to flowering, days to maturity, grain-filling duration and temperature depression were also mapped to detect QTL regions that were pleiotropically associated with these plant developmental traits and what, if any, influence they have on reproductive stage heat tolerance in wheat.

Fig. 3.5 Linkage map and quantitative trait loci (QTL) for heat susceptibility index (HSI) of main spike yield components and phenotypic traits in Halberd x Karl 92 recombinant inbred lines. QTL detected for both yield components (dark bars) and other agronomic and physiological traits (hatched bars) are presented as 2LOD intervals. Markers positions were calculated using the Kosambi mapping function and are listed in cm position from the top of each linkage group. QTL are annotated based on trait, linkage group and relative position on each chromosome. Details for QTL are presented in Table 3.4. Abbreviations: H (heat susceptibility index), Kwm (kernel weight), Knm (kernel number), Skm (single kernel weight), Dtf (days to flowering), grain-filling duration (Gfd), Fll (flag-leaf length), Flw (flag-leaf width), Tdl (temperature depression of the leaf), Tds(Temperature depression of the main spike).

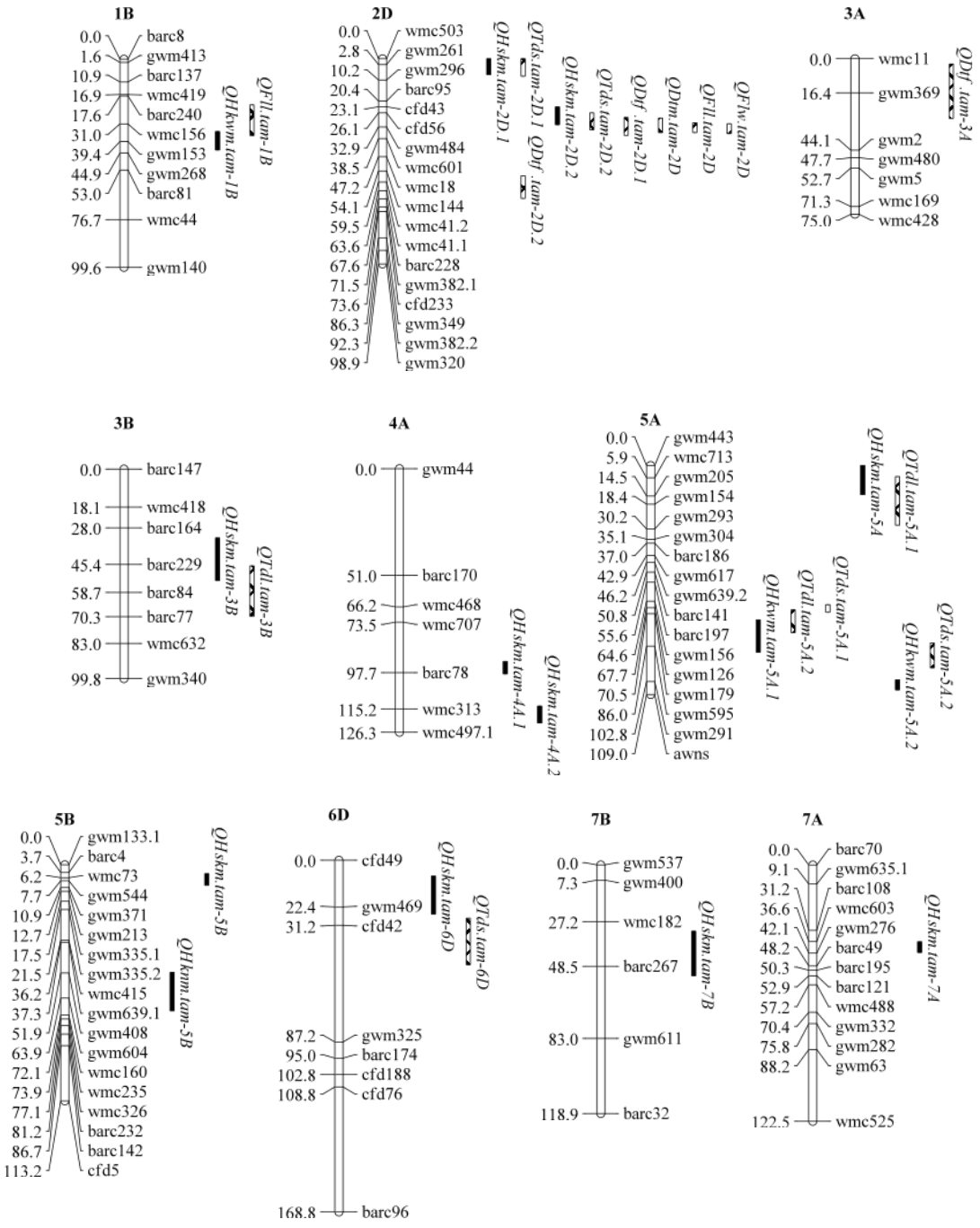


Fig. 3.5 Continued

Table 3.4 QTL detected in the Halberd x Karl 92 mapping population (n=121) for heat susceptibility index and developmental traits in the greenhouse in 2008

QTL (LOD threshold[a])	Marker	LOD	R^2	Additive[b]	Positive allele
HSI_Kernel number of main spike (3.03)					
QHknm.tam-5B	gwm408	3.05	0.134	1.28	Karl 92
HSI_Kernel weight of main spike (3.25)					
QHkwm.tam-1B	gwm153	3.93	0.101	-1.19	Halberd
QHkwm.tam-5A.1	gwm179	3.95	0.122	-1.49	Halberd
QHkwm.tam-5A.2	gwm291	3.81	0.114	1.38	Karl 92
HSI_Single kernel weight main spike (3.15)					
QHskm.tam-2D.1	gwm261	11.77	0.193	0.92	Karl 92
QHskm.tam-2D.2	cfd56	3.61	0.052	-0.49	Halberd
QHskm.tam-3B	barc229	3.17	0.045	-0.41	Halberd
QHskm.tam-4A.1	wmc707	5.50	0.096	-0.79	Halberd
QHskm.tam-4A.2	wmc313	7.55	0.123	0.87	Karl 92
QHskm.tam-5A	gwm443	4.04	0.058	0.47	Karl 92
QHskm.tam-5B	wmc73	4.08	0.062	-0.50	Halberd
QHskm.tam-6D	cfd49	6.01	0.147	-0.75	Halberd
QHskm.tam-7A	wmc603	4.27	0.093	-0.76	Halberd
QHskm.tam-7B	wmc182	3.79	0.055	-0.47	Halberd
Days to flowering (9.05)					
QDtf.tam-2D.1c	gwm484	3.80	0.152	4.02	Halberd
QDtf.tam-2D.2c	wmc41.1	4.15	0.095	-3.34	Karl 92
QDtf.tam-3Ac	gwm369	3.87	0.096	-3.22	Karl 92
Days to maturity (5.1)					
QMat.tam-2D	cfd56	5.92	0.235	4.05	Halberd

[a]LOD thresholds were estimated in QTL Cartographer v2.0 using 1000 permutation

[b]Additive effect of allele substitution

[c]Putative QTL

In total, 14 significant QTL associated with HSI were detected using CIM (Table 3.4). These QTL were located on nine of the 21 wheat chromosomes, including 1B, 2D, 3B, 4A, 5A, 5B, 6D, 7A, and 7B (Fig. 3.5). This included one QTL for HSI of kernel number, three QTL for HSI of kernel weight, and ten QTL for HSI of single kernel weight. No QTL were identified for HSI of grain-filling duration or days to maturity.

The single QTL for HSI of kernel number, *QHknm.tam-5B.1,* was located on chromosome 5B at *gwm408* and explained 13% of the phenotypic variation. The susceptible line, Karl 92, contributed the favorable allele at this locus. QTL identified for HSI of kernel weight were located on chromosomes 1B and 5A. The favorable allele at *QHkwm.tam-1B* was contributed by Halberd and explained 10.1% of the phenotypic variation. This QTL mapped to an 8.8 cm region near *wmc153* on the long arm of chromosome 1BL and confirms a QTL detected for the same trait in the Halberd x Cutter (H/C) population. The two other QTL for HSI of kernel weight were located on chromosome 5A, localizing to the distal region of the long arm. *QHkwm.tam-5A.1* was most closely linked to *gwm126/gwm179* and explained 12% of the phenotypic variation, while *QHkwm.tam-5A.1* was associated with *gwm291* and explained 11% of the phenotypic variation. These two loci are within 20cm of each other and are linked in repulsion, as the favorable allele for *QHkwm.tam-5A.1* is contributed by Halberd and the favorable allele for *QHkwm.tam-5A.2* contributed by Karl 92. These QTL are a confirmation of two QTL identified in H/C, located on 5AL. *QHkwm.tam-5A.1* is closely associated with the *awn*/*B1* locus, which may be pleiotropically associated with and contribute to improved heat tolerance at this locus.

Ten QTL were identified for HSI of single kernel weight including single QTL on chromosomes 3B, 5A, 5B, 6D, 7A, and 7B, as well as two QTL on each of both 2D and 4A (Table 3.4). The large number of QTL detected for HSI of single kernel weight compared to other traits is in agreement with the results of SMA, which detected 20

markers significantly associated with the trait at P=0.05. QTL for HSI of single kernel weight explained from 4.5% to 19.3% of the phenotypic variation with LOD values ranging from 3.17 to 11.77. Seven of the ten beneficial alleles for these loci were contributed by Halberd.

Temperature depression and flag leaf dimension QTL

QTL detected for temperature depression (Td) and flag leaf dimensions are presented in Table 3.5. For the purposes of this paper, only those QTL for Td and flag leaf dimensions that showed co-localization with heat tolerance QTL are presented herein. Details on other QTL detected can be found in the Appendix of this thesis.

QTL for Td co-localized with HSI at seven genome regions, including two loci for Td of the flag leaf and four loci for Td of the main spike and one locus where both flag leaf and spike temperature co-localized with HSI. At all loci, a cooler spike and/or flag-leaf temperature was associated with improved heat tolerance. Co-localization between HSI of single kernel weight and Td occurred at five regions, while Td and HSI of kernel weight co-localized at three region. Both QTL located on the distal end of chromosome 5AS, *QHkwm.tam-5A.1 and QHkwm.tam-5A.2* showed co-localization between TD and HSI of main spike kernel weight.

Flag leaf length co-localized with two heat tolerance QTL, including *QHkwm.tam-1B*, and *QHskm.tam-2D.2*. Flag leaf width co-localized at one locus, *QFlw.tam-2D/QHskm.tam-2D.2*. At all of the genetic loci, the Halberd allele was favorable for a longer/wider flag leaf and improved heat tolerance. It should be noted that only the *QTds.tam-2D.2* locus was associated with a difference in flowering time as the photoperiod locus, *Ppd-D1* is known to be located in this region and was confirmed by the presence of *Qdtf.tam-2D.1* for flowering time detected in this region.

Table 3.5 Summary of QTL detected in the Halberd x Karl 92 mapping population (n=121) for temperature depression, flag leaf length, and flag leaf width co-localizing with heat susceptibility index QTL

QTL (LOD threshold[a])	Co-localization	Marker	LOD	R^2	Additive[b]	Positive allele
Temperature depression of flag leaf (2.93)						
QTdl.tam-3Bc	*QHskm.tam-3B*	barc84	2.75	0.093	0.44	Halberd
QTdl.tam-5A.1	*QHskm.tam-5A*	gwm154	3.17	0.113	-0.60	Karl 92
QTdl.tam-5A.2	*QHkwm.tam-5A.1*	gwm179	3.18	0.093	0.46	Halberd
Temperature depression of main spike (2.89)						
QTds.tam-2D.1	*QHskm.tam-2D.1*	gwm261	5.46	0.110	-0.51	Karl 92
QTds.tam-2D.2	*QHskm.tam-2D.2*	cfd56	4.07	0.122	0.55	Halberd
QTds.tam-5A.1	*QHkwm.tam-5A.1*	gwm126	6.74	0.146	0.68	Halberd
QTds.tam-5A.2	*QHkwm.tam-5A.2*	gwm595	4.61	0.088	-0.56	Karl 92
QTds.tam-6D	*QHskm.tam-6D*	cfd42	9.71	0.321	0.81	Halberd
Flag leaf length (3.35)						
QFll.tam-1B	*QHkwm.tam-1B*	wmc156	3.08	0.038	1.31	Halberd
QFll.tam-2D	*QHskm.tam-2D.2*	gwm484	18.37	0.328	3.85	Halberd
Flag leaf width (3.37)						
QFlw.tam-2D	*QHskm.tam-2D.2*	gwm484	9.84	0.177	0.07	Halberd

[a]LOD thresholds were estimated in QTL Cartographer v2.0 using 1000 permutation

[b]Additive effect of allele substitution

[c]Putative QTL

Confirmation of QTL from the Halberd x Cutter mapping study

The present study confirms a number of QTL from the Halberd x Cutter study (Table 3.6). One hundred and eight SSR markers were common between the two genetic maps (Appendix), allowing for resolution of common QTL to the chromosome, chromosome arm, and general marker region, but not always to the same molecular marker, as not all markers were presented on homologous chromosomes across maps. Seven QTL present on six of the 21 wheat chromosomes were detected for HSI in both populations. This included QTL on chromosomes 1B, 3B, 4A, 5A, 5B, and 6D.

Table 3.6 Summary of QTL detected in homologous regions for both the Halberd x Karl 92 mapping population (n=121) and the Halberd x Cutter (n=64) population for heat susceptibility index

Population	Chromosome	QTL	Marker	LOD	R^2	Additive[b]	Positive allele
H/C	1B	*QHkwm.tam-1B*	gwm268	2.9	0.106	-0.95	Halberd
H/K	1B	*QHkwm.tam-1B*	gwm153	3.93	0.101	-1.19	Halberd
H/C	3B	*QHkwm.tam-3B*	wmc527	4.8	0.190	1.36	Cutter
H/K	3B	*QHskm.tam-3B*	barc229	3.17	0.045	-0.41	Halberd
H/C	4A	*QHskm.tam-4A*	barc170	4.6	0.135	-0.66	Halberd
H/K	4A	*QHskm.tam-4A.1*	wmc707	5.50	0.096	-0.79	Halberd
H/C	5A	QHknm.tam-5A.2	gwm126	3.8	0.321	-1.61	Halberd
H/K	5A	*QHkwm.tam-5A.1*	gwm126	3.95	0.122	-1.49	Halberd
H/C	5A	*QHkwm.tam-5A*	gwm291	3.5	0.219	1.26	Cutter
H/K	5A	*QHkwm.tam-5A.2*	gwm291	3.81	0.114	1.38	Karl 92
H/C	5B	*QHknm.tam-5B*	gwm213	5.7	0.246	4.80	Cutter
H/K	5B	*QHskm.tam-5B*	wmc73	4.08	0.062	-0.50	Halberd
H/C	6D	*QHkwm.tam-6D*	gwm325	6.0	0.386	-1.74	Halberd
H/K	6D	*QHskm.tam-6D*	cfd49	6.01	0.147	-0.75	Halberd

[a]LOD thresholds were estimated in QTL Cartographer v2.0 using 1000 permutation

[b]Additive effect of allele substitution

[c]Putative QTL

Only two of these QTL were detected for the same trait in both studies, located on chromosome 1B and 4A. QTL were often detected in both studies but for different traits, such as on chromosome 5A where *gwm126* was linked to *QHknm.tam-5A.2* for HSI of kernel number in the H/C population and to *QHkwm.tam-5A.1* for HSI of kernel weight in the Halberd x Karl 92 (H/K) population. This is not unexpected given the correlations observed between these traits in both studies. The favorable alleles QTL were mostly consistent, with the exception of the 3B and 5B loci in which the Cutter allele was favorable in H/C and the Halberd allele was favorable in the H/K population.

Discussion

Physiological analysis of wheat cultivars

The phenotypic response of three wheat cultivars, Halberd, Cutter, and Karl 92, to short-term heat shock during early reproductive development was measured. Subsequent analysis of HSI for main spike yield components, leaf and spike temperature depression, and flag leaf dimensions in a RIL population derived from Halberd x Karl 92 identified genetic loci associated with improved heat tolerance and confirmed QTL previously identified in Halberd x Cutter.

All cultivars maintained flag leaf and main spike temperatures cooler than that of the treatment temperature. While mostly small significant differences of $< 1.0°C$ were

observed between cultivars, Halberd consistently had an equally or significantly cooler flag leaf and spike temperature compared to the other two cultivars at the 7hr time-point. The difference was most evident for spike temperature, with Halberd having a 2-3°C cooler main spike compared to Cutter and Karl 92 at the 7hr time-point on d1 and d2 of heat stress. Cooler organ temperatures late in the treatment day suggests an acclimation mechanism in Halberd that might not be expressed in Cutter and Karl 92, as these cultivars tended to have cooler organ temperatures early in the day that subsequently increased. Photosynthetic rate of all cultivars showed nearly complete inhibition during day-one of heat stress with some small yet significant increases and differences observed between the cultivars during day-two heat stress. Results from day-one are in agreement with a previous study in wheat showing complete inhibition of photosynthesis at temperatures approaching 40°C (Law and Crafts-Brandner 1999) while the results from day-two suggest some level of acclimation, particularly in Cutter, to heat stress as observed previously for both photosynthesis (Law and Crafts-Brandner 1999) and yield components (Hays et al. 2007a). Previous studies of photosynthetic rate in wheat have shown genetic differences in net photosynthesis between cultivars under heat stress and correlations with yield (Blum 1986; Reynolds et al. 2000). Despite the significant differences for photosynthetic rate observed between cultivars in this study it is unlikely that stable photosynthesis would be the main source of photoassimilates given the low level of relative photosynthesis. It has been reported that some heat tolerant cultivars contain high levels of stem reserves that contribute to yield stability under heat stress (Yang et al. 2002a), which would be in better agreement with the low level of

photosynthesis yet high yield stability observed for Halberd in this study. This was not a detailed characterization of photosynthesis, only an attempt to identify large differences, which were not present. Future studies would need to use a more moderate treatment level (≤30°C) in order to detect more significant variation between these cultivars.

Cutter and Karl 92 showed large increases in both conductance and transpiration on day-two of heat stress that was in contrast to the decrease observed for Halberd. High stomatal conductance under conditions of both heat and drought stress is well documented and has been correlated with net photosynthesis and yield under hot, irrigated conditions (Reynolds et al. 2000). Despite lower conductance, Halberd maintained an equally cool flag leaf and significantly cooler spike temperature at the time when these measurements were taken (5hr to 7hr). Halberd has been shown to have high levels of epicuticular wax compared to both Cutter (Mason et. al. in review) and Karl 92 (Mondal et. al. in prep) that serves as a reflective mechanism to dissipate excesses light and heat. This may work in combination with stomatal conductance to cool organ temperatures. Halberd is adapted to warm growing regions of Australia, where heat is often accompanied by extreme drought, so it could be hypothesized that Halberd may possess an intrinsic genetic response to keep stomatal conductance low to prevent water loss under high-temperature and accompanying drought stress.

Effect of heat stress on yield components

Large and significant reductions in yield and yield components were observed in the susceptible cultivars, Cutter and Karl 92, following heat stress. This was in contrast to Halberd, which showed minimal non-significant reductions. Targeting a treatment to the main spike is a well established method for assessing heat tolerance in wheat and has previously been used for assessing HSI of both synthetic wheat (Yang et al. 2002b) and bread wheat (Yang et al. 2002a) as well as for identifying genetic loci associated with grain-filling duration under chronic heat stress (Yang et al. 2002c). Previous studies using these cultivars have shown similar levels of heat tolerance in Halberd (Hays et al. 2007a), and susceptibility in Cutter (Mason et al. in review) and Karl 92 (Hays et al. 2007a; Yang et al. 2002a).

The mean treatment effect on yield components of the Halberd x Karl 92 RIL population ranged from 0.0 to 10.4% (Table 3.2). Most traits had normal distribution and ranged from highly heat tolerant (negative) to highly heat susceptible (positive), with transgressive segregation present (Fig. 3.4).

Temperature depression

Spike temperatures were higher than those of the flag leaf, as has been previously reported (Ayeneh et al. 2002) but cooler than ambient temperature at 7hr after initial heat

stress. Temperature depression (Td) of the flag leaf during d1 of heat stress was the only organ and time-point favorably correlated with HSI. This result suggests that initial leaf cooling may be the most important component of heat tolerance, possibly by protection against irreparable damage to important enzymes and the photosynthetic apparatus as has been observed for both starch synthesis enzymes in barley (Savin et al. 1997) and rubisco activase in wheat (Ristic et al. 2009). Under field conditions, spike, flag leaf, and peduncle temperature collectively contribute to crop canopy temperature and a cooler crop canopy has been correlated with both yield and HSI under heat stress (Ayeneh et al. 2002), in agreement with the results presented here.

Main spike HSI QTL

QTL mapping was focused on two types of traits, those that serve as a direct measure of heat tolerance (i.e. HSI) and those associated with other phenotypic and adaptive traits that might influence heat tolerance (i.e. temperature depression, days to flowering, flag leaf traits, etc). In total, 14 QTL were identified for HSI of main spike yield components (Table 3.4). Both parents contributed favorable loci, in agreement with previous studies showing heat tolerance to be quantitatively inherited (Frova and Sarigorla 1994; Yang et al. 2002c) and with the transgressive segregation observed for HSI (Fig. 3.4). However, Halberd contributed the majority of QTL and total variance to HSI, including two of the three favorable alleles for HSI of kernel weight and seven of the ten QTL for HSI of single kernel weight.

In general, QTL for HSI had low to moderate genetic effects. The variance explained by individual QTL ranged from 4.5% to 19.3% as is common for a quantitative trait. This in contrast to the H/C population where most loci detected for HSI had moderate to large genetic effects but in agreement with a previous study estimating heat tolerance to be polygenic with small coefficient of variances (Yang et al. 2002c). Higher QTL effects in H/C would be attributable to the smaller population size (n=64) not to the QTL detection or accuracy of the phenotypic data, as a small population size lends itself to the overestimation of additive effects and phenotypic variances (Asins 2002). Without accounting for epistasis, QTL detected in this study collectively explained from 13%, 34% and 92% of the phenotypic variance for HSI of kernel number, kernel weight, and single kernel weight of the main spike, respectively.

It should be noted that only one of the fourteen main-spike QTL detected was pleiotropically associated with flowering time. *QHskm.tam-2D.2,* co-localized with *QDtf.tam-2D.1*, a QTL for days to flowering that is in close proximity to *Ppd-D1*, one of the major photoperiod loci in wheat. This region was pleiotropic and associated with six traits including flag leaf length, width, spike temperature depression and days to maturity in addition to HSI and days to flowering. Similar pleiotropy has been observed in both wheat (McCartney et al. 2005) and sorghum (Murray et al. 2008) in regions containing loci involved in flowering time. The experimental design here was such that stress was applied at a uniform developmental stage, 10 DAP, to account for differences in flowering time within the population. The lack of co-localization between QTL for

HSI and flowering time, with *QDtf.tam-2D.1* the exception, confirms that the experimental design was sound and that most QTL identified in this study should be independent of flowering time.

Co-localization between HSI and temperature depression QTL

QTL for HSI and temperature depression showed co-localization at seven loci including QTL on chromosomes 2D, 3B, 5A, and 6D (Table 3.5). At all loci, a cooler flag leaf or spike temperature was associated with improved heat tolerance. The benefits of a cooler crop canopy and yield are well documented (Ayeneh et al. 2002; Hede et al. 1999) and some correlation with plant morphology has been observed (Balota et al. 2008). Canopy temperature depression shows promise for use as a physiological marker for genetic gain in yield stress conditions (Reynolds et al. 2007b; Richards 2000). However, this is the first report identifying loci that co-localized for both Td and improved heat tolerance.

Co-localization between Td and HSI of single kernel weight occurred at five genetic loci. The largest effect locus for spike Td, *QTds.tam-6D,* and a QTL for HSI of single kernel weight, *QHskm.tam-6D,* co-localized to the same region on chromosome 6D. This locus mapped to a 22cm region, explaining 32.1% and 14.7% of the phenotypic variation for spike TD and HSI of single kernel weight, respectively.

HSI of kernel weight and Td showed co-localization at two loci, both located on the distal end of chromosome 5AL. *QHkwm.tam-5A.1/QTdl.tam-5A.2/QTds.tam-5A.1* was associated with leaf and spike TD as well as HSI of kernel weight, explaining 9.3%, 14.6%, and 12.2% of the phenotypic variation for these traits, respectively. The Halberd allele was favorable for all traits at this locus. A second QTL on the distal end of 5AS, *QHkwm.tam-5A.2/QTds.tam-5A.2,* was associated with HSI of kernel weight and spike Td with the favorable allele contributed by Karl 92. This QTL was not present with single marker analysis but was revealed using CIM and is tightly linked to the *B1* locus for awns. The presence of awns has previously been significantly associated with yield gains (Cuthbert et al. 2008) but has not with heat tolerance. It should be noted that the vernalization gene, *VrnA1,* does reside on 5AL, although no markers on chromosome 5A were significantly associated with flowering time, and it is unlikely that vernalization requirement would influence Td given the experimental design. It is also possible that heat tolerance loci in this region would be in linkage with the *VrnA1*, with improved heat tolerance associated with the allele for spring-type. However, given the marker density and the lack of a perfect marker for *VrnA1* on the Halberd x Karl 92 genetic map, it is unclear at this point how tightly linked these loci might be or if it would be possible to break this linkage via marker assisted selection. Future research and the addition of perfect markers for *VrnA1* in this region will be necessary to further understand the pleiotropy in this region.

Combining favorable alleles for heat tolerance and temperature depression

While single QTL moderately influence heat tolerance, combining multiple favorable alleles into a single genotype could prove more beneficial. Coleoptile vigor in wheat has been shown to be controlled by many small effect QTL which when combined may increase coleoptile length up to 50 mm (Rebetzke et al. 2007). The genetic control of yield in wheat is polygenic and regulated by many small effect loci. Cuthbert et al. (2008) found that the top ten yielding lines from a RIL population segregating for yield potential had a combination of the same favorable alleles at four of the five yield QTL detected.

The mean allele effects of markers most closely associated with three prominent QTL for HSI, *QHkwm.tam-5A.1, QHskm.tam-*6D*, and QHkwm.tam-1B,* are presented in Fig. 3.6. *QHkwm.tam-5A.1 and QHskw.tam-6D* are associated with both HSI and temperature depression, while *QHkw.tam-1B* was significant for HSI only. Combining the Halberd alleles for *QHkwm.tam-5A.1/QHskm.tam-6D* resulted in a decrease in HSI (improved heat tolerance) below that of the either single allele for both HSI of kernel number and kernel weight, with the mean for HSI of single kernel weight equal to the largest effect QTL. Mean HSI for *QHkwm.tam-5A.1/QHskm.tam-6D* decreased kernel number by -1.19, kernel weight by -1.27, and single kernel weight by -0.20 compared to the average mean of both single Halberd alleles. Similar results were observed for *QHkw.tam-5A.1/QHwm.tam-1B,* which produced the lowest values for HSI of kernel

number and kernel weight, decreasing HSI for these traits by -1.18 and -1.24 compared to the mean of single alleles. This is in agreement with the genetic effects of these QTL as both *QHkw.tam-5A.1* and *QHwm.tam-1B* are associated with HSI of kernel weight. For the *QHwm.tam-1B/QHskm.tam-6D* combination the result was similar for HSI of kernel weight and kernel number, with gains of -0.87 a -0.70 in HSI, compared to the mid-allele value for Halberd, respectively, and a large increase in both of these traits compared to the single *QHskm.tam-6D* locus. Differences were not significant for all allele combinations and traits, due in part to limited statistical power given the limited number of lines having both favorable alleles (i.e. on average ~30 lines would contain both alleles) but the benefit of combining favorable loci is obvious. Interestingly, increased susceptibility was also observed when unfavorable alleles from Karl 92 were combined, particularly for the *QHkw.tam-5A.1/QHwm.tam-1B* and *QHwm.tam-1B/QHskm.tam-6D* combinations (Fig. 3.6).

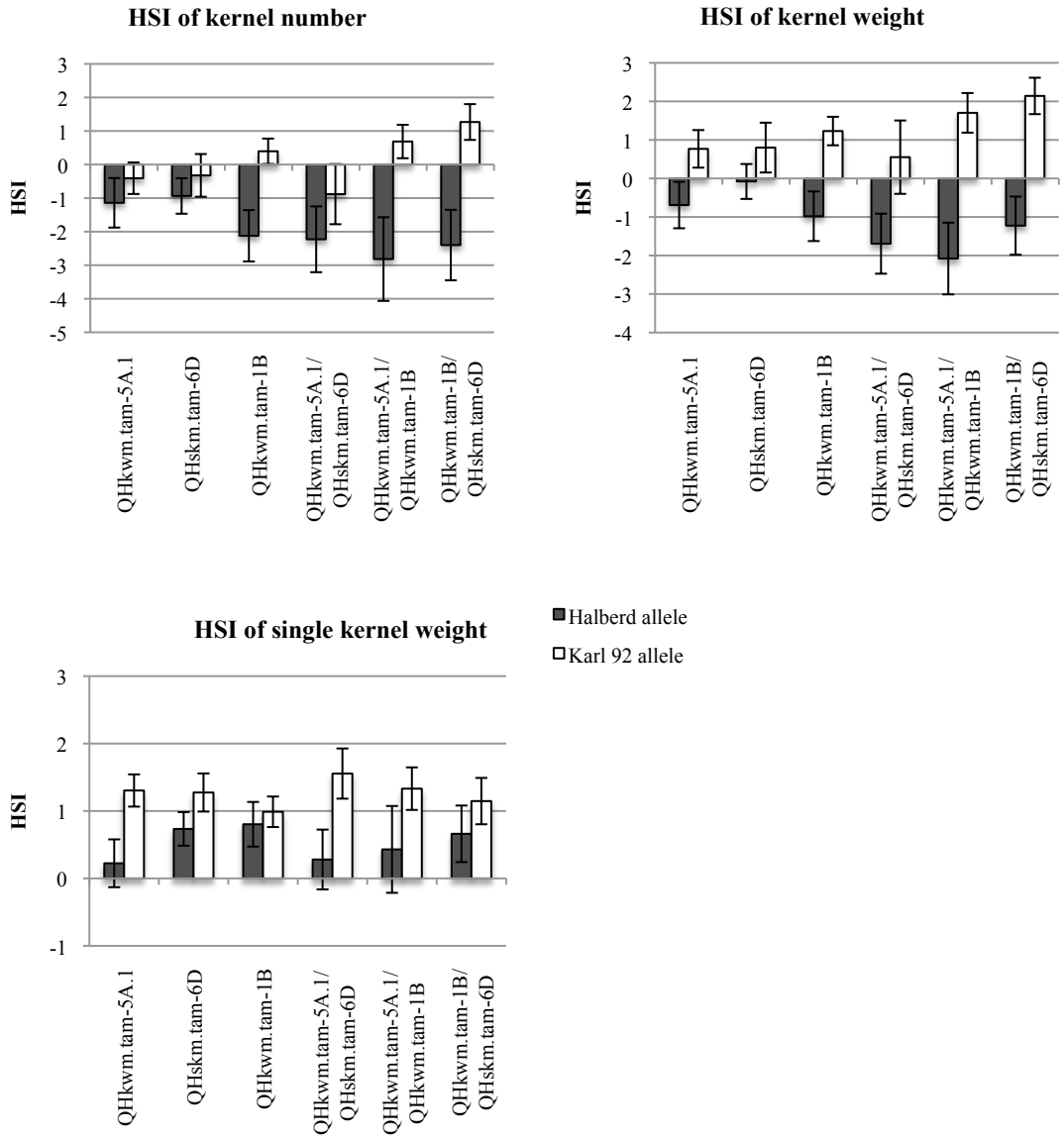


Fig. 3.6. Mean allele values for heat susceptibility index of Halberd x Karl 92 RILs having either the Halberd or Karl 92 allele for markers most closely associated with *QHkw.tam-5A.1, QHskm.tam-6D,* and *QHkwm.tam-1B* as well as combinations of these loci. Markers *gwm126,* cfd42 and *gwm153* were used for the QTL on 5A, 6D, and 1B, respectively. A negative HSI is associated with higher heat tolerance.

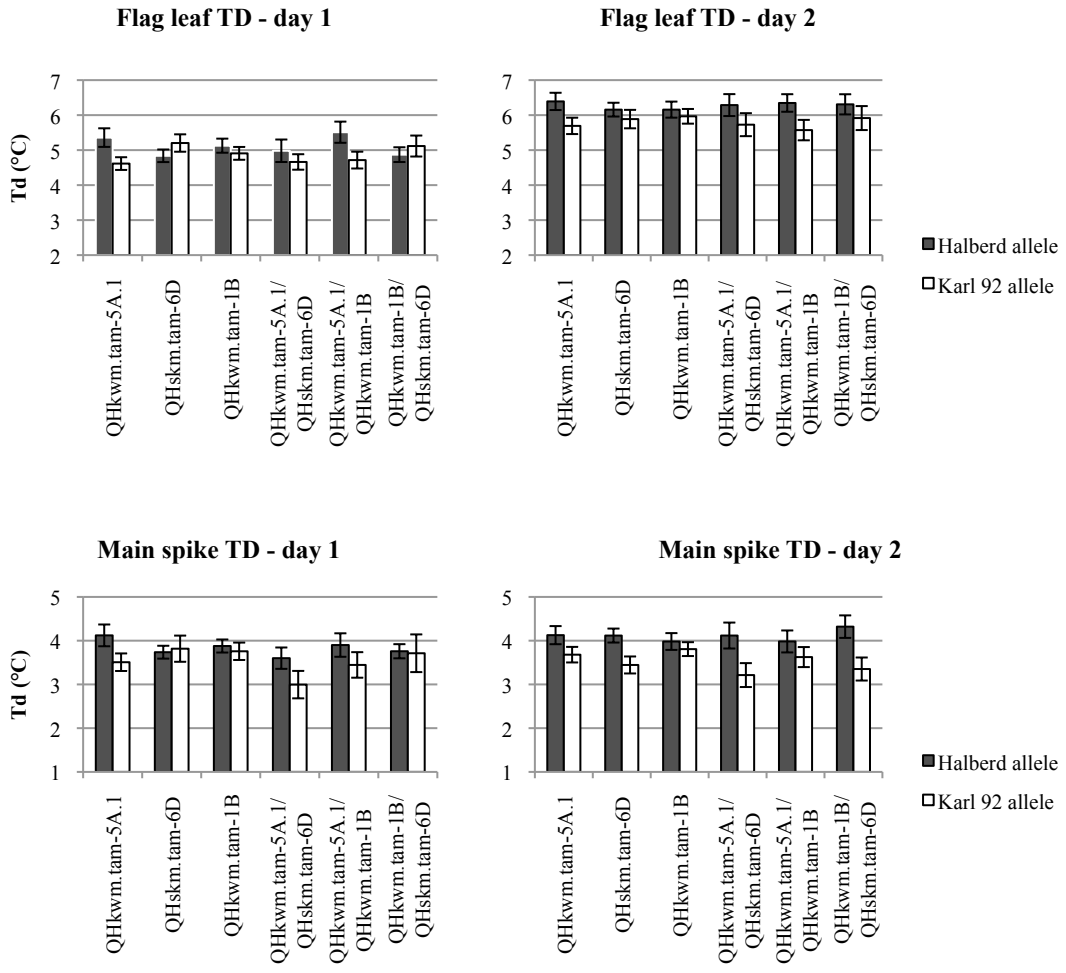


Fig. 3.7 Mean allele values for temperature depression of the main spike and flag leaf of Halberd x Karl 92 RILs having either the Halberd or Karl 92 allele for markers most closely associated with *QHkw.tam-5A.1, QHskm.tam-6D,* and *QHkwm.tam-1B* as well as combinations of these loci. Markers *gwm126,* cfd42 and *gwm153* were used for the QTL on 5A, 6D, and 1B, respectively.

The gain from combining favorable alleles for temperature depression was less pleiotropic but still evident (Fig. 3.7). A significant increase in Td of the flag leaf was observed for the *QHkw.tam-5A.1/QHwm.tam-1B* combination, with Td increasing by

0.27°C compared to the mean of single Halberd alleles. A gain of 0.27°C was also observed for spike Td on day two in the *QHwm.tam-1B/QHskm.tam-6D* combination.

These are both promising results in that they both show the ability to combine a QTL for HSI with a QTL for Td and get a positive increase for both traits. Collectively, this data points toward the potential benefits of marker assisted selection aimed at combining not only single QTL, but sets of QTL which in combination result in a higher level of heat tolerance compared to single alleles.

Confirmation of QTL from the Halberd x Cutter mapping study

QTL identified in this study confirm previous results from the Halberd x Cutter population (Mason et al. in review, Chapter II). Seven QTL regions were found to be common between the two studies, although lack of marker density prevents adequate resolution of some QTL (Table 3.6). Both the H/C and H/K studies identified QTL on the distal end of 5AL that were linked in repulsion. As previously mentioned, this region is known to contain both *Vrn1A* as well as the *B1/awn* locus. The QTL detected on 5AL were not present in both years in H/C, although they had relatively large effects for the years in which they were detected (Table 3.6). The pleiotropic association between HSI and temperature depression as well as the possible interaction or linkage to *Vrn1A* at this locus is deserving of future analysis.

The stable QTL on chromosome 1B was detected for HSI of kernel weight in both studies, with similar additive effects and phenotypic variances explained (Table 3.6 and Fig. 3.8). Although this QTL only explains 10% of the additive variance in these studies, independent confirmation of this region for both grain-filling duration (Yang et al. 2002c) and yield under high temperature (Kuchel et al. 2007b) as well as in the studies presented here, makes this a significant and intriguing locus.

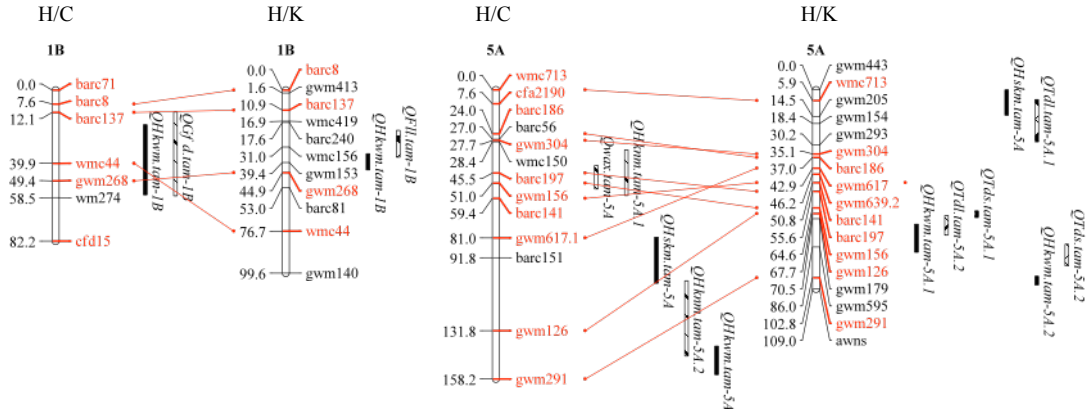


Fig. 3.8 Comparative map for chromosomes 1B and 5A for both the Halberd x Cutter (H/C) and Halberd x Karl 92 (H/K) RIL populations. Homologous markers are represented in red.

The experiment conduct by Yang et al. (2002c) was similar to those presented here, targeting heat stress to the main spike at 10 DAP. However, no yield components were measured and only single marker analysis was used to detect QTL in an F_2 population. Kuchel et al. (2007b) concluded that the QTL on 1B would be a prime target for marker-

assisted selection for improved adaptability and stress tolerance in wheat. Given the consistent detection of this QTL and its effect not only as a single locus, but also in combination with other QTL (Fig. 3.5 and Fig. 3.6) makes it a prime target for future targeted studies aimed at fine-mapping of this region.

Conclusions

The results of this study both confirm previous QTL identified in the Halberd x Cutter population and identify novel QTL associated with heat susceptibility index and organ temperature depression in response to reproductive stage heat stress at 10 DAP. At least seven loci were detected that co-localized for both HSI and temperature depression, with favorable alleles at five of these loci contributed by Halberd. At all loci, a cooler flag leaf or main spike temperature was associated with improved heat tolerance. Based on a mean allele contrast analysis for three of these QTL, it was determined that loci associated with HSI and temperature depression can be effectively combined, resulting in even larger gains in heat tolerance. Future studies to elucidate the causal effects of these loci, such as those that co-localized with stomatal conductance, reflective waxes, or other morphological characters will work to better understand their influence and regulation of heat tolerance in wheat.

CHAPTER IV

QUANTITATIVE TRAIT LOCI ASSOCIATED WITH YIELD AND YIELD COMPONENTS IN THE FIELD AND CONCLUSIONS ON THE POTENTIAL OF MARKER-ASSISTED SELECTION FOR IMPROVED HEAT TOLERANCE IN WHEAT

Introduction

The genetic control of yield in wheat is quantitatively inherited and is strongly influenced by environment as well as genotype x environment effects. Historically, the bulk of research related to yield in wheat, through either traditional or molecular breeding strategies, has focused on resistance to biotic stresses including development of markers associated with resistance to *Puccinia* spp., *Fusarium* head blight, and other diseases prevalent in the wheat growing regions of the world. More recently, focus has shifted toward improving wheat production in marginal environments as the available land for agricultural use continues to diminish and the impact of global climate change on agriculture continues to grow (Reynolds et al. 2007a). A thorough understanding of the loci that are involved in yield potential in wheat under conditions of abiotic stress, including those related to both direct gene signaling pathways and those which influence yield pleiotropically will prove beneficial for a continued and accelerated improvement in wheat yields.

A number of recent studies have utilized QTL mapping to understand the genetic control of yield in both bread wheat (Cuthbert et al. 2008; Groos et al. 2003; Hai et al. 2008; Huang et al. 2006; Kuchel et al. 2007a; Marza et al. 2006; McCartney et al. 2005; Raman et al. 2009) and durum wheat (Maccaferri et al. 2008; Peleg et al. 2009). In a select few of these studies, fungicide was used to focus specifically on identifying QTL independent of disease resistance (Cuthbert et al. 2008; Huang et al. 2006). Cuthbert et al. 2008 identified five stable QTL for yield in a spring wheat population that were also associated with individual yield components.

Recent studies have also identified loci associated with improved grain yield under drought conditions. A QTL on chromosome 4A was associated with drought susceptibility index, increased yield, biomass, weight per spike, earliness per se, and other agronomic traits under severe and moderate drought stress (Kirigwi et al. 2007). Quarrie et al. 2005 characterized a number of QTL present across a range of environments including salinity, nutrient, and drought. In durum wheat, QTL for yield under drought have been shown to co-localize with plant height (Maccaferri et al. 2008). In Australia, where drought during planting is a limiting factor to wheat establishment, a focus has been put on identifying loci associated with improved coleoptile vigor (Rebetzke et al. 2005; Rebetzke et al. 2007).

Conditions of drought stress are often accompanied by extreme heat stress. Two marker loci linked to the genetic control of grain-filling duration under long-term heat stress in winter wheat were detected on chromosomes 1B and 5A, explaining 23% of the total variation (Yang et al. 2002c). The same marker on chromosome 1B, *gwm11*, was associated with a QTL for yield that was present in environments with the highest number of days with maximum temperature (T_{max}) above 30°C across 18 site-years (Kuchel et al. 2007b). In this same study, T_{max} was found to be the only significant environmental covariable affecting yield potential.

In addition to development of molecular genetic based tools for improving abiotic stress tolerance in wheat, physiological approaches have also proved useful. The benefits of a cooler crop canopy, influenced by a combination of greater temperature depression in leaf, spike, and have been correlated with yield in the field (Ayeneh et al. 2002; Gutierrez-Rodriguez et al. 2000; Reynolds et al. 2000) and under controlled conditions (Hede et al. 1999) and associations with differences in plant morphology have been documented (Balota et al. 2008). Canopy temperature depression has shown promise for use as a physiological marker for genetic gains in yield under conditions of stress given its robustness, its correlation with yield and its ease of measurement (Reynolds et al. 2007b; Richards 2000).

The previous two studies presented in this dissertation have focused on dissecting reproductive stage heat tolerance into its basic components. These include yield

stability, measured in terms of a heat susceptibility index (HSI), organ temperature under heat stress, and morphological characters associated with improved heat tolerance. Genetic loci associated with and/or regulating these traits have been identified within the wheat genome and the effects of these loci have been determined. These experiments were carried out under controlled environmental conditions, where heat stress could be applied at a pre-determined point of development of 10 days after pollination (DAP).

The objective of the final study of this dissertation is to characterize the Halberd x Karl 92 recombinant inbred line (RIL) population under abiotic stress in the field and to determine the phenotypic expression and heritability of yield and yield components. Stable loci associated with yield, stress tolerance, and morphological characters that contributed to improved yield will be identified though QTL mapping. It will be determined which loci detected for improved heat tolerance, as previously present in the first two studies herein, contribute to improved yield under field conditions. Finally, the potential utilization of these loci and loci identified in the previous two studies for marker-assisted selection and future research for improving heat tolerance in wheat will be discussed.

Materials and methods

Line development

A population of 121 recombinant inbred lines (RILs) was derived from a cross between 'Halberd' and 'Karl 92'. Halberd is a heat tolerant Australian hard white spring wheat cultivar. Karl 92 is a heat susceptible hard red winter wheat developed at Kansas State University. A cross between the two parents was carried out in 2003 in the greenhouse in College Station, TX. Lines were advance by single seed descent in the greenhouse to the F_5 generation and seed from individual F_5 plants were bulked to create 121 F_2F_6 lines. These F_6 and F_7 lines were evaluated in the field in 2008 and 2009.

Field trials

Halberd, Karl 92, and a subset of the Halberd x Karl 92 RILs (n=118) were grown in the field in College Station, TX in 2008 and 2009 growing seasons under non-irrigated conditions. Lines were sown under early and late sowing conditions in both years to push lines into heat stress during reproductive development. In 2008, lines were sown on December 1 and December 21. In 2009, lines were sown on December 7 and January 7. Lines were planted in a randomized complete block design, with two replications per RIL per sowing date. Plot size was 10ft x 5ft plots with 7 rows per plot, seeded at 45 grams/plot. Pre-plant fertilizer (16-20-00) was applied at 300lbs/acre and top dressed

with 15 gallons of N32 per acre two months after planting. In order to eliminate the effect of rust stress on yield potential, the fungicide TILT® (Syngenta Crop Protection, Inc., Greensboro, NC) was applied as recommended to control for leaf rust disease. Due to uneven stand in the late sowing date in 2009, plots were not harvested, resulting in at total of three site-years.

Table 4.1 Field environments and phenotypic traits evaluated for Halberd x Karl 92 RIL population

Trait	Symbol	Environments[1]	Method of Measurement
Biomass (kg/ha)	Bio	CS08-1, CS08-2	Amount of above ground biomass estimated from $1ft^2$ sample
Kernel Diameter (mm)[2]	Kdi	CS08-1, CS08-2, CS09-1	Average diameter of 300 individual kernels measured using SKHT
Days to heading (days)	Hdg	CS08-1, CS08-2, CS09-1	Period of time from planting to emergence of 50% of inflourescences in each plot
Days to maturity (days)	Mat	CS08-1, CS08-2, CS09-1	Period of time from planting to 90% senescence in each plot
Grain-filling duration (days)	Gfd	CS08-1, CS08-2, CS09-1	Period of time from heading to 90% senescence in each plot
Kernel hardness (hardness index)[2]	Kha	CS08-1, CS08-2, CS09-1	Average hardness of 300 individual kernels using SKHT
Harvest index (%)	Hi	CS08-1, CS08-2	Calculated as grain yield/total above ground biomass
Kernel number per spike	Kns	CS08-1, CS08-2, CS09-1	Estimated from $1ft^2$ sample (08) or 50 individual spikes (09) from each plot
Kernel weight per spike (g)	Kws	CS08-1, CS08-2, CS09-1	Estimated from $1ft^2$ sample (08) or 50 individual spikes (09) from each plot
Single kernel weight (mg)[2]	Skw	CS08-1, CS08-2, CS09-1	Average kernel weight of 300 individual kernels using SKHT
Spike density (spike/m^2)	Sm2	CS08-1, CS08-2, CS09-1	Estimated from $1ft^2$ (08) or 50 individual spikes (09) from each plot
Thousand kernel weight (g)	Tkw	CS08-1, CS08-2, CS09-1	Weight of 1000 kernels
Yield (kg/ha)	Yld	CS08-1, CS08-2, CS09-1	Total plot yield
Canopy temperature depression (°C)	CTD	CS08-2, CS09-1	Difference between air temperature and canopy temperature, measured on May 14 and 15 from 2-4pm
Plant height (cm)	Hgt	CS08-1, CS08-2,CS09-1	Average plant height from soil surface to tip of spike
Test weight (g)	Twgt	CS09-1	Measure on a volume basis

[1]CS08-1, College Station, TX sown on December 1 2007, CS08-2, College Station, TX sown on December 30, 2007,CS09-1, College Station, TX sown on December 7, 2008

[2] Measured using a Single kernel hardness tester

Phenotypic trait measurement

Prior to whole plot harvest, samples were taken to estimate yield components. Details of traits, symbols, and method of measurement are presented in Table 4.1. In 2008, yield components were estimated by harvesting a $1ft^2$ subplot from each main plot, encompassing four $1ft^2$ rows to estimate spike density (Sm2), kernel number per spike (Kns), kernel weight per spike (Kws), biomass (Bio), and harvest index (Hi). A seed counter was used to calculate the weight of 1000 kernels (Tkw).

In 2009, 50 spikes were pulled from each plot and used to estimate the same yield components with the exception of biomass and harvest index, which were not estimated. Test weight (Twgt) by volume was also calculated in 2009. Kernel diameter (Kdi), kernel hardness (Kha), and single kernel weight (Skw) were determined using a Single Kernel Characteristic System (SKCS) 4100 (Perten Instruments). Plant height (Hgt) was measured from the soil surface to the top of each plot, not including awns. Days-to-heading (Hdg) was measured as the number of days from planting to emergence of 50% of inflorescences within each plot. Grain-filling duration (Gfd) was measured as the date of heading to date of senescence of 90% of inflorescences. Days-to-maturity (Mat) was measured as the time from planting to 90% senescence of inflorescences in each plot. Canopy temperature depression (CTD) was measured during a heat stress episode on May 14 and May 15 in both 2008 and 2009 using a handheld thermometer (Model AG-42, Teletemperature Corp, Fullerton, CA) with an adjustable field of view.

Measurements were taken on the front and back of each plot and averaged to produced one measurement per plot. CTD was calculated as the difference between the air temperature (T_{air}) and canopy temperature (T_{canopy}).

A heat susceptibility index (HSI) for each individual RIL was calculated using the equation by Fisher and Maurer (Fischer and Maurer 1978): $HSI = (1-Y_h/Y)/(1-X_h/X)$, where Y_h and Y are the phenotypic means for each genotype under control and heat stressed conditions, respectively, and X_h and X are the phenotypic means for all lines under control and heat stressed conditions, respectively. Yield from the CS08-1 environment was used as the control and CS09-1 was used as the heat stressed environment. A heat susceptibility index was used to be consistent with previous studies presented here.

Statistical analysis

Each year and sowing date was treated as a separate environment. All traits were analyzed using the MIXED procedure (SAS v8.2, SAS Institute Inc., Cary, NC, USA) with all effects in the model (genotype, environment, replication and genotype x environment) treated as random. In addition, all environments were analyzed together using the restricted maximum likelihood (REML) method to produced best linear unbiased predictors (BLUP) for each trait across the three environments. Broad sense

heritability was estimated from the variance component estimates using TYPE3 sum of squares method.

Genetic map construction and QTL analysis

A genetic linkage map for the Halberd x Karl 92 population consisting of 189 SSR markers and one phenotypic marker was constructed as previously described in Chapter III of this dissertation. Data from each individual environment was used for QTL mapping in addition to BLUP estimates for the entire data set in a combined analysis. The BLUP estimates were used instead of overall means for the combined data set due to better consistency in detecting QTL in multiple years as has previously been documented in the literature (Cuthbert et al. 2008; Kuchel et al. 2007a).

QTL Cartographer version 2.5 (WINQTL) (Wang et al. 2007) was used for QTL detection. Initially, single marker analysis was used to identify genetic markers significantly associated with phenotypic traits. Composite interval mapping (CIM) was then used to determine likely QTL positions and a 1000 permutation test at a significance level of P=0.05 was used to determine the LOD threshold for each trait. For CIM, forward regression with backward elimination was used to identify background cofactors at P=0.05. A 10 CM window was used for CIM. A QTL was declared for a trait when it was significantly detected in at least one environment as well as in the combined analysis using the BLUP trait values. For discussion purposes, putative QTL

below the 1000 permutation threshold that are present in at least one other environment may be included in the tables and discussion. QTL were designated based on the nomenclature in the catalog for gene symbols for wheat (http://wheat.pw.usda.gov/ggpages/wgc/98/), consisting of a "Q" followed by the trait name, institution designation, and chromosome assignment.

Results

Environmental conditions

With the objective of detecting QTL associated with yield, yield components and agronomic traits under abiotic stress, the Halberd x Karl 92 RIL population was grown in College Station, TX under ideal and late sowing conditions over two growing seasons. Early and late sowing treatments were used to push the population into late season heat stress during reproductive development. College Station has a subtropical and temperate climate with mild winters and hot summers, adequate moisture for wheat production and unfavorably high temperatures during the grain-filling period. The population was disease free, being so through the regular application of fungicide.

Weather data for periods of vegetative and reproductive growth are presented in Table 4.2. The late sowing in both 2008 and 2009 resulted in an average delay in heading date of 15 days. Notable differences in growing conditions across the three environments

included both low precipitation during vegetative growth and higher number of days with T_{max}>30°C in CS09-1. This low precipitation in 2009 resulted in unequal plot stand in the late sowing date, which was not harvested. Rainfall during reproductive stage growth was comparable across the three environments, ranging from 60.96 mm to 91.4 mm. No days with T_{max}>30°C were observed during vegetative phase growth for CS08-1, and only one day with T_{max}>30°C was observed each for CS08-2 and CS09-1.

Table 4.2 Summary of weather data for vegetative and reproductive stage growth phases of wheat for the 2007-2008 and 2008-2009 growing seasons in College Station, TX

Environment	Phenology	Duration (Days)	GDD (°C)	Tmax (°C)	Tmin (°C)	>30°C Days	Max RH (%)	Min RH (%)	Precip (mm)	Solar Radiation
CS08-1	Sowing: December 1, 2007									
	Vegetative	1-114	1411.1	19.4	5.3	1	90.9	44.1	330.2	3403.3
	Reproductive	115-176	1313.9	27.6	15.5	15	89.4	44.1	91.4	5344.2
CS08-2	Sowing: December 21, 2007									
	Vegetative	1-108	1411.7	20.0	6.1	1	90.4	43.1	276.86	3703.8
	Reproductive	109-160	1144.4	28.6	16.3	19	89.4	42.0	60.96	5650.5
CS09-1	Sowing: December 7, 2008									
	Vegetative	1-113	1471.1	20.3	5.8	0	87.1	39.7	86.36	3357.6
	Reproductive	114-187	1645.0	28.7	16.4	32	89.7	40.0	78.74	5859.9

CS08-1, College Station, TX 2007-2008 Early sowing, CSO8-2 College Station, TX Late sowing

CS09-1 College Station, TX 2008-2009 Early sowing

GDD, growing degree days ((T_{max}+T_{min}/2)*Days))

T_{max}, maximum daily temperature, T_{min}, minimum daily temperature RH, relative humidity

Days with T_{max}>30°C during the reproductive stage were 15, 19, and 32 for CS08-1, CS08-2, and CS09-1, respectively. Growing degree days (GDD) were comparable

during vegetative growth, but varied widely during reproductive stage growth (Table 4.2).

Phenotypic analysis of quantitative traits

Phenotypic data collected for the Halberd x Karl 92 RIL population and the parental lines is presented as both individual environments (Table 4.3) and BLUP adjusted means across all environments (Table 4.4). Large variation was observed between parental lines for most traits with Halberd having higher yield, thousand-kernel weight, kernel diameter, and biomass compared to Karl 92. Halberd was also taller and had earlier ear emergence than Karl 92. On average, kernel number per spike, kernel weight per spike, spike density, and harvest index were comparable between parents, but varied depending on environment. For example, in CS09-1, Karl 92 produced 48.0 kernels per spike versus 33.2 kernels per spike for Halberd. In two of the three environments, Halberd had lower kernel number per spike, but had a much higher thousand-kernel weight contributing to higher kernel weight per spike. Phenotypic traits within RILs showed significant genetic variation for most traits and were normally distributed with the exception of heading (Fig. 4.1). High and low transgressive segregation was observed for all traits with the exception of test weight.

Table 4.3 Mean and range of trait values for parental cultivars and Halberd x Karl 92 RILs measured in College Station, Texas under early and late sowing conditions in 2008 and 2009

Trait	Early Sowing 2008 (CS08-1)						Late sowing 2008 (CS08-2)						Early sowing 2009 (CS09-1)					
	Halberd	Karl 92	RILs	sd	Min	Max	Halberd	Karl 92	RILs	sd	Min	Max	Halberd	Karl 92	RILs	sd	Min	Max
Days to heading	121.0	129.0	122.0	7.8	115.0	151.0	116.0	121.0	117.3	8.4	109.0	131.0	126.0	142.0	125.1	12.5	114.0	155.0
Grain filling Duration	39.0	35.0	38.3	4.6	23.0	47.0	37.0	35.0	32.3	4.6	24.0	47.0	42.0	31.0	42.9	9.2	25.0	73.0
Days to maturity	160.0	164.0	160.3	7.3	151.0	176.0	153.0	156.0	149.5	7.3	135.0	160.0	168.0	173.0	167.9	7.5	157.0	187.0
Thousand kernel weight (g)	33.3	24.5	27.8	2.9	21.5	35.1	28.8	22.9	27.3	3.8	20.2	39.4	33.1	25.5	29.8	3.2	22.3	39.1
Yield (kg/ha)	3030.5	2476.6	2751.5	829.7	902.9	5542.7	3241.6	2541.4	2736.5	1117.1	499.5	5450.7	2979.0	3043.3	2637.9	749.9	878.4	4503.4
Spike density (m^{-2})	313.5	395.0	360.4	57.0	219.5	513.1	356.3	311.4	288.7	49.0	143.2	417.0	271.8	250.6	252.5	75.1	82.1	498.2
Kernel weight per spike (g)	1.0	0.7	0.76	0.2	0.4	1.3	0.9	0.8	0.95	0.4	0.2	1.7	1.10	1.22	0.99	0.2	0.48	1.5
Kernel number per spike	29.0	26.8	27.3	5.7	12.8	40.0	32.4	36.0	34.2	10.2	8.0	55.0	33.2	48.0	33.3	6.4	19.5	47.6
Kernel hardness	81.7	83.4	75.5	8.4	53.0	94.0	83.2	93.1	84.0	8.5	59.3	100.4	67.0	67.1	68.4	7.7	51.0	89.5
Single kernel weight (mg)	32.5	24.6	27.0	2.7	21.3	33.7	27.5	23.4	27.2	3.3	21.7	36.6	33.0	27.2	30.4	2.9	24.8	39.5
Kernel diameter (mm)	2.8	2.4	2.5	0.1	2.3	2.9	2.6	2.4	2.5	0.1	2.3	2.9	2.80	2.50	2.65	0.10	2.40	2.9
Height (cm)	89.5	85.0	82.6	13.2	53.5	111.0	80.0	75.0	75.1	13.3	47.5	105.0	85.0	80.0	78.6	13.2	50.0	107.0
Biomass (kg/ha)	10659.0	8374.6	9244.4	2014.4	4449.6	15064.7	11064.5	7927.4	8925.8	2123.4	4056.7	14868.3	-	-	-	-	-	-
Harvest index	0.28	0.30	0.30	0.06	0.17	0.51	0.29	0.32	0.30	0.08	0.10	0.49	-	-	-	-	-	-
CTD (°C) (Heat)	-	-	-	-	-	-	2.7	2.0	3.1	0.9	0.9	5.1	3.05	2.55	2.66	1.1	-1.1	4.7
CTD (°C) (Recov)	-	-	-	-	-	-	1.1	2.0	1.7	0.7	0.0	3.0	4.45	2.55	2.37	1.3	-1.4	5.1
Test weight	-	-	-	-	-	-	-	-	-	-	-	-	55.1	55.0	54.5	1.6	47.5	57.5

CS08-1, College Station, TX 2007-2008 Early sowing, CS08-2 College Station, TX Late sowing, CS09-1 College Station, TX 2008-2009 Early sowing

Table 4.4 Mean and range of the BLUP adjusted trait values of the Halberd x Karl 92 RIL population across all environments

Trait	Parents		RIL population		
	Halberd	Karl 92	Mean	Min	Max
Days to heading	121.3	130.0	121.7	113.8	139.5
Grain filling Duration	38.9	35.5	38.1	31.1	46.6
Days to maturity	160.7	164.4	159.7	149.4	175.0
Thousand kernel weight (g)	31.1	24.9	28.3	23.0	34.2
Yield (kg/ha)	2949.7	2683.2	2688.2	1645.9	3696.1
Spike density (m^{-2})	305.5	307.8	299.3	249.5	361.3
Kernel weight per spike (g)	0.92	0.90	0.90	0.81	1.00
Kernel number per spike	31.5	33.9	31.5	26.7	36.0
Kernel hardness	75.0	79.3	75.8	59.3	90.3
Single kernel weight (mg)	29.3	25.6	28.2	24.3	33.6
Kernel diameter (mm)	2.64	2.47	2.57	2.43	2.75
Biomass (kg/ha)	10008.6	8554.8	9045.1	7135.2	10989.9
Harvest index	0.29	0.31	0.30	0.19	0.43
Height (cm)	84.7	80.0	78.8	51.1	107.1

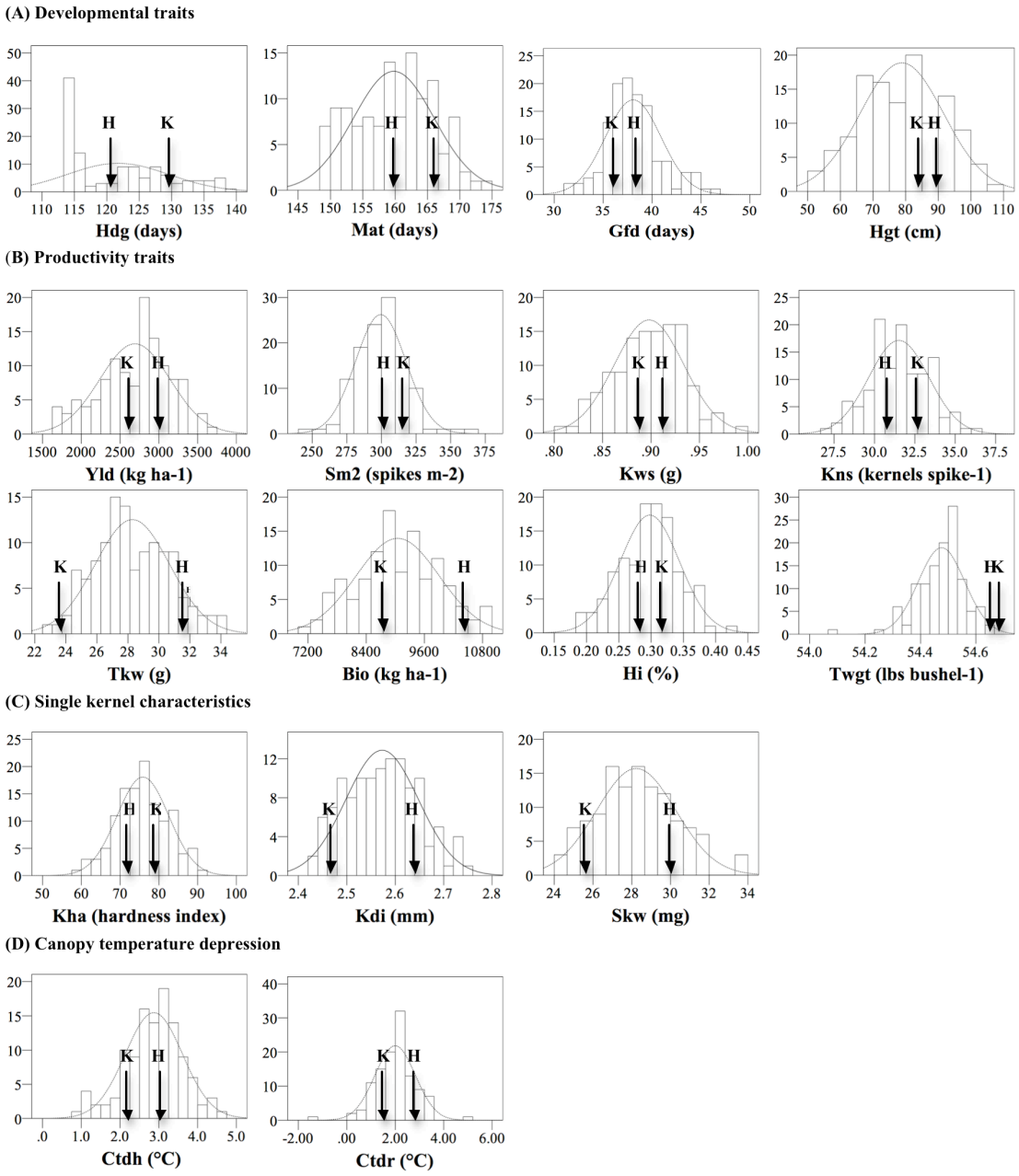


Fig. 4.1 Frequency distribution of phenotypic characters in the Halberd x Karl 92 recombinant inbred line (RIL) population. Data includes plant developmental traits (A) including heading (Hdg), maturity (Mat), grain-filling duration (Gfd) and plant height (Hgt), plant productivity traits (B) including grain yield (Yld), spikes per m^2 (Sm2), kernel weight per spike (Kws), kernel number per spike (Kns), thousand-kernel weight (Tkw), biomass (Bio), harvest index (Hi), and test weight (Twgt), single kernel characteristics (C), kernel hardness (Kha), kernel diameter (Kdi), and single kernel weight (Skw) and canopy temperature depression (D) under heat (Ctdh) and recovery (Ctdr). Phenotypic values are based on BLUP adjusted means across three-site years. Approximate positions of parents Halberd (H) and Karl 92 (K) are also presented.

Thousand kernel weight, kernel diameter, and single kernel weight showed low levels of transgressive segregation, with Halberd at the high end and Karl 92 at the low end for these traits (Fig. 4.1). Average grain yield was highest in CS08-1 at 2751.5 kg^{-1} compared to 2736.5 kg ha^{-1} and 2637.9 kg ha^{1} in CS08-2 and CS09-1, respectively, although these differences were not significant. Increased yield in CS08-1 was due to high spike m^{-2} compared to the other environments, as most yield components were actually lower compared to CS08-2 and CS09-1. The late sowing date in CS08-2 resulted in a shortening of the vegetative stage that led to a reduction in mean spike m^{-2}. A similar effect was seen in CS09-1, most likely due to lack of moisture during vegetative growth. A compensatory response to the reduction in mean spikes m^{-2} was observed in both CS08-2 and CS09-1 in terms of more kernels per spike and heavier spikes but fewer spike m^{-2}, which resulted in comparable total yield across all three environments.

Variance components and broad-sense heritability

Contributions of genetic, environmental, genotype x environment (GxE) and other sources of variance are presented in Table 4.5. The amount of variation (%) attributed to genetic effects was generally large compared to environmental and GxE. Plant height, heading, and thousand-kernel weight had the highest genetic variance, while kernel number per spike, CTD-heat, and CTD-recovery had non-significant genetic variances. Significant environmental variation was observed for heading, grain-filling duration,

maturity, kernel number per spike, and kernel weight per spike. Since biotic stress effects were minimized with agronomic practices, factors influencing environmental variance should be limited to abiotic stresses, including growing-degree-days, heat stress, and precipitation. Significant GxE effects were observed for yield and most yield components, including thousand-kernel weight, spike density, kernel weight per spike, kernel number per spike and biomass, as well as other agronomic traits.

Table 4.5 Broad sense heritability estimates and percentage of variance components attributable to genetic, environment, genotype x environmental and other effects

	Variance component[a]					
Trait	H^2	G	E	GxE	Rep (E)[d]	Residual
Heading (days)	0.90	0.62***	0.14***	0.20***	0.00	0.04
Grain-filling duration (day)	0.60	0.19***	0.35**	0.30*	0.02***	0.15
Maturity (days)	0.92	0.29***	0.60***	0.05***	0.01***	0.05
Thousand kernel weight (g)	0.86	0.50***	0.10	0.14***	0.06***	0.20
Yield (kg/ha)	0.68	0.32***	0.00	0.27***	0.03***	0.37
Spike density (spike/m^2	0.45	0.09***	0.33*	0.14***	0.06***	0.38
Kernel weight per spike (g)	0.25	0.07*	0.14**	0.52***	0.01***	0.25
Kernel number per spike	-	0.00	0.15**	0.50***	0.01**	0.34
Kernel hardness	0.89	0.32***	0.44	0.10	0.12	0.01
Single kernel weight (mg)	0.98	0.30***	0.33	0.00	0.00	0.37
Kernel diameter (mm)	0.79	0.12***	0.27	0.00	0.00	0.61
Biomass (kg/ha)	0.55	0.25***	0.00	0.10*	0.02*	0.62
Harvest index (%)	0.75	0.44***	0.00	0.05	0.03***	0.48
Height (cm)	0.99	0.78***	0.06	0.00	0.00*	0.16
CTD-heat (°C)[b]	-	0.00	0.14	0.59***	0.00	0.27
CTD-recovery (°C)[c]	-	0.00	0.08	0.31***	0.01	0.60

$H^2 = \sigma_g^2 / \sigma_g^2 + \sigma^2_{gxe/e} + \sigma^2_{error/re}$

[a]Variance component of each effect divided by the total of all variance components

G, genotype, E, environment, GxE, genotype x environment

[b]CTD-heat, Canopy temperature depression taken on day of heat stress

[c]CTD-recovery, Canopy temperature depression taken on day following heat stress

[d]Replications nested within environment

*Signicant a P=.05, **Significant at P=.01, ***Significant at P=.001

Single kernel characters, including single kernel weight, kernel diameter, and kernel hardness did not show significant GxE variation. Broad sense heritability estimates were low to intermediate (H^2=0.0 to 0.68) for Kns, Kws, Sm2, Yld, and Bio and high (H^2=0.75 to 0.99) for Hdg, Mat, Tkw, Kha, Skw, Kdi, Hi, and Hgt (Table 4.5). Kernel weight per spike had the lowest estimated heritability at H^2 = 0.25 and the highest variation explained by GxE effects.

Correlations

Correlations among yield components are presented in Table 4.6. As expected, yield was highly correlated with spike density, thousand-kernel weight, kernel weight per spike and kernel number per spike. Heading was negatively correlated with most yield components and agronomic traits, as lines that flowered later tended to yield less than early flowering lines. This is an expected result given that the main yield limitations in all three environments would be high temperature and water limitations that, in general, increase in severity throughout the growing season. Heading was lowly negatively correlated with spike density, but highly correlated with per spike characteristics suggesting that reduction in yield of later flowering lines is due more to limitations in grain set and filling of developing grains per spike rather than from spike production. Kernel hardness was negatively correlated with all yield components, but was positively correlated with both height and heading. It is plausible that the positive correlation between kernel hardness and heading could be due to a decrease in the ratio of starch to

protein in the grain as a result of high temperature stress late in the growing season which would negatively impact starch content. However, these grain characteristics were not investigated in this study.

Table 4.6 Pearson correlation coefficients of yield, yield components, and agronomic traits based on BLUP trait values

	Gfd	Mat	Tkw	Yld	Sm2	Kws	Knw	Kha	Skw	Kdi	Bio	Hi	Hgt	Twgt	Ctd-h	Ctd-r
Hdg	-0.66**	0.84**	-0.60**	-0.74**	-0.25**	-0.73**	-0.52**	0.66**	-0.64**	-0.60**	-0.50**	-0.74**	0.72**	-0.03	0.35**	0.14
Gfd	-	-0.15	0.34**	0.34**	0.05	0.48**	0.40**	-0.25**	0.32**	0.30**	0.20*	0.45**	-0.32**	-0.02	-0.09	-0.14
Mat		-	-0.55**	-0.73**	-0.29**	-0.62**	-0.40**	0.69**	-0.61**	-0.57**	-0.53**	-0.64**	0.71**	-0.06	0.41**	0.08
Tkw			-	0.55**	0.06	0.69**	0.19*	-0.47**	0.95**	0.90**	0.39**	0.45**	-0.25**	0.02	-0.15	-0.00
Yld				-	0.63**	0.81**	0.69**	-0.58**	0.56**	0.51**	0.78**	0.67**	-0.56**	-0.05	-0.24**	-0.06
Sm2					-	0.15	0.18*	-0.26**	0.06	0.03	0.69**	0.13	-0.16	-0.14	-0.03	-0.07
Kws						-	0.83**	-0.52**	0.68**	0.64**	0.50**	0.73**	-0.49**	0.04	-0.19*	-0.04
Knw							-	-0.32**	0.22*	0.19*	0.41**	0.63**	-0.44**	0.02	-0.15	-0.08
Kha								-	-0.53**	-0.41**	-0.40**	-0.57**	0.64**	-0.05	0.29**	0.06
Skw									-	0.92**	0.42**	0.49**	-0.34**	0.05	-0.24*	-0.02
Kdi										-	0.39**	0.44**	-0.31**	0.00	-0.22*	-0.07
Bio											-	0.28**	-0.33**	-0.03	-0.18	-0.07
Hi												-	-0.74**	0.06	-0.18	-0.11
Hgt													-	-0.07	0.27**	0.07
Twgt														-	-0.10	-0.07
Ctd-h															-	0.32**
Ctd-r																-

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Abbreviations: heading (Hdg), maturity (Mat), grain-filling duration (Gfd) and plant height (Hgt), yield (Yld), spikes per m^2 (Sm2), kernel weight per spike (Kws), kernel number per spike (Kns), thousand-kernel weight (Tkw), biomass (Bio), harvest index (Hi), and test weight (Twgt), single kernel characteristics (C), kernel hardness (Kha), kernel diameter (Kdi), and single kernel weight (Skw) and canopy temperature depression under heat (Ctdh) and recovery (Ctdr).

Canopy temperature depression (CTD) was lowly and negatively correlated with yield. We hypothesized that the benefits of a cooler canopy temperature under stress conditions were masked due to the positive correlation between CTD and heading (r=0.35) and the negative correlation between heading and most yield components. To test this hypothesis, a subset of RILs (n=46) with a three day window of days to heading (Hdg = 114 days to 116 days) were analyzed separately to reveal which, if any significant correlations might be present. In this sub-population, a positive and significant correlation was observed between CTD-heat (day 1, extreme heat) and thousand-kernel weight (r=0.40, Fig. 4.2), kernel weight per spike (r=0.30), and single kernel weight (r=0.31) as well as between CTD-recovery (day 2, mild heat) and thousand-kernel weight (r=0.40, Fig. 4.2), single kernel weight (r=0.48) and kernel diameter (r=0.48).

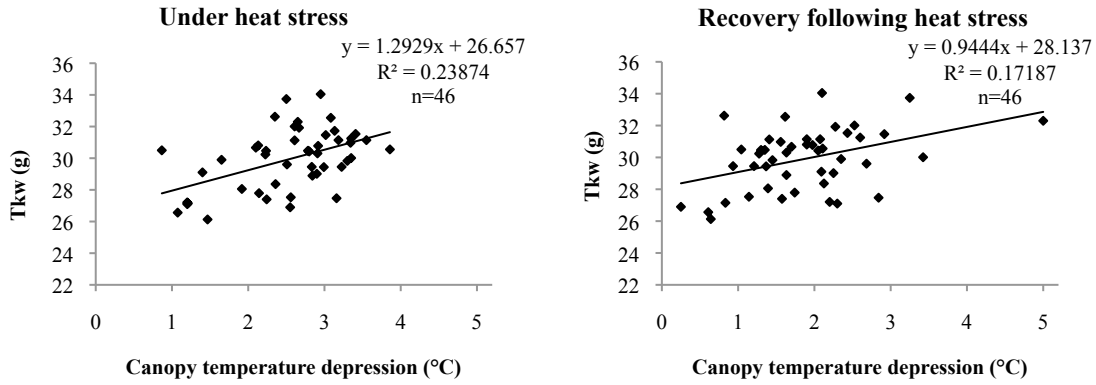


Fig. 4.2 Relationship between canopy temperature depression and thousand-kernel weight. Data was recorded on a subset of Halberd x Karl 92 RILs (n=46) with a three day window of heading date (Hdg = 114-116 days) under heat stress and recovery in the field.

Genetic linkage map construction

A genetic linkage map incorporating 189 SSR and one phenotypic marker (*B1/awns*) was used to identify QTL associated with yield, yield components, and agronomic traits. The constructed genetic map consisted of 21 linkage groups spanning a total of 2343.6 CM with an average distance of 12.3 CM/maker. Markers were unevenly distributed between linkage groups with marker numbers per chromosome ranging from three on chromosome 4D to eighteen on chromosomes 2D and 5B. Notable gaps (>40 CM) were present on chromosomes 3D, 4A, 4D, and 6D. Large gaps within linkage groups in wheat is not uncommon, as markers on each of the large chromosome arms often map as separate linkage groups. In general, marker order was conserved with previous wheat genetic maps and consensus SSR maps (Somers et al. 2004).

QTL mapping

For QTL mapping, data sets from individual environments as well as a combined analysis using the BLUP estimates across all environments was used for QTL mapping. A QTL was declared significant if it was identified in the combined analysis and in at least one individual environment. QTL detection using the BLUP estimates detected all QTL which were present in two or three environments, and it would be assumed based on the use of this method in previous reports (Cuthbert et al. 2008; Kuchel et al. 2007a) that it would also identify the most prominent QTL that were detected in only a single

environment. Single marker analysis (SMA) in QTL Cartographer was initially used to identify significant single marker QTL associated with yield components (Appendix). Composite interval mapping (CIM) was then used to determine final QTL, peak positions, and significant QTL LOD intervals. In general, SMA and CIM were in good agreement, although more significant QTL were identified using SMA versus CIM because of less stringency in declaring a QTL significant.

QTL number per trait varied, ranging from zero to eleven, as did the number and effect of QTL identified in any single environment (Appendix). At least one QTL was identified for each trait in each environment that it was measured with the exception of grain-filling duration in CS08-1, spike density in CS08-2, and CTD-recovery in CS09-2. QTL effects, LOD values, nearest marker, and environments detected for the combined analysis using the BLUP estimates is presented in Table 4.7. In general, the expected parent contributed favorable alleles for QTL, although often there were at least one favorable QTL that was contributed by an unexpected parent.

Table 4.7 Effects of QTL detected in the Halberd x Karl 92 mapping population (n=121) for yield, yield components and agronomic traits based on BLUP trait values across all environments

QTL (LOD threshold[a])	Marker	LOD	R^2	Additive[b]	Positive allele	Environments detected[c]
Biomass (3.00)						
QBiom.tam-3B	barc229	6.87	0.190	444.5	Halberd	CS08-1, CS08-2
Days to Heading (3.00)						
QHdg.tam-2D	wmc601	3.01	0.067	2.3	Halberd	CS08-1, CS09-1
QHdg.tam-5B	gwm408	4.11	0.072	2.3	Karl 92	CS08-1
QHdg.tam-7A.1	gwm276	5.40	0.091	2.6	Halberd	CS09-1
QHdg.tam-7A.2	wmc525	5.59	0.120	2.9	Karl 92	CS08-2, CS09-1
QHdg.tam-7D	wmc14	3.54	0.060	2.1	Halberd	CS08-2, CS09-1
Days to maturity (4.18)						
QMat.tam-2D	gwm484	8.61	0.180	2.9	Halberd	CS08-1, CS08-2, CS09-1
QMat.tam-4A	barc78	6.63	0.115	2.6	Halberd	CS08-1
QMat.tam-5B	gwm639.1	4.24	0.090	2.0	Karl 92	CS08-1
QMat.tam-7A	wmc603	5.54	0.125	3.6	Halberd	CS08-1, CS08-2
QMat.tam-7D	wmc14	6.83	0.120	2.4	Halberd	CS08-1, CS08-2
Grain-filling duration (2.93)						
QGfd.tam-5A	gwm205	6.61	0.142	1.2	Halberd	CS09-1
QGfd.tam-7A	wmc525	5.28	0.180	1.3	Halberd	CS09-1
Harvest indext (3.08)						
QHi.tam-2D	gwm484	9.74	0.271	0.025	Karl 92	CS08-1, CS08-2
QHi.tam-5B.1	barc4	3.37	0.054	0.012	Karl 92	CS08-2
QHi.tam-5B.2	gwm408	5.08	0.106	0.016	Halberd	CS08-1
QHi.tam-7D	gwm130	3.51	0.057	0.012	Karl 92	CS08-1, CS08-2
Height (3.42)						
QHt.tam-2D	cfd56	6.28	0.210	6.1	Halberd	CS08-1, CS08-2, CS09-1
QHt.tam-4B	wmc89.1	4.89	0.128	4.7	Halberd	CS08-1, CS08-2, CS09-1
Kernel diameter (3.22)						
QKdi.tam-2D	gwm484	6.63	0.164	0.031	Karl 92	CS08-1, CS08-2
QKdi.tam-5A	barc186	4.40	0.102	0.025	Halberd	CS08-2*, CS09-1
Kernel hardness (3.05)						
QKha.tam-2D.2	gwm484	3.33	0.058	1.7	Halberd	CS08-1, CS08-2
QKha.tam-3A	gwm480	7.19	0.062	1.8	Karl 92	CS08-1
QKha.tam-4A	barc78	3.72	0.038	1.4	Halberd	CS08-1
QKha.tam-4B	wmc89.1	3.49	0.035	1.4	Halberd	CS08-1
QKha.tam-5B	gwm639.1	4.39	0.059	1.9	Karl 92	CS08-1, CS09-1
QKha.tam-5D	gwm292	7.80	0.071	2.1	Karl 92	CS08-1
QKha.tam-7D	wmc14	8.59	0.080	2.2	Halberd	CS08-1, CS08-2
Kernel number per spike (3.02)						
QKns.tam-4B	gwm251	4.16	0.082	0.6	Karl 92	CS08-1, CS08-2
QKns.tam-7A	gwm282	5.01	0.097	0.6	Karl 92	CS08-1, CS08-2
QKns.tam-7D	barc76	4.69	0.104	0.6	Karl 92	CS08-2
Kernel weight per spike (3.10)						
QKws.tam-2D	gwm484	4.45	0.103	0.012	Karl 92	CS08-1, CS08-2, CS09-1
QKws.tam-5A	gwm291	3.77	0.093	0.012	Karl 92	CS09-1
QKws.tam-7D	barc76	3.59	0.088	0.012	Karl 92	CS08-1
Single kernel weight (3.06)						
QSkw.tam-2D	gwm484	6.78	0.197	0.9	Karl 92	CS08-1, CS08-2
Spike density (3.00)						
QSm2.tam-1D	cfd15	3.78	0.038	4.0	Halberd	CS09-1
QSm2.tam-2A	gwm294	3.05	0.049	4.6	Karl 92	CS09-1
QSm2.tam-3B	barc229	10.43	0.135	8.3	Halberd	CS09-1
QSm2.tam-5B	gwm639.1	6.04	0.106	6.7	Halberd	CS09-1
QSm2.tam-6B	gwm193	7.84	0.098	6.6	Halberd	CS08-1
QSm2.tam-7B	gwm400	6.49	0.097	6.2	Karl 92	CS09-1
QSm2.tam-7D	wmc634	4.79	0.053	4.8	Karl 92	CS09-1
Thousand kernel weight (3.16)						
QTkw.tam-2D	gwm484	7.45	0.152	0.98	Karl 92	CS08-1, CS08-2
QTkw.tam-5A	barc186	4.07	0.078	0.73	Halberd	CS08-1, CS08-2, CS09-1

Table 4.7 Continued

QTL (LOD threshold[a])	Marker	LOD	R^2	Additive[b]	Positive allele	Environments detected[c]
Test weight (2.82)						
QTwgt.tam-1A	gwm135	4.92	0.118	0.03	Karl 92	CS09-1
QTwgt.tam-5A	gwm179	3.23	0.082	0.03	Karl 92	CS09-1
Yield (3.18)						
QYld.tam-1D	gwm136	3.76	0.074	135.5	Halberd	CS09-1
QYld.tam-2D	gwm484	10.43	0.219	226.5	Karl 92	CS08-1, CS08-2
QYld.tam-3B	barc229	3.80	0.070	145.0	Halberd	CS09-1
QYld.tam-5D	cfd29	3.39	0.080	142.1	Halberd	CS08-1, CS09-1
QYld.tam-7D	barc76	3.60	0.065	126.9	Karl 92	CS09-1

[a]LOD thresholds were estimated in QTL Cartographer v2.0 using 1000 permutation

[b]Additive effect of allele substitution

[c] CS08-1, Early sowing 2007-08, CS08-2, Late sowing 2007-08, CS09-1, Early sowing 2008-09

*****Putative QTL with LOD value below 1000 permutation threshold

Fig. 4.3 Linkage map and quantitative trait loci (QTL) for yield, yield components and agronomic traits for the Halberd x Karl 92 recombinant inbred lines. QTL detected for both yield components, yield components, and agronomic traits (dark bars) as well as canopy temperature depression and heat susceptibility index (hatched bars) are presented as 2LOD intervals. Markers positions were calculated using the Kosambi mapping function and are listed in CM position from the top of each linkage group. QTL are annotated based on trait, linkage group and relative position on each chromosome. Details for QTL are presented in Table 4.7. Abbreviations: QTL (Q) heading (Hdg), maturity (Mat), grain-filling duration (Gfd) and plant height (Hgt), grain yield (Yld), spikes per m^2 (Sm2), kernel weight per spike (Kws), kernel number per spike (Kns), thousand-kernel weight (Tkw), biomass (Bio), harvest index (Hi), test weight (Twgt), kernel hardness (Kha), kernel diameter (Kdi), single kernel weight (Skw) and canopy temperature depression under heat (Ctdh) and recovery (Ctdr).

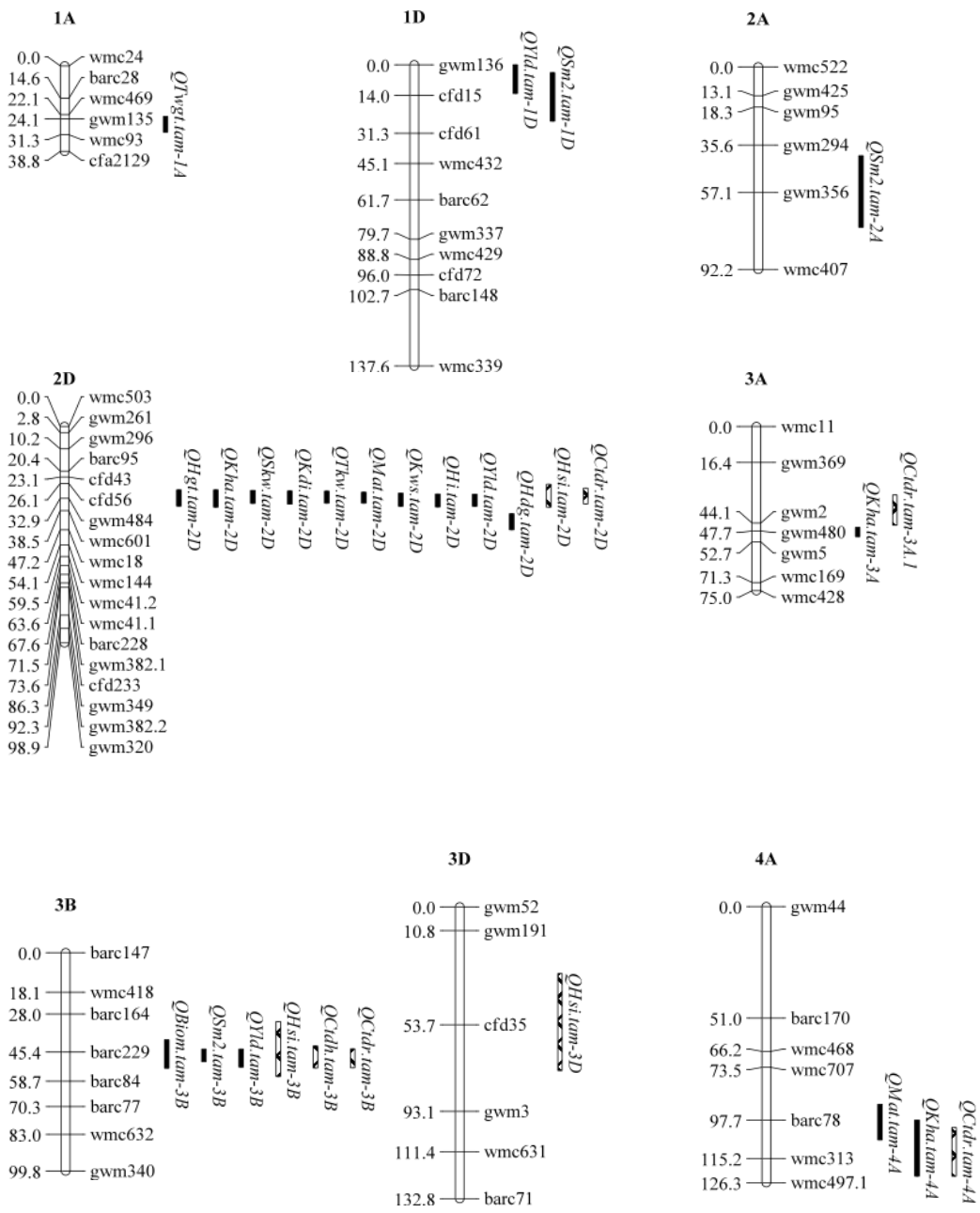


Fig 4.3 Continued

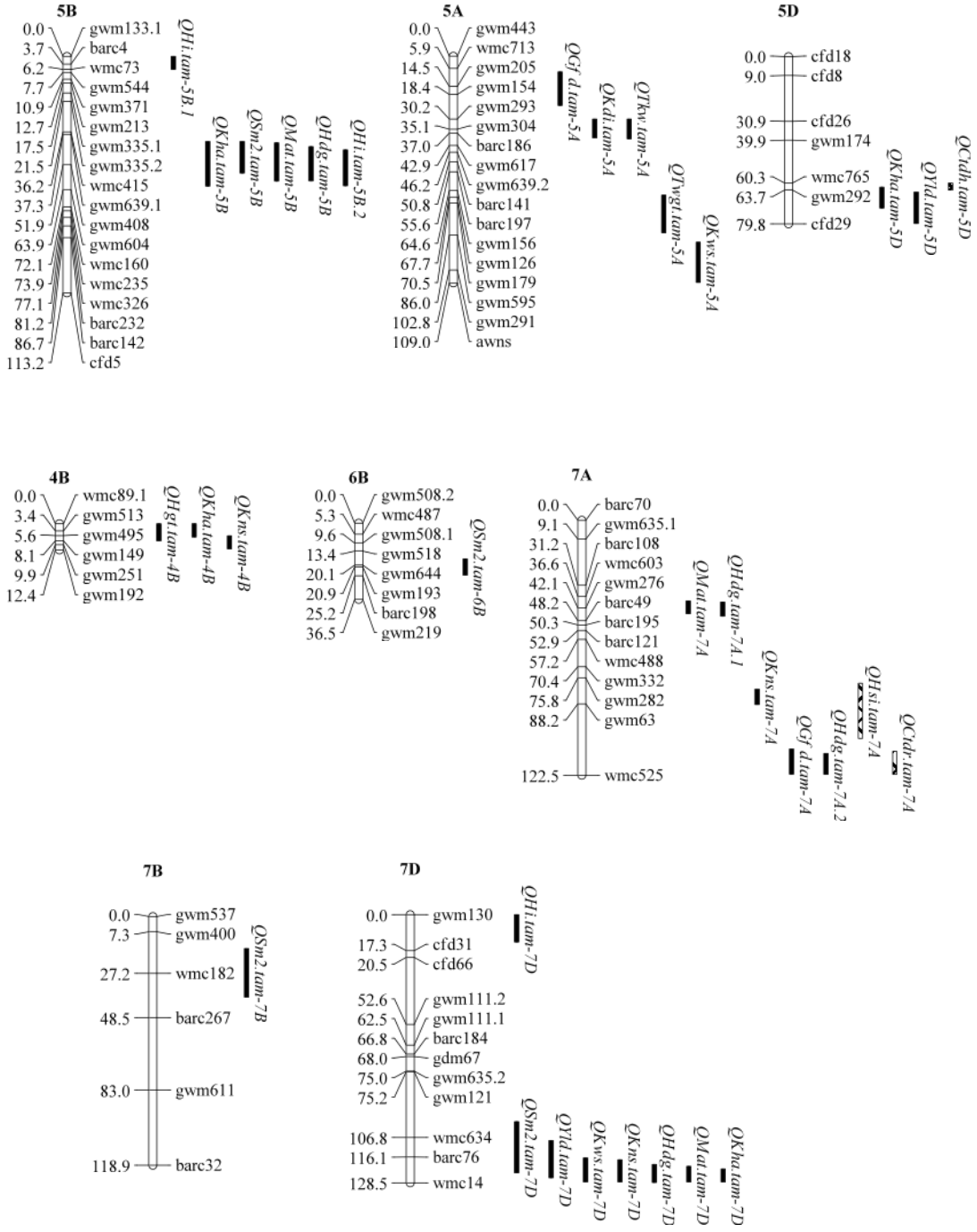


Fig 4.3 Continued

Significant QTL were detected on 16 of the 21 wheat chromosomes (Fig. 4.3). For most traits at least one "stable" QTL was detected that was present in more than one environment (Table 4.7). Exceptions include grain-filling duration and spike density. Traits with the highest heritability and highest percentage of genetic variance (Table 4.5) generally had the greatest number of QTL detected in multiple environments. These include heading, days to maturity, height, and thousand-kernel weight. An exception would be kernel number per spike, which was lowly heritable but had two QTL which were present in two environments. Due to the effectiveness of detecting QTL present in multiple environments using the BLUP estimates and the general lack of large environmental variances for most traits, further discussion will focus mostly on QTL results from this combined analysis, as it incorporates QTL detected from all environments. Details of QTL identified in individual environments and details on QTL positions for each analysis can be found in the Appendix of this dissertation.

Yield QTL

Both parents contributed favorably to total yield, with Halberd contributing three QTL and Karl 92 contributing two QTL (Table 4.7). *QYld.tam-1D, QYld.tam-3B, and QYld.tam-5D* from Halberd explained 7.4%, 7.0%, and 8.0% of the phenotypic variation for yield, respectively, increasing the additive genetic effect of the each loci increasing yield 135.5 to 145.0 kg/ha. *QYld.tam-2D* and *Qyld.tam-7D* from Karl 92 explained 21.9% and 6.5% of the phenotypic variation in yield, respectively, increasing yield by

226.5 and 126.5 kg/ha. *QYld.tam-2D* co-localized with *QHdg.tam-2D*, a QTL for days to heading, as did *QYld.tam-7D*. The *QHdg.tam-2D* is most likely the *Ppd-D1* locus, the major photoperiod gene in wheat. *QHdg.tam-2D* was negatively pleiotropic with most yield components and agronomic traits, with lower yields and generally lower yield component measurements for lines with a later heading date (see discussion section).

Yield component and plant productivity QTL

Karl 92 contributed all favorable alleles for kernel number and kernel weight per spike in the combined analysis. This was unexpected given that Halberd had higher kernel weight per spike in both CS08-1 and CS08-2. Each parent contributed one favorable QTL for thousand-kernel weight. The favorable QTL from Halberd, *QTkw.tam-5A* was stable across all three environments. *QTkw.tam-5A* explained 7.8% of the phenotypic variation for thousand-kernel weight in the combined analysis, increasing the mean of the trait by 0.73 grams. In the individual environments, *QTkw.tam-5A* explained 4.6% to 14.6% of the phenotypic variation for thousand-kernel weight. Halberd contributed seven of the ten favorable alleles detected for thousand-kernel weight in the individual environments, which is in better agreement with the phenotypic data for the parental lines (Tables 4.3). QTL detected for spike density were inconsistent across environments with no stable QTL. The majority of QTL detected in the combined analysis for spike density were from the CS09-1 environment. One stable QTL was

detected for biomass, *QBio.tam-3B*. The favorable allele for this QTL was contributed by Halberd and explained 19.0% of the phenotypic variation.

Plant development and phenological QTL

Five QTL were identified for heading date, with three QTL detected in at least two environments. *QHdg.tam-2D/Ppd-D1* explained only 6.7% of the variation for flowering time in the combined analysis, but explained 16.5% and 16.7% of the phenotypic variation in CS08-1 and CS09-1, respectively (Appendix). *QHdg.tam-2D* was not detected in CS08-2, which may be the result of the delayed planting and other factors such as temperature contributing to growing degree-days affecting emergence of inflorescences. *QHdg.tam-5B* is in the proximity of *VrnB1*, one of three vernalization genes on the group 5 chromosomes, but was only detected in CS08-1.

QTL for days to maturity were highly coincident with those for days to heading, with co-localization present at four of the five QTL regions. The lone QTL unique for days to maturity was *QMat.tam-4A* and was only detected in CS08-1. Both QTL for grain-filling duration were detected in CS09-1 only, with both positive alleles contributed by Halberd. These QTL, *QGfd.tam-5A* and *QGfd.tam-7A* increased grain-filling duration by 1.2 and 1.3 days, respectively, explaining 14.2% and 18.0% of the phenotypic variation.

Two QTL for plant height were detected and were present in all three environments. The largest QTL affecting plant height, *QHgt.tam-2D* explained 21.0% of the phenotypic variation for height and co-localized with the photoperiod locus, *QHdg.tam-2D.* The allele from Halberd contributed more days to heading and increased plant height at this locus, in agreement with the high correlation that is observed between these two traits (Table 4.6). *QHdg.tam-4B* is in proximity to *Rht-B1*, one of the major dwarfing genes in wheat, with the Halberd allele again contributing to taller plant height at this locus.

Single kernel characteristic QTL

Kernel hardness, kernel diameter, and single kernel weight were measured using a single kernel characteristic system and data was used for QTL mapping of single kernel characteristics. Kernel diameter and single kernel weight were highly correlated traits (r=0.92) and were highly correlated with thousand-kernel weight (r=0.95 and r=0.90), respectively (Table 4.6). Both traits are also negatively correlated with kernel hardness (r= -0.53 and r=-0.41) for single kernel weight and kernel diameter, respectively. In the combined analysis one, two, and seven QTL were identified for single kernel weight, kernel diameter, and kernel hardness, respectively. The only QTL detected for single kernel weight, *QSkw.tam-2D* co-localized with a QTL for kernel diameter, *QKdi.tam-2D,* and a QTL for kernel hardness, *QKha.tam-2D*. The Karl 92 allele for *QSkw.tam-2D* and *QKdi.tam-2D* increased these trait values, while the Halberd allele contributed to

increased kernel hardness, in agreement with the correlation data between these traits. QTL for kernel hardness generally had low genetic effects, explaining only 3.5% to 8.0% of the phenotypic variance. This evidence in combination with the phenotypic distribution of kernel hardness (Fig. 4.1) and previous characterization of both parental lines as "hard wheat" lend to the conclusion that no major genes for kernel hardness are segregating within this population. The three known major genes influencing kernel hardness and texture, *Puroindoline a* and *Puroindoline b* (*Pina-D1* and *Pinb-D1*), as well as *Grain softness protein-1* (*Gsp-1),* are known to reside in tight linkage on the short-arm of chromosome 5D but do not appear to be segregating within this population (reviewed by (Bhave and Morris 2008).

Stress tolerance and canopy temperature depression QTL

Data from CS08-1 (control) and CS09-1 (stressed) was used to calculate a heat susceptibility index for each RIL based on reduction in yield across the two environments (Table 4.8). Five QTL were detected for HSI, including two putative QTL. Four of the five beneficial alleles for these QTL were contributed by the stress tolerant parent Halberd, and explained 8.5% to 13.0% of the phenotypic variance in HSI. *QHsi.tam-2D* co-localized with the photoperiod locus, *QHdg.tam-2D*. *QHsi.tam-3B* was a putative QTL with a LOD value of 2.57, explaining 9.7% of the phenotypic variation for HSI. *QHsi.tam-3B* was pleiotropic and co-localized with *QBio.tam-3B, QSm2.tam-*

3B, QYld.tam-3B, and *QCtdh.tam-3B,* significant for biomass, spike density, yield, and canopy temperature depression under heat stress, respectively.

Table 4.8 Effects of QTL detected in the Halberd x Karl 92 mapping population (n=121) for heat susceptibility index under field conditions

QTL (LOD threshold[a])	Marker	LOD	R^2	Additive[b]	Positive allele	Environments observed
Heat susceptibility index (3.12)						
QHsi.tam-2D[d]	gwm484	2.79	0.130	-2.64	Halberd	CS08/CS09-1
QHsi.tam-3B[d]	barc229	2.57	0.097	-2.44	Halberd	CS08/CS09-1
QHsi.tam-3D	cfd35	3.40	0.086	-2.32	Halberd	CS08/CS09-1
QHsi.tam-5D	cfd18	3.10	0.124	2.66	Karl 92	CS08/CS09-1
QHsi.tam-7A	gwm63	3.23	0.085	-2.14	Halberd	CS08/CS09-1

[a]LOD thresholds were estimated in QTL Cartographer v2.0 using 1000 permutation

[b]Additive effect of allele substitution

[c]Putative QTL below 1000 permutation threshold

QTL detected for canopy temperature depression that co-localized with yield or yield components are presented in Table 4.9. Measurements were taken on the delayed planting in both years over a two-day period during intense heat stress on day 1 (heat, 32°C-34°C) followed more moderate temperatures on day 2 (recovery, 28°C-30°). Preliminary evidence in our lab has shown plasticity in the response of wheat to extreme temperature stress versus more moderate temperatures, so measurements taken during heat and recovery were treated as different phenotypic traits.

Table 4.9 Effects of QTL detected in the Halberd x Karl 92 mapping population (n=121) for canopy temperature depression in College Station, TX 2008 and 2009

QTL (LOD threshold[a])	Marker	LOD	R^2	Additive[b]	Positive allele	Environments observed
Canopy temperature depression heat (3.0)						
QCtdh.tam-3A.1	gwm369	7.29	0.129	0.42	Halberd	CS09-1-2
QCtdh.tam-3B	barc229	3.94	0.150	0.39	Halberd	CS08-2
QCtdh.tam-3B	barc229	3.83	0.055	0.33	Halberd	CS09-1-2
QCtdh.tam-4A	barc78	4.15	0.072	0.33	Karl92	CS09-1-2
QCtdh.tam-5D	wmc765	3.10	0.066	0.30	Halberd	CS08-2
Canopy temperature depression recovery (3.0)						
QCtdr.tam-2D	cfd56	4.88	0.147	0.28	Halberd	CS08-2
QCtdr.tam-7A	wmc525	6.11	0.200	0.32	Halberd	CS08-2

[a]LOD thresholds were estimated in QTL Cartographer v2.0 using 1000 permutation

[b]Additive effect of allele substitution

[c]Increase (%) represents the improvement of allele substitution in the trait over the mean of the population

[d]Putative QTL

Only one QTL was consistent between the two years, *QCtdh.tam-3B*. This QTL explained 5.5% and 15.0% of the phenotypic variation for CTD in 2008 and 2009 respectively, with the favorable allele contributed by Halberd. This QTL also co-localized with *QHsi.tam-3B* for HSI, and was associated with biomass, spike density, and yield as discussed above. Co-localization between CTD-heat and yield was also observed at the stable yield *QYld.tam-5D*, with the Halberd allele associated with both a cooler crop canopy and higher yield. This co-localization between QTL for CTD with both yield and heat susceptibility index is consistent with results from the greenhouse showing a strong association between improved heat tolerance and organ temperature depression.

Discussion

Phenotypic variation

Detection of QTL is dependent on allele polymorphisms linked to phenotypic variability being both present in parental lines and heritable within a segregating population. The Halberd x Karl 92 population used in this study had heritable and significant genetic variation for most yield components as was expected based on the phenotypic differences observed between parental lines (Table 4.2). In addition to these phenotypic characters, Halberd and Karl 92 have been well characterized as having differences in their response to stress, particularly heat stress, with Karl 92 previously described as heat susceptible (Yang et al. 2002a) and Halberd previously characterized as heat tolerant (Hays et al. 2007a; Panozzo et al. 1999; Yang et al. 2002a). This characterization is in strong agreement with the results presented in the previous two studies of this dissertation. While neither of these lines is necessarily adapted to the growing conditions in College Station, TX, the environment is ideal for identifying QTL associated with yield potential under abiotic stress. Similar to other QTL studies, fungicide was applied in each environment to reduce the effect of leaf rust and other fungal pathogens on yield potential (Cuthbert et al. 2008; Huang et al. 2006) and isolate those of abiotic stress.

Most of the traits measured in this study had moderate to high heritability with a high proportion of this variance explained by genetic effects (Table 4.5). Previous studies using mapping populations have found similar results for broad-sense heritability of yield and yield components (Cuthbert et al. 2008; Marza et al. 2006). One exception would be kernel number per spike, which showed no significant genetic variance and hence no heritability in this study.

RILs showed transgressive segregation for most traits (Fig. 4.1). A notable level of transgressive segregation was observed for days to heading, where RILs ranged from 113.8 days to 139.5 days, despite only a nine day difference in heading time between parental lines. QTL for heading and yield components often co-localize in QTL studies and it is important to differentiate those QTL associated with actual gains in yield and yield components from those QTL associated with gains in yield through pleiotropy with other phenotypic characters. Previous QTL studies have identified QTL associated with yield, grain protein, thousand-kernel weight and other components, but failed to account for those QTL that could be explained by pleiotropy with heading date (Groos et al. 2003; Hai et al. 2008; Huang et al. 2006; Kumar et al. 2007; McCartney et al. 2005; Raman et al. 2009). Other QTL studies that documented heading date accounted for QTL co-localizing for both heading and yield (Cuthbert et al. 2008; Marza et al. 2006).

QTL clusters and pleiotropy

QTL are often present in clusters when a major gene or major phenotypic character is segregating within a population. QTL clusters associated with five or more traits were present on chromosomes 2D, 3B, 5B, and 7D in the present study. With the exception of 3B, all other QTL clusters were pleiotropically associated with days to heading QTL, including *QHdg.tam-2D, QHdg.tam-5B,* and *QHdg.tam-7D.* As mentioned previously, *QHdg.tam-2D* is most likely *Ppd-D1*, the major photoperiod gene which is known to be closely linked to gwm484 on 2DS (Hanocq et al. 2004). This region was associated with 10 of the 16 traits measured, with early heading resulting in higher yield, kernel weight per spike, and thousand-kernel weight. This is characteristic of a heat avoidance response and has been well documented in the literature (See review by (Araus et al. 2008).

In addition to *QHdg.tam-2D*, yield was pleiotropically associated with flowering time at *QHdg.tam-7D/QYld.tam-7D.* Other traits co-localizing to this region on the distal end of 7DS included spikes m^{-2}, kernel number per spike, kernel weight per spike, days to maturity and kernel hardness. This region has previously been associated with QTL for yield and thousand-kernel weight (Groos et al. 2003; Hai et al. 2008) but in neither of these studies did the authors account for phenotypic differences in heading date. It is therefore possible that this represents a novel QTL involved in ear emergence that appears to also have pleiotropic effects on a number of yield components.

Yield QTL and co-localization with previous reports

This study identified five main QTL for yield that were present in the combined analysis. As mentioned previously, two of these QTL were pleiotropically associated with both yield and days to heading, *QYld.tam-2D/QHdg.tam-2D* and *QYld.tam-7D/QHdg.tam-7D* As this study and the previous results presented in this dissertation are focused on identifying QTL for heat tolerance, independent of avoidance or flowering time, these QTL are not of importance.

QYld.tam-5D was the most stable QTL for yield not associated with flowering time and was detected in both CS08-1 and CS09-1. The Halberd allele at *QYld.tam-5D* increased mean yield across environments by 142.1 kg ha^{-1}, explaining 8.0% of the phenotypic variance. For individual environments, *QYld.tam-5D* increased yield by 230.6 kg ha^{-1} and 214.4 kg ha^{-1} in CS08-1 and CS09-1, respectively. *QYld.tam-5D* also co-localized with a QTL for CTD-heat, with the Halberd allele with a cooler crop canopy. Previous studies have shown this region to be associated with spike length (Kumar et al. 2007), test weight (McCartney et al. 2005) and grain number per ear (Hai et al. 2008), but no total grain yield QTL have previously been reported in this region or on chromosome 5D. The detection of this QTL in multiple years and the co-localization of CTD at this locus make this a significant and novel QTL not previously reported in the literature that could have benefits to wheat adaptation in stressed environments.

QYld.tam-3B was detected for yield in both the combined analysis and in CS09. The QTL explained 7.0% of the phenotypic variance for yield in the combined analysis, increasing yield by 145 kg ha^{-1}. In addition to yield, this region also co-localized with a stable QTL for biomass explaining 19.0% of the phenotypic variation, a stable QTL for CTD-heat, as well as with QTL for spikes m^{-2}, and thousand-kernel weight. When grain-yield of the most heat stressed environment (CS09-1) and least heat stressed environment (CS08-1) was used to calculate a heat susceptibility index (HSI), a putative QTL for HSI was also found to co-localize in this region (Table 4.8). This QTL, *QHsi.tam-3B,* had a putative LOD value of 2.57 and explained 9.7% of the phenotypic variance for HSI. A mean allele contrast analysis for traits significantly associated with this QTL region are presented in Fig. 4.4. The Halberd allele at this locus was associated with higher values for all traits in all environments, although not always significant.

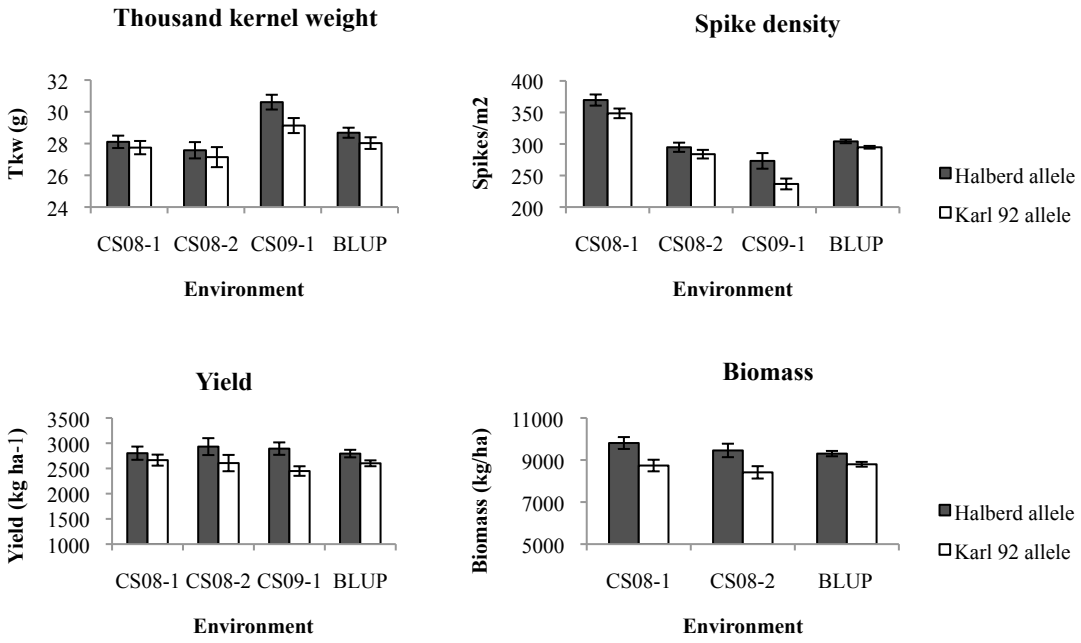


Fig. 4.4 Mean allele contrast analysis of trait values for barc229 on chromosome 3B. Dark bars are the mean trait values of Halberd x Karl 92 RILs that possess the Halberd allele for barc229 and the light bars are the mean trait values of RILs that possess the Karl 92 allele at barc229. Error bars are ±SE.

Co-localization of QTL for above ground biomass and yield in durum wheat under drought stress have been reported on 2A (Peleg et al. 2009) as well as between biomass, grain yield and drought susceptibility index in hexaploid wheat on 4A (Kirigwi et al. 2007). Chromosome 3B is known to contain *Fhb1*, a major QTL for *Fusarium* head blight resistance from the Chinese wheat cultivar Sumai 3 (Cuthbert et al. 2006), but this region is located on the distal end of 3BS, approximately 50 CM from the QTL identified here. Peleg et al. 2009 identified a region ~15 CM proximal to the centromere on 3B to be associated with QTL for osmotic potential under drought conditions in

durum wheat, but not with total yield. Previous studies have also associated the centromeric region on 3B with thousand-kernel weight and quality parameters including mixing developmental time and energy to peak (Huang et al. 2006), yield and tiller number (Kumar et al. 2007), and yield, thousand kernel weight, kernel number per spike, and spike m^{-2} (Cuthbert et al. 2008). In the study by Cuthbert et al. 2008, the 3B QTL was associated with increases in yield, thousand kernel weight and kernel number per spike with fewer spike m^{-2}. This is in contrast to the data presented here, as the favorable Halberd allele is associated with increases in spikes m^{-2}, thousand-kernel weight, and yield. The benefits in yield and biomass associated with the Halberd allele at this locus in combination with the production of a cooler crop canopy under heat stress could make this a potentially beneficial target for marker-assisted-selection for stress tolerance by breeders.

Stable yield component QTL

QTkw.tam-5A was a stable QTL for thousand-kernel weight and was detected in all environments. In the combined analysis, *QTkw.tam-5A* explained 7.8% of the phenotypic variation for thousand-kernel weight and varied from 4.6% to 14.6% in the individual environments. A stable QTL for kernel diameter, *QKdi.tam-5A,* also co-localized in this region, and explained 10.2% of the phenotypic variation. This region has previously been associated with QTL for yield in at least five studies (Cuthbert et al. 2008; Groos et al. 2003; Marza et al. 2006; Quarrie et al. 2005; Wang et al. 2009) and is

often associated with thousand-kernel weight or other kernel and spike characters. The *QTkw.tam-5A/QKdi.tam-5A* region has not previously been associated with ear emergence or flowering-time, nor was it associated with those traits in this study. Other QTL influencing grain yield and yield components located on 5A included *VrnA1,* involved in vernalization and the timing of ear emergence (Galiba et al. 1995; Law et al. 1976) and the *B1* locus controlling the presence of awns (Kato et al. 2000). Both of these loci are located on the distal end of 5AL where as *QTkw.tam-5A/QKdi.tam-5A* is located proximal to the centromere on 5AS and are not the QTL identified here.

Co-localization with previously identified heat tolerance QTL

Problems arise under field conditions when trying to impose stress. Differences in flowering time can pose major problems assuring that each line receives equal and adequate levels of stress. Previous characterization of the Halberd x Karl 92 population for reproductive stage heat tolerance indentified 14 loci associated with heat susceptibility index of main spike yield components following a three day, 38°C heat stress treatment, including QTL on 2D, 3B, 4A, 5A, 5B, 6D, 7A, and 7D. Only one QTL in this study was associated with flowering time, as all lines were treated uniformly at 10 DAP. Co-localization between QTL identified in the greenhouse analysis of the H/K population and QTL detected under field conditions occurred at three genetic loci, located on chromosomes 3BS and opposite arms of 5A (Table 4.10). The general lack of detection of common QTL can be directly related to the effects of segregating flowering

time, which was present in this population and influenced nearly every yield component measured.

Table 4.10 Summary of QTL detected in the Halberd x Karl 92 mapping population (n=121) for yield and yield components in the field that show co-localization with heat susceptibility index from the greenhouse

Chromosome	Environments	Marker	Traits	Positive allele
3B	CS08-1, CS08-2, CS09	barc229	Yield, biomass, spike density, thousand kernel weight, Ctd, HSI	Halberd
3B	Greenhouse 2008	barc229	HSI of single kernel weight, flag leaf temperature depression	Halberd
5AS	CS08-1, CS08-2, CS09	barc186	Thousand kernel weight, kernel diameter	Halberd
5AS	Greenhouse	gwm443	HSI of single kernel weight	Karl 92
5AL	CS09-1	gwm291	Kernel weight per spike	Karl 92
5AL	Greenhouse	gwm291	HSI kernel weight per spike	Karl 92

[1]CS08-1, College Station, TX sown on December 1 2007, CS08-2, College Station, TX sown on December 30, 2007,CS09-1, College Station, TX sown on December 7, 2008

HSI, heat susceptibility index, CTD, canopy temperature depression

Despite the influence of flowering time, the QTL detected across studies for both 3B and 5AL were consistent for both the favorable parental allele and for the traits associated with these regions. The region on 5AL was a favorable allele for both kernel weight per spike in the field and HSI of kernel weight in the greenhouse. In the field, this QTL was only detected in CS09-1, which was the most stressed environment, in agreement with the detection of this locus for HSI in the greenhouse. The favorable allele at this locus was contributed by Karl 92, and is in close linkage to the *B1/awn* allele. This QTL was also detected in the Halberd x Cutter greenhouse study. These results support the

hypothesis that awns contribute some measure of heat tolerance in wheat and confirms previous reports of higher yields in awned wheat (Cuthbert et al. 2008).

The 3B region was associated with temperature depression, single kernel weight, and HSI under both control and field conditions. In the greenhouse, 3B was associated with HSI of single kernel weight, involved in maintaining a higher single kernel weight under heat stress, albeit a small effect, as it only explained 4.5% of the phenotypic variance. In the field, this region was associated with increased thousand-kernel weight, yield, biomass and spike density as well as HSI. It is plausible that the pleiotropic determinate at this locus may be increased biomass, which would influence yield by increasing tiller number and providing a larger source of photo-assimilates during grain-filling to both increase and maintain kernel weight under heat stress. As mentioned previously, co-localization of QTL for above ground biomass and yield in durum wheat under drought stress have been reported on 2A (Peleg et al. 2009) as well as between biomass, grain yield and drought susceptibility index in hexaploid wheat on 4A (Kirigwi et al. 2007). While not previously associated with abiotic stress or heat stress per se, the combined results presented here as well as previous reports of this region being associated with yield and yield components point to the importance of this locus for stress tolerance and in wheat.

Conclusions and the potential of marker-assisted selection for improved heat tolerance in wheat

Analysis of the Halberd x Karl 92 population under field conditions identified important and novel QTL involved in yield, thousand-kernel weight, biomass, stress adaptability and other beneficial phenotypic traits. These results, in combination with those presented in the previous two chapters of this dissertation represent a comprehensive analysis of both the phenotypic response of wheat to high temperature stress and a robust analysis of the genetic loci associated with and regulating reproductive stage heat tolerance and yield potential. In a comparison across two populations of recombinant inbred lines, seven common QTL regions were identified, with inheritance of the favorable allele stable at most loci (Table 3.6). Subsequent analysis of temperature depression of both the main spike and flag leaf in the Halberd x Karl 92 population identified seven loci that co-localized for both cooler organ temperature and improved heat tolerance. Analysis under abiotic stress conditions in the field confirmed chromosome regions for two of these seven loci to be involved in wheat yield potential in the field and/or cooler canopy temperatures. An allele contrast analysis of the markers associated with all fourteen QTL identified in the H/K greenhouse study showed very little linkage drag associated with the favorable allele for any of these heat tolerance QTL (Appendix). So while the effects of these loci may not be detectable given the variable environmental conditions in the field, selection for these loci could

still be feasible and important given their importance under controlled conditions and lack of any detectable drag on yield potential under field conditions.

The QTL detected on chromosome 1B in both the H/C and H/K greenhouse studies is an intriguing locus, with the potential to utilized for improving heat tolerance in wheat. *QHkwm.tam-1B* was involved in the maintenance of kernel weight under heat stress and showed appears to be combinable with other heat tolerance QTL, resulting in even higher levels of heat tolerance (Fig. 3.6 and Fig. 3.7). This QTL is significant, not only because of its genetic effect and its detection across populations, but also because of the determination of this region on 1B to be important for heat tolerance in independent studies at other institutions (Kuchel et al. 2007a, b; Yang et al. 2002c). I believe that this region is deserving of future characterization and that this QTL could potentially be a valid target for marker assisted selection for heat tolerance.

The region on chromosome 3B, associated with both heat tolerance and temperature depression in the greenhouse and yield, yield components, biomass, and canopy temperature depression in the field is also deserving of future analysis. In addition to the genetic importance of this locus, the recent completion of a physical map for and current sequencing or the 3B chromosome in wheat will provide the necessary genetic tools to make fine-mapping of this locus very feasible. The relative ease of measuring temperature depression in both the field and greenhouse could also provide a rapid assay

for analyzing a large segregating population, fine mapping of this locus, and the potential to map-based clone this locus.

In conclusion

It is my belief that the results presented in this dissertation provide the most thorough analysis of heat stress tolerance in wheat to date. The data, results, and conclusions presented herein will be valuable to both wheat breeders and geneticists and to the scientific community as a whole, as we continue the struggle of increasing crop yields, feeding the hungry, and adapting to the changing global landscape of the present day and in future generations.

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APPENDIX

Table A.1 Position of QTL detected in the Halberd x Cutter mapping population (n=64) for heat susceptibility index of yield components in the greenhouse, 2005

QTL	Marker	Position[a]	2LOD Left	1LOD Left	1LOD Right	2LOD Right
HSI_kernel number of main spike (2.91)						
QHknm.tam-1A	cfa2129	24.9	12.2	15.2	36.2	36.2
QHknm.tam-2B	barc200.2	46.9	40.1	42.3	53.0	55.0
QHknm.tam-3B	barc147	0.0	0.0	0.0	2.4	5.2
QHknm.tam-4A	wmc89	24.0	10.3	14.4	30.0	31.2
QHknm.tam-5B	gwm213	13.2	0.0	4.6	17.2	18.4
HSI_kernel weight of main spike (2.84)						
QHkwm.tam-1B	gwm268	39.9	18.8	27.1	52.5	57.0
QHkwm.tam-2B	gwm111.2	40.9	31.2	33.9	48.3	51.4
QHkwm.tam-3B	wmc527	89.8	77.8	81.6	95.4	99.8
QHkwm.tam-5A	gwm291	149.8	140.2	135.8	155.8	155.8
QHkwm.tam-6D	gwm325	64.4	54.7	57.7	72.9	75.9
HSI_single kernel weight of main spike (2.91)						
QHskm.tam-1A	cfa2129	38.2	29.6	35.4	41.6	44.4
QHskm.tam-2A	gwm356	127.0	133.2	140.8	144.2	144.2
QHskm.tam-2B	barc200.2	26.7	14.2	17.7	30.8	33.1
QHskm.tam-4A	barc170	2.5	0.0	0.0	6.0	13.8
QHskm.tam-5A	barc151	89.0	80.6	82.6	103.8	105.8
Days to flowering						
QDtf.tam-2D	wmc601	57.0	50.4	55.0	64.6	70.9
QDtf.tam-7D	wmc438	69.2	39.6	50.4	83.7	89.4

[a]QTL positions, 2LOD, and 1LOD intervals calculated with QTL cartographer v.2.0

*Putative QTL below 1000 permutation threshold

Table A.2 Position of QTL detected in the Halberd x Cutter mapping population (n=64) for heat susceptibility index of yield components in the greenhouse, 2006

QTL	Marker	Positiona	2LOD Left	1LOD Left	1LOD Right	2LOD Right
HSI_kernel number of main spike (2.50)						
QHknm.tam-2A	wmc407	0.0	0.0	0.0	3.8	7.7
QHknm.tam-2B	gwm111.2	36.9	24.7	29.9	46.8	52.4
QHknm.tam3B	barc147	8.8	0.0	2.2	11.3	16.1
QHknm.tam-5A.1	barc197	47.5	32.8	38.7	53.7	57.8
QHknm.tam-5A.2	gwm126	121.8	104.5	109.0	140.2	145.7
HSI_kernel weight of main spike (2.86)						
QHkwm.tam-3B	wmc326	123.6	117.4	120.2	127.4	129.1
HSI_single kernel weight of main spike (2.89)						
QHskm.tam-1A	cfa2129	38.2	34.9	34.9	41.8	44.8
QHskm.tam-2A	gwm294	109.4	92.7	100.6	111.4	112.6
QHskm.tam-7A	gwm282	138.4	119.3	126.1	140.4	140.4
HSI_grain filling duration (2.59)						
QHgfd.tam-1D	gwm337	46.5	33.4	37.6	58.2	70.0
QHgfd.tam-2A	wmc407	0.0	0.0	0.0	4.3	9.0
QHgfd.tam-6D	gwm325	35.3	22.3	23.5	47.5	54.8
Flag leaf length (2.85)						
QFll.tam-3B	barc147	8.8	3.6	5.8	52.2	11.2
QFll.tam-5B	wmc160	98.9	100.8	104.4	104.4	104.4
Flag leaf width (2.50)						
QFlw.tam-1D	wmc336	111.6	85.8	96.6	117.7	122.4
QFlw.tam-2B	barc200.2	24.7	17.5	20.8	27.6	29.9
QFlw.tam-7A	gwm60	10.0	0.0	0.0	19.4	21.6
Grain-filling duration (2.83)						
QGfd.tam-1B	barc137	32.1	11.9	19.0	47.8	57.6
QGfd.tam-2A	wmc407	10.0	2.6	4.5	14.5	18.1
QGfd.tam-2D	cfd43	51.1	39.0	43.1	53.1	53.1
Visual wax score (3.30)						
Qwax.tam-5A	wmc150	49.4	41.4	43.4	52.1	54.3
Days to flowering(3.00)		40.4	28.6	33.6	44.9	46.3
QDtf.tam-4B	gwm251	11.4	8.1	9.6	15.0	16.6
QDtf.tam-5B	wmc160	97.6	99.3	105.0	105.0	105.0

[a]QTL positions, 2LOD, and 1LOD intervals calculated with QTL cartographer v.2.0

*Putative QTL below 1000 permutation threshold

Table A.3 Position of QTL detected in the Halberd x Karl 92 mapping population (n=121) for heat susceptibility index of yield components and temperature depression in the greenhouse, 2008.

QTL	Marker	Position[a]	2LOD Left	1LOD Left	1LOD Right	2LOD Right
Kernel number of main spike						
Qhsiknms.tam-5B	gwm408	59.9	51.6	52.1	67.0	70.0
Kernel weight of main spike						
QHkwm.tam-1B	gwm153	39.4	34.6	36.4	42.9	43.4
Qhsikwms.tam-5A.1	gwm179	84.5	73.5	77.0	86.7	88.9
Qhsikwms.tam-5A.2	gwm291	102.8	102.0	102.0	105.9	106.8
Single kernel weight of ma						
Qhsiskw.tam-2D.1	gwm261	2.8	0.0	0.0	6.3	7.7
Qhsiskw.tam-2D.2	cfd56	26.1	23.2	24.4	29.5	31.8
Qhsiskw.tam-3B	barc229	45.4	32.8	37.3	50.0	53.1
Qhsiskw.tam-4A.1	wmc707	97.5	92.3	94.7	97.9	98.3
Qhsiskw.tam-4A.2	wmc313	115.3	113.7	113.7	118.9	121.8
Qhsiskw.tam-5A	gwm443	0.0	0.0	0.0	9.3	13.9
Qhsiskw.tam-5B	wmc73	6.2	4.3	5.2	8.0	9.8
Qhsiskw.tam-6D	cfd49	16.0	7.6	10.6	23.0	25.8
Qhsiskw.tam-7A	wmc603	38.7	36.7	36.7	40.2	41.8
Qhsiskw.tam-7B	wmc182	47.2	31.7	37.9	61.9	53.0
Single kernel weight of main spike						
Qhsiskw.tam-2A	wmc522	10.0	0.0	1.7	15.2	16.0
Qhsiskw.tam-2B	barc55	94.8	83.8	86.4	102.1	105.9
Qhsiskw.tam-5A	gwm443	0.0	0.0	0.0	5.1	11.6
Temperature depression of flag leaf day 1						
Qctdlf.tam-3B[c]	barc84	64.7	46.1	50.8	69.3	70.1
Qctdlf.tam-5A	gwm179	70.5	68.7	67.1	75.3	79.5
Temperature depression of flag leaf day 2						
Qctdlf.tam-3D	gwm191	10.8	0.0	1.1	28.2	36.6
Qctdlf.tam-5A	gwm154	20.4	5.3	8.4	26.2	28.4
Temperature depression of main spike day 1						
Qctdspk.tam-2D-1	gwm261	2.8	0.0	0.0	6.3	8.4
Qctdspk.tam-2D-3	cfd56	28.1	26.1	26.9	32.3	34.2
Qctdspk.tam-3A	gwm480	47.7	45.0	45.6	50.9	50.1
Qctdspk.tam-4A	gwm44	0.0	0.0	0.0	14.4	20.5
Qctdspk.tam-5A-1	gwm126	67.7	66.2	66.6	69.7	69.9
Qctdspk.tam-5A-2	gwm595	86.0	84.5	84.5	92.8	96.3
Qctdspk.tam-7A	wmc525	121.4	100.9	106.5	121.4	121.4
Temperature depression of main spike day 2						
Qctdspk.tam-2B	wmc25	39.5	29.3	34.3	52.1	57.3
Qctdspk.tam-2D-2	gwm296	12.2	5.8	8.1	20.1	20.3
Qctdspk.tam-3A	gwm2	44.1	25.0	31.5	46.1	46.1
Qctdspk.tam-3D	gwm191	14.8	4.4	8.6	38.0	48.2
Qctdspk.tam-6D	cfd42	37.2	27.8	30.0	45.3	50.1
Qctdspk.tam-7D	gwm121	95.2	91.9	81.4	114.2	115.3

Table A.3 Continued

QTL	Marker	Position[a]	2LOD Left	1LOD Left	1LOD Right	2LOD Right
Days to flowering						
Qdtf.tam-2D.1[c]	gwm484	34.9	28.3	29.6	36.7	36.9
Qdtf.tam-2D.2[c]	wmc41.1	63.7	56.4	57.9	65.8	67.3
Qdtf.tam-3A[c]	gwm369	16.4	2.6	7.2	23.7	28.7
Days to maturity						
Qdtm.tam-2D	cfd56	32.1	28.6	29.7	35.0	35.6
Flag leaf length (3.35)						
QFll.tam-1B	wmc156	31.0	21.9	25.7	34.5	36.5
QFll.tam-2A	gwm645	34.3	30.0	31.5	53.1	42.0
QFll.tam-2D.1	gwm484	32.9	30.9	32.1	34.3	35.6
QFll.tam-2D.2	barc228	69.6	63.7	69.1	71.5	73.4
QFll.tam-3D	wmc631	115.4	111.4	112.6	122.2	125.7
QFll.tam-6A	gwm427	84.7	72.2	75.5	98.2	107.8
QFll.tam-7D	wmc634	112.8	106.8	107.8	118.6	122.7
Flag leaf width (3.37)						
QFlw.tam-1D	barc148	110.7	92.2	99.1	120.7	124.8
QFlw.tam-2A.1	wmc522	12.0	4.1	7.3	14.2	15.3
QFlw.tam-2A.2	gwm294	39.6	35.5	37.6	43.8	45.9
QFlw.tam-2D.1	gwm484	32.9	31.3	32.0	34.5	36.1
QFlw.tam-2D.2	wmc41.2	59.6	57.1	57.9	62.8	66.2
QFlw.tam-3A	gwm369	42.4	22.6	29.8	45.2	46.1
QFlw.tam-6A	wmc417	80.8	71.7	74.4	82.8	82.8
QFlw.tam-6D	barc174	101.0	97.0	98.4	105.8	106.8

[a]QTL positions, 2LOD, and 1LOD intervals calculated with QTL cartographer v.2.0

Table A.4 Effects of QTL detected in the Halberd x Karl 92 mapping population (n=121) for yield, yield components and agronomic traits in early sowing date, College Station 2008

QTL (LOD threshold[a])	Marker	LOD	R^2	Additive[b]	Positive allele
Biomass (3.14)					
QBio.tam-2D.1	gwm484	3.34	0.084	598.8	Karl 92
QBio.tam-3B.2	barc164	4.10	0.114	717.7	Halberd
QBio.tam-7D	barc76	3.90	0.092	653.9	Karl 92
Days to heading (2.93)					
QHdg.tam-2D	gwm484	7.17	0.165	3.4	Halberd
QHdg.tam-3D	wmc631	3.22	0.092	2.5	Karl 92
QHdg.tam-5B	gwm408	5.24	0.137	3.1	Karl 92
QHdg.tam-5D	cfd8	3.57	0.083	2.3	Karl 92
Days to maturity (3.38)					
QMat.tam-1B	gwm413	3.70	0.068	2.1	Halberd
QMat.tam-1D	gwm136	4.26	0.064	2.1	Karl 92
QMat.tam-2D	gwm484	9.47	0.184	3.4	Halberd
QMat.tam-3D	wmc631	3.59	0.087	2.3	Karl 92
QMat.tam-4A	barc78	7.23	0.160	3.3	Halberd
QMat.tam-5B	gwm639.1	7.65	0.152	3.0	Karl 92
QMat.tam-7A	gwm276	5.82	0.090	2.5	Halberd
QMat.tam-7D	wmc14	4.29	0.070	2.3	Halberd
Harvest index (3.00)					
QHi.tam-2D	gwm484	3.33	0.119	0.021	Karl 92
QHi.tam-5B	gwm6391.1	3.57	0.110	0.021	Halberd
QHi.tam-7D.1	gwm130	3.97	0.093	0.019	Karl 92
Height (3.39)					
QHt.tam-2D	cfd56	6.34	0.206	6.1	Halberd
QHt.tam-4B	wmc89.1	5.13	0.136	5.0	Halberd
Kernel diameter (3.12)					
QKdi.tam-1D	gwm136	3.98	0.046	0.025	Halberd
QKdi.tam-2D.2	cfd56	16.09	0.276	0.060	Karl 92
QKdi.tam-3D	gwm3	5.11	0.099	0.038	Halberd
QKdi.tam-5B	gwm639.1	5.34	0.079	0.043	Halberd
QKdi.tam-5D.1	cfd8	5.14	0.113	0.039	Halberd
QKdi.tam-5D.2	gwm174	7.92	0.151	0.046	Halberd
QKdi.tam-6D.1	gwm325	5.02	0.072	0.039	Karl 92
QKdi.tam-6D.2	cfd76	10.08	0.268	0.065	Halberd
QKdi.tam-7D	barc184	5.07	0.057	0.028	Karl 92
Kernel hardness (3.15)					
QKha.tam-1B	barc8	8.53	0.110	3.5	Halberd
QKha.tam-2D.2	gwm484	3.93	0.052	2.1	Halberd
QKha.tam-3A	gwm480	3.49	0.063	2.2	Karl 92
QKha.tam-4A.2	wmc313	3.47	0.041	1.9	Halberd
QKha.tam-5B.1	gwm408	5.35	0.068	2.5	Karl 92
QKha.tam-5D	gwm292	4.81	0.057	2.2	Karl 92

Table A.4 Continued

QTL (LOD threshold[a])	Marker	LOD	R^2	Additive[b]	Positive allele
QKha.tam-7A.1	barc70	4.00	0.044	1.9	Karl 92
QKha.tam-7A.1	gwm276	3.37	0.037	1.8	Halberd
QKha.tam-7D	wmc14	8.01	0.100	2.9	Halberd
Kernel number per spike (3.07)					
QKns.tam-1D	gwm136	3.10	0.071	1.8	Halberd
QKns.tam-2D.1	gwm484	3.25	0.075	1.6	Karl 92
QKns.tam-4B	gwm251	3.96	0.082	1.7	Karl 92
QKns.tam-5D	gwm292	4.72	0.128	2.2	Halberd
QKns.tam-7A	gwm63	3.32	0.067	1.6	Karl 92
Kernel weight per spike (3.10)					
QKws.tam-2D	gwm484	5.09	0.148	0.076	Karl 92
QKws.tam-7A.2	gwm282	4.00	0.132	0.071	Karl 92
Single kernel weight (3.14)					
QSkw.tam-2D.1	gwm484	6.70	0.205	1.2	Karl 92
QSkw.tam-3D.2	gwm3	3.21	0.090	0.8	Halberd
Spike density (3.16)					
QSm2.tam-2A.1	gwm425	4.05	0.072	16.3	Karl 92
QSm2.tam-2D	barc228	4.57	0.080	18.0	Halberd
QSm2.tam-4A.1	gwm44	5.93	0.214	27.1	Karl 92
QSm2.tam-5A.1	gwm639.2	3.25	0.064	15.3	Karl 92
QSm2.tam-6B	gwm193	4.25	0.075	17.2	Halberd
Thousand kernel weight (3.04)					
QTkw.tam-2D.1	cfd56	6.00	0.164	1.4	Karl 92
QTkw.tam-5A	barc186	4.20	0.099	1.0	Halberd
Yield (3.01)					
QYld.tam-2B.1	gwm148	3.07	0.061	305.8	Halberd
QYld.tam-2B.2	barc91	3.31	0.089	338.7	Karl 92
QYld.tam-2D	gwm484	9.79	0.190	374.4	Karl 92
QYld.tam-3A	wmc11	4.37	0.092	261.6	Halberd
QYld.tam-4B	gwm251	3.38	0.056	201.3	Karl 92
QYld.tam-5D	cfd29	3.74	0.070	230.6	Halberd
QYld.tam-7A	gwm63	4.78	0.078	251.1	Karl 92
QYld.tam-7B	wmc182	4.46	0.119	294.5	Karl 92

[a]LOD thresholds were estimated in QTL Cartographer v2.0 using 1000 permutation

[b]Additive effect of allele substitution

Table A.5 Effects of QTL detected in the Halberd x Karl 92 mapping population (n=121) for yield, yield components and agronomic traits in late sowing date, College Station 2007-2008

QTL (LOD threshold[a])	Marker	LOD	R^2	Additive[b]	Positive allele
Biomass (3.08)					
QBio.tam-2A	wmc407	3.17	0.060	530.0	Halberd
QBio.tam-2B	wmc25	5.74	0.090	764.4	Karl 92
QBio.tam-2D.2	wmc144	5.94	0.134	816.1	Karl 92
QBio.tam-3B.1	wmc418	4.05	0.059	625.7	Karl 92
QBio.tam-3B.2	barc229	5.60	0.092	811.7	Halberd
QBio.tam-3B.3	gwm340	5.31	0.090	699.7	Halberd
QBio.tam-3D	gwm52	3.41	0.059	541.9	Halberd
QBio.tam-5B	gwm133.1	5.07	0.076	656.2	Karl 92
QBio.tam-6D	barc174	4.61	0.070	607.8	Karl 92
QBio.tam-7A	barc108	3.68	0.055	577.0	Karl 92
QBio.tam-7D	wmc14	3.56	0.060	621.1	Halberd
Canopy temperature depression - heat (3.00)					
QCtd.tam-3B	barc229	3.94	0.150	0.39	Karl 92
Canopy temperature depression - recovery (3.00)					
QCtd.tam-1D.1	barc62	4.00	0.095	0.25	Karl 92
QCtd.tam-1D.2	wmc339	4.15	0.250	0.35	Halberd
QCtd.tam-2D	cfd56	4.88	0.147	0.28	Halberd
QCtd.tam-7A	wmc525	6.11	0.200	0.32	Halberd
Days to heading (3.00)					
QHdg.tam-7A	wmc525	3.78	0.110	3.1	Halberd
QHdg.tam-7D	wmc14	4.02	0.100	2.8	Halberd
Days to maturity (3.00)					
QMat.tam-2D	gwm484	5.81	0.132	3.0	Halberd
QMat.tam-7A	gwm635.1	3.08	0.087	2.4	Halberd
QMat.tam-7D	wmc14	4.16	0.090	2.4	Halberd
Grain-filling duration (3.05)					
Qgfd.tam-1B.1	barc137	3.46	0.091	1.5	Halberd
Qgfd.tam-5A.2	gwm595	3.71	0.093	1.5	Karl 92
Qgfd.tam-5B.1	gwm639.1	3.43	0.086	1.5	Karl 92
Harvest index (3.00)					
QHi.tam-1B	barc8	4.63	0.070	0.024	Karl 92
QHi.tam-2D	gwm484	7.40	0.175	0.037	Karl 92
QHi.tam-6A	wmc179	10.79	0.317	0.049	Karl 92
QHi.tam-7D.1	gwm130	6.14	0.095	0.029	Karl 92
QHi.tam-7D.2	wmc14	6.97	0.110	0.030	Halberd
Height (3.48)					
QHt.tam-2D	cfd56	6.57	0.204	6.2	Halberd
QHt.tam-4B	wmc89.1	4.84	0.122	4.7	Halberd
QHt.tam-5B	gwm639.1	3.39	0.109	4.5	Karl 92
Kernel diameter (3.03)					
QKdi.tam-2B	gwm148	3.52	0.077	0.039	Halberd
QKdi.tam-2D.2	gwm484	6.90	0.231	0.064	Karl 92

Table A.5 Continued

QTL (LOD threshold[a])	Marker	LOD	R^2	Additive[b]	Positive allele
QKdi.tam-4B	wmc89.1	3.17	0.065	0.035	Halberd
Kernel hardness (3.11)					
QKha.tam-2D.2	cfd56	4.20	0.128	3.1	Halberd
QKha.tam-7D	wmc14	3.28	0.080	2.4	Halberd
Kernel number per spike (3.10)					
QKns.tam-2D.1	gwm484	14.02	0.226	5.3	Karl 92
QKns.tam-3B	barc229	7.46	0.105	4.2	Halberd
QKns.tam-4B	gwm495	6.78	0.084	3.5	Karl 92
QKns.tam-5B.1	gwm133.1	4.25	0.049	2.6	Karl 92
QKns.tam-5B.2	gwm408	3.73	0.044	2.5	Halberd
QKns.tam-6A	gwm570	3.02	0.037	2.2	Karl 92
QKns.tam-7A	gwm282	3.56	0.043	2.6	Karl 92
QKns.tam-7D	wmc14	4.10	0.050	2.7	Karl 92
Kernel weight per spike (3.10)					
QKws.tam-2D	gwm484	9.47	0.325	0.215	Karl 92
QKws.tam-5A.1	barc186	4.97	0.101	0.136	Halberd
QKws.tam-5B.1	gwm133.1	3.54	0.067	0.102	Karl 92
QKws.tam-7A.1	barc108	4.11	0.083	0.113	Karl 92
QKws.tam-7A.3	wmc525	4.05	0.110	0.120	Halberd
Single kernel weight (3.35)					
QSkw.tam-1D	gwm136	7.04	0.115	1.2	Halberd
QSkw.tam-2D.1	cfd56	13.36	0.253	2.0	Karl 92
QSkw.tam-2D.2	wmc41.1	6.21	0.078	1.1	Halberd
QSkw.tam-3B	barc77	3.79	0.049	0.8	Halberd
QSkw.tam-3D.1	gwm191	9.38	0.240	1.9	Halberd
QSkw.tam-5A	barc186	3.77	0.052	1.0	Halberd
QSkw.tam-5B	barc142	5.39	0.127	1.3	Karl 92
QSkw.tam-6A	barc146	8.18	0.110	1.3	Halberd
QSkw.tam-6D	cfd76	6.24	0.250	1.9	Halberd
QSkw.tam-7D.1	cfd31	8.74	0.116	1.3	Karl 92
QSkw.tam-7D.2	gwm111.1	5.35	0.076	1.0	Karl 92
Thousand kernel weight (3.15)					
QTkw.tam-2D.1	gwm484	14.27	0.332	2.5	Karl 92
QTkw.tam-2D.2	barc228	4.12	0.066	1.1	Halberd
QTkw.tam-3D	gwm191	3.37	0.253	2.1	Halberd
QTkw.tam-5A	gwm304	2.7	0.046	0.9	Halberd
QTkw.tam-6A	barc146	4.84	0.090	1.2	Halberd
QTkw.tam-6D	cfd76	4.93	0.320	2.5	Halberd
QTkw.tam-7D	gdm67	4.53	0.116	1.4	Karl 92
Yield (3.02)					
QYld.tam-2D	gwm484	5.58	0.208	514.7	Karl 92
QYld.tam-5B	gwm133.1	3.52	0.098	362.8	Karl 92

[a]LOD thresholds were estimated in QTL Cartographer v2.0 using 1000 permutation

[b]Additive effect of allele substitution

Table A.6 Effects of QTL detected in the Halberd x Karl 92 mapping population (n=121) for yield, yield components and agronomic traits in early sowing date, College Station 2008-2009

QTL (LOD threshold[a])	Marker	LOD	R^2	Additive[b]	Positive allele
Canopy temperature depression - heat (3.18)					
QCtd.tam-1B	gwm153	4.14	0.052	0.28	Karl 92
QCtd.tam-3A.1	gwm369	7.29	0.129	0.42	Halberd
QCtd.tam-3A.2	wmc169	6.12	0.083	0.34	Karl 92
QCtd.tam-3B	barc229	3.83	0.055	0.33	Halberd
QCtd.tam-4A	barc78	4.15	0.072	0.33	Karl 92
QCtd.tam-6A	wmc179	4.77	0.085	0.34	Karl 92
Days to heading (3.00)					
QHdg.tam-2D	gwm484	7.92	0.167	5.3	Halberd
QHdg.tam-5A	gwm205	3.00	0.053	3.1	Karl 92
QHdg.tam-7A	wmc525	6.15	0.150	4.6	Halberd
Days to maturity (5.94)					
QMat.tam-2D	gwm484	7.60	0.357	4.5	Halberd
Grain-filling duration (3.06)					
Qgfd.tam-1B.2	gwm268	3.95	0.056	2.4	Halberd
Qgfd.tam-2D.1	gwm296	4.58	0.090	2.9	Karl 92
Qgfd.tam-2D.2	cfd56	4.29	0.079	2.7	Karl 92
Qgfd.tam-3A	gwm5	5.62	0.140	3.6	Halberd
Qgfd.tam-4B	gwm495	5.18	0.077	2.9	Karl 92
Qgfd.tam-5A.1	wmc713	5.64	0.106	3.3	Halberd
Qgfd.tam-5B.2	gwm408	3.30	0.051	2.3	Halberd
Qgfd.tam-6B	gwm508.1	5.76	0.087	2.9	Karl 92
Qgfd.tam-7A	wmc525	9.08	0.200	4.2	Halberd
Height (3.37)					
QHt.tam-2D	gwm484	6.23	0.240	6.1	Halberd
QHt.tam-4B	wmc89.1	4.90	0.128	4.8	Halberd
QHt.tam-5B	gwm639.1	3.43	0.130	4.9	Karl 92
Kernel diameter (3.11)					
QKdi.tam-2D.1	wmc503	3.18	0.060	0.028	Karl 92
QKdi.tam-3A	gwm369	3.96	0.114	0.036	Halberd
QKdi.tam-5A.1	barc186	7.99	0.170	0.050	Halberd
QKdi.tam-5A.2	gwm156	3.08	0.057	0.031	Karl 92
Kernel hardness (3.09)					
QKha.tam-2D.1	gwm296	4.60	0.094	2.5	Halberd
QKha.tam-4A.1	gwm44	5.82	0.242	3.9	Halberd
QKha.tam-5B.1	gwm639.1	8.77	0.203	4.5	Karl 92
QKha.tam-5B.2	gwm604	4.64	0.082	3.1	Halberd
QKha.tam-7A.1	barc70	3.16	0.051	1.9	Karl 92
Kernel number per spike (3.19)					
QKns.tam-1B	gwm153	5.22	0.092	2.1	Halberd
QKns.tam-2B	barc55	4.62	0.088	2.1	Halberd

Table A.6 Continued

QTL (LOD threshold[a])	Marker	LOD	R^2	Additive[b]	Positive allele
QKns.tam-2D.1	gwm484	9.63	0.215	3.1	Halberd
QKns.tam-2D.2	wmc41.1	3.52	0.065	1.8	Karl 92
QKns.tam-3D.1	wmc631	3.62	0.095	2.1	Karl 92
QKns.tam-5A	gwm304	3.37	0.070	1.8	Karl 92
QKns.tam-5B.2	gwm408	5.68	0.147	2.6	Karl 92
Kernel weight per spike (3.19)					
QKws.tam-2B	barc91	3.23	0.057	0.054	Halberd
QKws.tam-2D	gwm484	6.89	0.204	0.096	Halberd
QKws.tam-5A.2	gwm291	5.12	0.100	0.070	Halberd
QKws.tam-5B.2	gwm408	4.91	0.116	0.072	Karl 92
QKws.tam-6B	gwm508.1	3.28	0.060	0.053	Karl 92
Single kernel weight (3.04)					
QSkw.tam-5A	barc186	3.70	0.085	0.9	Halberd
QSkw.tam-6B	gwm508.1	4.33	0.099	1.0	Karl 92
Spike density (3.04)					
QSm2.tam-1A	wmc93	4.79	0.081	25.1	Halberd
QSm2.tam-1D	cfd15	4.32	0.066	21.7	Halberd
QSm2.tam-2A.2	gwm294	4.37	0.105	26.1	Karl 92
QSm2.tam-3B	barc229	7.22	0.127	29.5	Halberd
QSm2.tam-4A.2	barc78	8.13	0.200	38.2	Karl 92
QSm2.tam-5A.2	gwm156	4.61	0.071	23.4	Karl 92
QSm2.tam-5B.1	gwm639.1	6.22	0.116	27.9	Halberd
QSm2.tam-5B.2	barc142	3.94	0.109	26.1	Karl 92
QSm2.tam-5D	cfd29	3.47	0.070	20.2	Halberd
QSm2.tam-7B	wmc182	3.10	0.067	20.6	Karl 92
QSm2.tam-7D	wmc634	7.34	0.142	41.3	Karl 92
Test weight (2.93)					
QTwgt.tam-1A	gwm135	4.92	0.118	0.6	Karl 92
QTwgt.tam-5A	gwm179	3.23	0.082	0.5	Karl 92
Thousand kernel weight (3.11)					
QTkw.tam-3B	barc164	3.50	0.089	1.0	Halberd
QTkw.tam-5A	barc186	7.25	0.146	1.5	Halberd
Yield (3.13)					
QYld.tam-1D	cfd15	3.31	0.058	197.4	Halberd
QYld.tam-3B	barc164	4.94	0.107	266.3	Halberd
QYld.tam-4A	barc78	7.50	0.161	346.6	Karl 92
QYld.tam-5A	gwm156	6.04	0.113	299.1	Karl 92
QYld.tam-5D	cfd29	3.47	0.080	214.4	Halberd
QYld.tam-7D	barc76	5.75	0.110	271.3	Karl 92

[a]LOD thresholds were estimated in QTL Cartographer v2.0 using 1000 permutation

[b]Additive effect of allele substitution

Table A.7 Position of QTL detected in the Halberd x Karl 92 mapping population (n=121) for yield, yield components and agronomic traits in early sowing date, College Station 2008

QTL	Marker	Position[a]	2LOD Left	1LOD Left	1LOD Right	2LOD Right
Biomass						
QBio.tam-2D.1	gwm484	32.9	29.2	30.8	36.6	35.5
QBio.tam-3B.2	barc164	44.0	33.6	37.1	51.0	54.0
QBio.tam-7D	barc76	116.1	98.9	109.5	123.9	126.1
Days to heading (2.93)						
QHdg.tam-2D	gwm484	32.9	30.9	31.8	35.2	35.8
QHdg.tam-3D	wmc631	129.4	115.5	120.1	131.4	131.4
QHdg.tam-5B	gwm408	53.9	45.9	48.3	58.4	60.6
QHdg.tam-5D	cfd8	29.0	13.2	17.3	38.9	39.9
Days to maturity (3.38)						
QMat.tam-1B	gwm413	1.6	0.0	0.0	7.3	9.6
QMat.tam-1D	gwm136	0.0	0.0	0.0	9.2	12.0
QMat.tam-2D	gwm484	32.9	31.4	32.0	36.1	35.0
QMat.tam-3D	wmc631	123.4	111.2	113.6	129.4	129.4
QMat.tam-4A	barc78	99.7	88.9	91.3	103.8	106.4
QMat.tam-5B	gwm639.1	49.3	41.3	43.6	51.8	55.1
QMat.tam-7A	gwm276	42.1	39.6	40.5	62.4	45.3
QMat.tam-7D	wmc14	128.1	117.4	120.9	128.0	128.0
Harvest index (3.00)						
QHi.tam-2D	gwm484	34.9	31.8	29.6	37.8	36.9
QHi.tam-5B	gwm639.1	49.3	40.9	43.8	59.8	62.5
QHi.tam-7D.1	gwm130	0.0	0.0	0.0	7.4	11.6
Height (3.39)						
QHt.tam-2D	cfd56	32.1	28.7	29.7	35.1	35.7
QHt.tam-4B	wmc89.1	0.0	0.0	0.0	6.3	8.1
Kernel diameter (3.12)						
QKdi.tam-1D	gwm136	0.0	0.0	0.0	6.2	10.3
QKdi.tam-2D.2	cfd56	32.1	30.1	30.1	33.3	33.8
QKdi.tam-3D	gwm3	105.1	95.4	98.9	111.1	111.4
QKdi.tam-5B	gwm639.1	51.3	46.2	48.3	54.9	58.4
QKdi.tam-5D.1	cfd8	27.0	19.1	22.0	30.9	30.9
QKdi.tam-5D.2	gwm174	43.9	40.8	34.9	49.2	51.4
QKdi.tam-6D.1	gwm325	93.2	89.7	91.2	94.0	94.7
QKdi.tam-6D.2	cfd76	120.8	108.4	111.1	130.3	135.7
QKdi.tam-7D	barc184	66.8	53.5	57.6	67.0	67.3
Kernel hardness (3.15)						
QKha.tam-1B	barc8	0.0	0.0	0.0	3.4	6.8
QKha.tam-2D.2	gwm484	32.9	29.1	30.6	35.6	36.6
QKha.tam-3A	gwm480	49.7	45.6	47.1	62.3	52.1
QKha.tam-4A.2	wmc313	115.3	97.6	100.1	123.3	123.3
QKha.tam-5B.1	gwm408	51.9	43.2	45.8	57.3	60.7

Table A.7 Continued

QTL	Marker	Position[a]	2LOD Left	1LOD Left	1LOD Right	2LOD Right
QKha.tam-5D	gwm292	63.7	63.5	62.3	71.7	76.4
QKha.tam-7A.1	barc70	0.0	0.0	0.0	6.6	13.1
QKha.tam-7A.1	gwm276	42.1	39.8	39.5	45.0	47.3
QKha.tam-7D	wmc14	128.0	121.5	123.8	128.0	128.0
Kernel number per spike (3.07)						
QKns.tam-1D	gwm136	0.0	0.0	0.0	9.9	12.0
QKns.tam-2D.1	gwm484	32.9	30.0	31.4	35.4	36.5
QKns.tam-4B	gwm251	9.9	3.4	6.1	11.9	11.9
QKns.tam-5D	gwm292	77.7	64.1	68.5	79.7	79.7
QKns.tam-7A	gwm63	88.2	75.8	80.5	101.2	107.2
Kernel weight per spike (3.10)						
QKws.tam-2D	gwm484	32.9	30.4	31.5	37.4	35.4
QKws.tam-7A.2	gwm282	83.9	76.0	78.6	93.6	100.3
Single kernel weight (3.14)						
QSkw.tam-2D.1	gwm484	32.9	29.8	31.0	35.2	37.6
QSkw.tam-3D.2	gwm3	111.1	92.7	98.3	118.8	123.8
Spike density (3.16)						
QSm2.tam-2A.1	gwm425	13.1	4.0	7.8	15.8	16.5
QSm2.tam-2D	barc228	67.6	63.7	66.5	69.6	69.6
QSm2.tam-4A.1	gwm44	8.0	0.0	0.0	20.9	26.5
QSm2.tam-5A.1	gwm639.2	46.2	44.9	44.9	48.8	50.2
QSm2.tam-6B	gwm193	20.9	11.1	15.9	25.2	25.2
Thousand kernel weight (3.04)						
QTkw.tam-2D.1	cfd56	32.1	30.0	30.1	33.3	34.3
QTkw.tam-5A	barc186	37.0	23.1	35.1	41.1	39.6
Yield (3.01)						
QYld.tam-2B.1	gwm148	77.5	58.1	66.9	79.7	81.3
QYld.tam-2B.2	barc91	97.4	94.9	95.2	104.3	109.0
QYld.tam-2D	gwm484	32.9	30.4	31.4	34.8	36.6
QYld.tam-3A	wmc11	14.0	0.0	4.1	22.9	28.6
QYld.tam-4B	gwm251	9.9	3.4	4.2	11.4	11.9
QYld.tam-5D	cfd29	79.7	65.4	71.2	79.7	79.7
QYld.tam-7A	gwm63	88.2	81.4	84.4	98.8	104.3
QYld.tam-7B	wmc182	41.2	28.2	32.9	48.3	61.9

[a]QTL positions, 2LOD, and 1LOD intervals calculated with QTL cartographer v.2.0

Table A.8 Position of QTL detected in the Halberd x Karl 92 mapping population (n=121) for yield, yield components and agronomic traits in late sowing date, College Station 2008

QTL	Marker	Position[a]	2LOD Left	1LOD Left	1LOD Right	2LOD Right
Biomass (3.08)						
QBio.tam-2A	wmc407	90.6	70.5	77.0	90.3	90.3
QBio.tam-2B	wmc25	39.5	25.4	29.1	45.4	51.0
QBio.tam-2D.2	wmc144	56.1	52.5	50.2	62.7	69.3
QBio.tam-3B.1	wmc418	18.1	7.3	10.5	21.9	24.8
QBio.tam-3B.2	barc229	45.4	44.0	44.0	49.2	51.2
QBio.tam-3B.3	gwm340	99.2	89.8	95.1	99.2	99.2
QBio.tam-3D	gwm52	8.0	0.0	0.0	32.0	37.8
QBio.tam-5B	gwm133.1	0.0	0.0	0.0	2.0	2.0
QBio.tam-6D	barc174	95.0	90.0	92.0	97.4	99.1
QBio.tam-7A	barc108	31.2	23.3	26.6	39.6	40.8
QBio.tam-7D	wmc14	128.1	118.9	122.0	128.1	128.1
Canopy temperature depression - heat (3.00)						
QCtd.tam-3B	barc229	45.4	42.6	44.0	49.8	52.7
Canopy temperature depression - recovery (3.00)						
QCtd.tam-1D.1	barc62	61.7	55.4	57.2	67.8	69.9
QCtd.tam-1D.2	wmc339	135.8	119.8	123.7	135.8	135.8
QCtd.tam-2D	cfd56	32.1	28.2	29.6	34.4	35.4
QCtd.tam-7A	wmc525	122.1	111.0	115.5	122.1	122.1
Days to heading (3.00)						
QHdg.tam-7A	wmc525	122.3	112.9	117.2	122.3	122.3
QHdg.tam-7D	wmc14	128.1	117.7	121.8	128.1	128.1
Days to maturity (3.00)						
QMat.tam-2D	gwm484	32.9	29.3	30.5	35.6	34.9
QMat.tam-7A	gwm635.1	29.1	19.0	22.2	39.6	41.1
QMat.tam-7D	wmc14	128.2	119.0	122.6	128.2	128.2
Grain-filling duration (3.05)						
Qgfd.tam-1B.1	barc137	10.9	4.3	7.0	13.3	14.9
Qgfd.tam-5A.2	gwm595	86.0	76.1	80.0	95.6	99.9
Qgfd.tam-5B.1	gwm639.1	37.3	28.2	36.2	40.5	43.2
Harvest index (3.00)						
QHi.tam-1B	barc8	0.0	0.0	0.0	7.3	9.7
QHi.tam-2D	gwm484	34.9	31.0	32.9	37.3	36.9
QHi.tam-6A	wmc179	52.4	39.4	42.9	59.2	61.8
QHi.tam-7D.1	gwm130	0.0	0.0	0.0	7.0	10.6
QHi.tam-7D.2	wmc14	128.2	119.9	122.6	128.2	128.2
Height (3.48)						
QHt.tam-2D	cfd56	32.1	28.9	29.9	35.4	36.1
QHt.tam-4B	wmc89.1	0.0	0.0	0.0	5.9	7.7
QHt.tam-5B	gwm639.1	49.3	39.2	42.3	57.9	61.9

Table A.8 Continued

QTL	Marker	Position[a]	2LOD Left	1LOD Left	1LOD Right	2LOD Right
Kernel diameter (3.03)						
QKdi.tam-2B	gwm148	77.5	58.0	62.9	84.4	92.2
QKdi.tam-2D.2	gwm484	34.9	31.5	32.9	36.9	36.9
QKdi.tam-4B	wmc89.1	0.0	0.0	0.0	2.0	3.4
Kernel hardness (3.11)						
QKha.tam-2D.2	cfd56	32.1	27.9	29.2	35.2	36.1
QKha.tam-7D	wmc14	128.3	117.2	122.0	128.1	128.1
Kernel number per spike (3.10)						
QKns.tam-2D.1	gwm484	32.9	31.5	32.1	35.2	37.4
QKns.tam-3B	barc229	45.4	44.0	44.0	55.9	48.6
QKns.tam-4B	gwm495	5.7	2.6	4.2	8.1	10.6
QKns.tam-5B.1	gwm133.1	0.0	0.0	0.0	4.2	6.2
QKns.tam-5B.2	gwm408	51.9	46.7	49.2	57.3	61.1
QKns.tam-6A	gwm570	13.7	1.5	6.9	20.4	20.4
QKns.tam-7A	gwm282	87.9	76.6	81.1	98.6	91.8
QKns.tam-7D	wmc14	128.1	119.3	122.7	128.1	128.1
Kernel weight per spike (3.10)						
QKws.tam-2D	gwm484	34.9	32.9	32.9	36.9	36.9
QKws.tam-5A.1	barc186	37.0	30.7	35.1	40.1	39.1
QKws.tam-5B.1	gwm133.1	0.0	0.0	0.0	6.2	6.2
QKws.tam-7A.1	barc108	31.1	21.3	23.9	39.3	41.0
QKws.tam-7A.3	wmc525	122.0	110.3	115.2	122.0	122.0
Single kernel weight (3.35)						
QSkw.tam-1D	gwm136	2.0	0.0	0.0	7.7	10.0
QSkw.tam-2D.1	cfd56	32.1	29.5	30.1	36.3	34.9
QSkw.tam-2D.2	wmc41.1	63.7	60.6	63.2	67.9	69.1
QSkw.tam-3B	barc77	70.3	61.4	65.0	74.9	77.8
QSkw.tam-3D.1	gwm191	42.8	31.3	35.2	50.8	53.7
QSkw.tam-5A	barc186	37.0	33.6	35.1	40.0	41.0
QSkw.tam-5B	barc142	106.7	95.5	98.7	110.7	110.7
QSkw.tam-6A	barc146	0.0	0.0	0.0	4.7	8.2
QSkw.tam-6D	cfd76	132.8	122.0	127.8	142.6	146.8
QSkw.tam-7D.1	cfd31	17.3	6.8	11.0	19.3	20.5
QSkw.tam-7D.2	gwm111.1	66.5	64.2	66.0	66.9	67.3
Thousand kernel weight (3.15)						
QTkw.tam-2D.1	gwm484	32.9	30.8	31.7	36.0	35.0
QTkw.tam-2D.2	barc228	67.6	62.0	65.0	69.1	70.6
QTkw.tam-3D	gwm191	34.8	19.9	26.4	66.3	77.6
QTkw.tam-5A	gwm304	19.8	31.2	40.1	41	19.8
QTkw.tam-6A	barc146	2.0	0.0	0.0	11.3	12.4
QTkw.tam-6D	cfd76	128.8	121.1	122.2	139.1	143.6
QTkw.tam-7D	gdm67	74.0	71.3	72.0	75.0	75.0
Yield (3.02)						

Table A.8 Continued

QTL	Marker	Position[a]	2LOD Left	1LOD Left	1LOD Right	2LOD Right
QYld.tam-2D	gwm484	34.9	31.1	32.9	37.3	36.9
QYld.tam-5B	gwm133.1	2.0	0.0	0.0	5.7	6.2

[a]QTL positions, 2LOD, and 1LOD intervals calculated with QTL cartographer v.2.0

Table A.9 Position of QTL detected in the Halberd x Karl 92 mapping population (n=121) for yield, yield components and agronomic traits in early sowing date, College Station 2009

QTL	Marker	Position[a]	2LOD Left	1LOD Left	1LOD Right	2LOD Right
Canopy temperature depression - heat (3.18)						
QCtd.tam-1B	gwm153	39.4	36.4	37.9	42.6	43.4
QCtd.tam-3A.1	gwm369	42.4	31.3	34.9	44.6	45.1
QCtd.tam-3A.2	wmc169	71.3	70.8	70.8	71.9	72.4
QCtd.tam-3B	barc229	45.4	43.9	44.0	49.4	52.6
QCtd.tam-4A	barc78	113.7	101.0	106.0	119.8	123.3
QCtd.tam-6A	wmc179	64.4	46.0	53.4	73.9	78.1
Days to heading (3.00)						
QHdg.tam-2D	gwm484	32.9	30.9	31.8	35.0	35.5
QHdg.tam-5A	gwm205	14.5	2.2	8.4	23.6	27.6
QHdg.tam-7A	wmc525	122.0	111.8	115.9	122.0	122.0
Days to maturity (5.94)						
QMat.tam-2D	gwm484	34.9	32.9	32.9	36.4	38.0
Grain-filling duration (3.06)						
Qgfd.tam-1B.2	gwm268	44.9	35.3	40.0	49.5	51.6
Qgfd.tam-2D.1	gwm296	14.2	10.2	10.2	20.0	20.2
Qgfd.tam-2D.2	cfd56	32.1	28.2	29.6	34.0	35.1
Qgfd.tam-3A	gwm5	56.8	52.8	52.8	61.0	63.2
Qgfd.tam-4B	gwm495	5.7	0.9	2.4	8.7	9.9
Qgfd.tam-5A.1	wmc713	12.0	5.5	6.3	16.5	18.4
Qgfd.tam-5B.2	gwm408	51.9	43.9	46.9	59.7	61.9
Qgfd.tam-6B	gwm508.1	9.6	2.1	5.5	12.9	11.6
Qgfd.tam-7A	wmc525	122.0	115.3	118.6	122.0	122.0
Height (3.37)						
QHt.tam-2D	gwm484	32.2	28.9	29.7	35.6	36.4
QHt.tam-4B	wmc89.1	0.0	0.0	0.0	7.0	8.1
QHt.tam-5B	gwm639.1	47.3	39.3	42.3	57.8	61.9
Kernel diameter (3.11)						
QKdi.tam-2D.1	wmc503	0.0	0.0	0.0	8.7	8.8
QKdi.tam-3A	gwm369	20.4	2.0	7.0	31.0	35.4
QKdi.tam-5A.1	barc186	37.0	35.1	35.1	41.0	39.4
QKdi.tam-5A.2	gwm156	64.6	59.2	61.4	82.5	67.5
Kernel hardness (3.09)						
QKha.tam-2D.1	gwm296	14.2	4.1	6.5	20.2	20.3
QKha.tam-4A.1	gwm44	10.0	0.0	0.0	20.5	25.4
QKha.tam-5B.1	gwm639.1	51.3	47.5	49.0	54.0	56.8
QKha.tam-5B.2	gwm604	63.9	63.0	63.5	66.3	67.5
QKha.tam-7A.1	barc70	0.0	0.0	0.0	4.8	8.0
Kernel number per spike (3.19)						
QKns.tam-1B	gwm153	39.4	35.5	37.0	42.0	43.4
QKns.tam-2B	barc55	94.8	85.8	88.8	104.5	107.5
QKns.tam-2D.1	gwm484	32.9	31.4	32.1	35.7	38.5

Table A.9 Continued

QTL	Marker	Position[a]	2LOD Left	1LOD Left	1LOD Right	2LOD Right
QKns.tam-2D.2	wmc41.1	65.7	57.5	61.7	68.5	69.5
QKns.tam-3D.1	wmc631	125.4	111.8	116.4	129.4	129.4
QKns.tam-5A	gwm304	32.3	20.7	24.0	39.8	41.4
QKns.tam-5B.2	gwm408	55.9	45.2	48.0	59.8	61.9
Kernel weight per spike (3.19)						
QKws.tam-2B	barc91	95.4	83.4	87.9	104.1	108.7
QKws.tam-2D	gwm484	34.9	32.1	32.7	36.0	37.1
QKws.tam-5A.2	gwm291	108.9	102.9	105.5	108.9	108.9
QKws.tam-5B.2	gwm408	57.9	51.8	53.1	63.9	65.6
QKws.tam-6B	gwm508.1	11.6	5.3	10.6	19.5	19.8
Single kernel weight (3.04)						
QSkw.tam-5A	barc186	37.0	24.5	33.3	40.2	41.0
QSkw.tam-6B	gwm508.1	9.6	5.3	8.7	12.9	17.9
Spike density (3.04)						
QSm2.tam-1A	wmc93	33.3	26.2	28.6	35.3	35.3
QSm2.tam-1D	cfd15	14.0	4.9	8.7	22.4	26.4
QSm2.tam-2A.2	gwm294	53.6	41.6	46.1	64.4	69.9
QSm2.tam-3B	barc229	45.4	39.4	42.1	48.5	50.7
QSm2.tam-4A.2	barc78	101.7	92.5	94.9	106.4	109.1
QSm2.tam-5A.2	gwm156	64.6	63.0	63.6	66.7	68.9
QSm2.tam-5B.1	gwm639.1	51.3	43.7	45.8	56.0	58.5
QSm2.tam-5B.2	barc142	106.7	91.5	96.7	110.7	110.7
QSm2.tam-5D	cfd29	79.5	66.9	72.2	79.5	79.5
QSm2.tam-7B	wmc182	33.2	16.6	22.0	45.7	47.9
QSm2.tam-7D	wmc634	114.8	107.8	109.8	117.2	119.6
Test weight (2.93)						
QTwgt.tam-1A	gwm135	24.1	23.0	23.5	28.2	30.1
QTwgt.tam-5A	gwm179	72.5	66.8	67.6	81.9	84.9
Thousand kernel weight (3.11)						
QTkw.tam-3B	barc164	34.0	11.5	23.0	42.0	44.5
QTkw.tam-5A	barc186	37.0	35.1	35.1	39.7	40.5
Yield (3.13)						
QYld.tam-1D	cfd15	14.0	2.6	7.0	24.4	29.2
QYld.tam-3B	barc164	44.0	33.2	36.7	49.7	52.0
QYld.tam-4A	barc78	97.7	92.7	95.0	105.1	110.0
QYld.tam-5A	gwm156	64.6	63.3	63.6	65.3	65.9
QYld.tam-5D	cfd29	79.6	66.7	71.5	79.6	79.6
QYld.tam-7D	barc76	116.1	106.0	109.8	121.0	123.4

[a]QTL positions, 2LOD, and 1LOD intervals calculated with QTL cartographer v.2.0

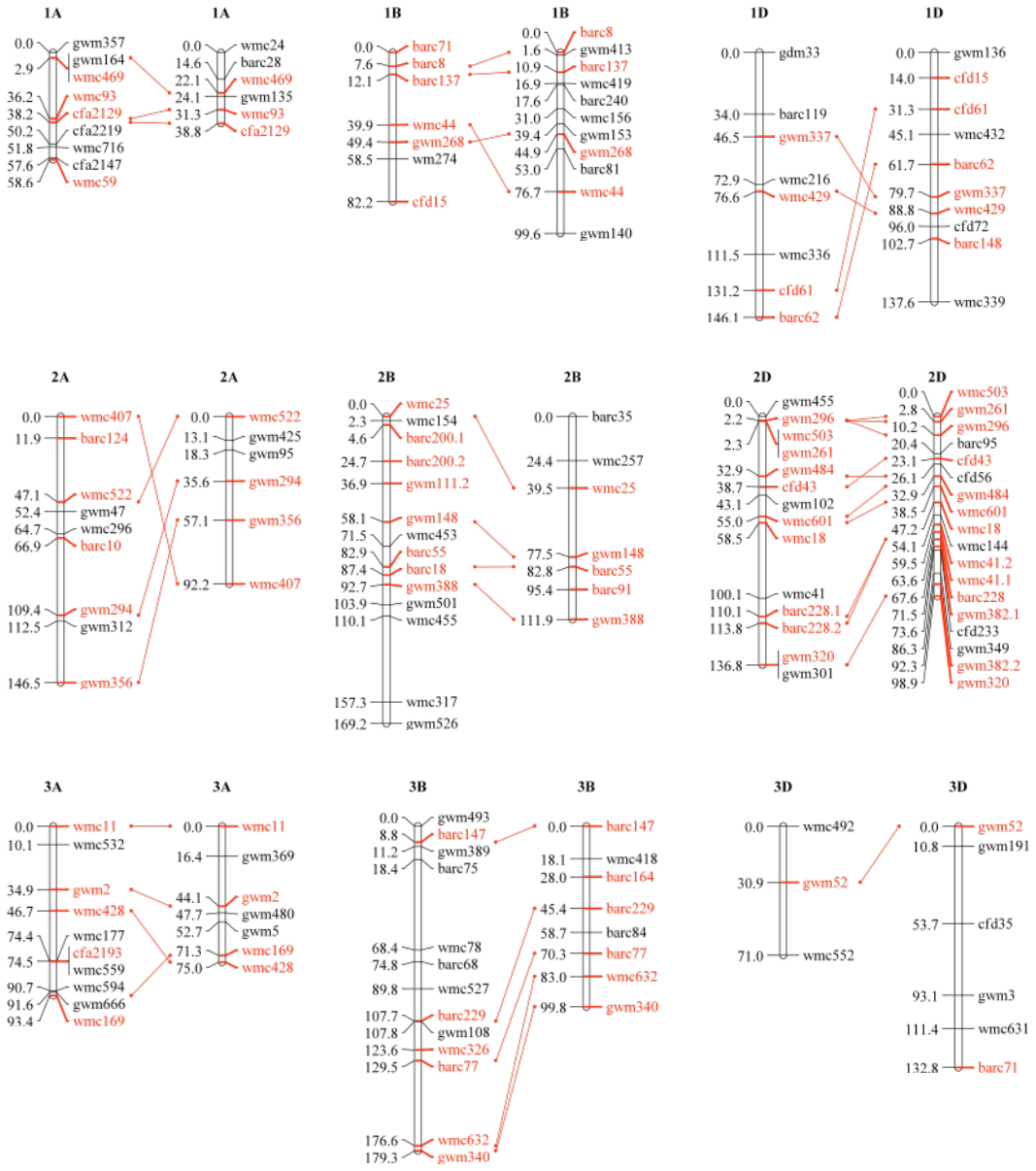

Fig A.1 Comparison of genetic linkage maps for Halberd x Cutter (left group) and Halberd x Karl 92 (right group) recombinant inbred line populations. Homologous markers and relative order are present in red text and red lines, respectively.

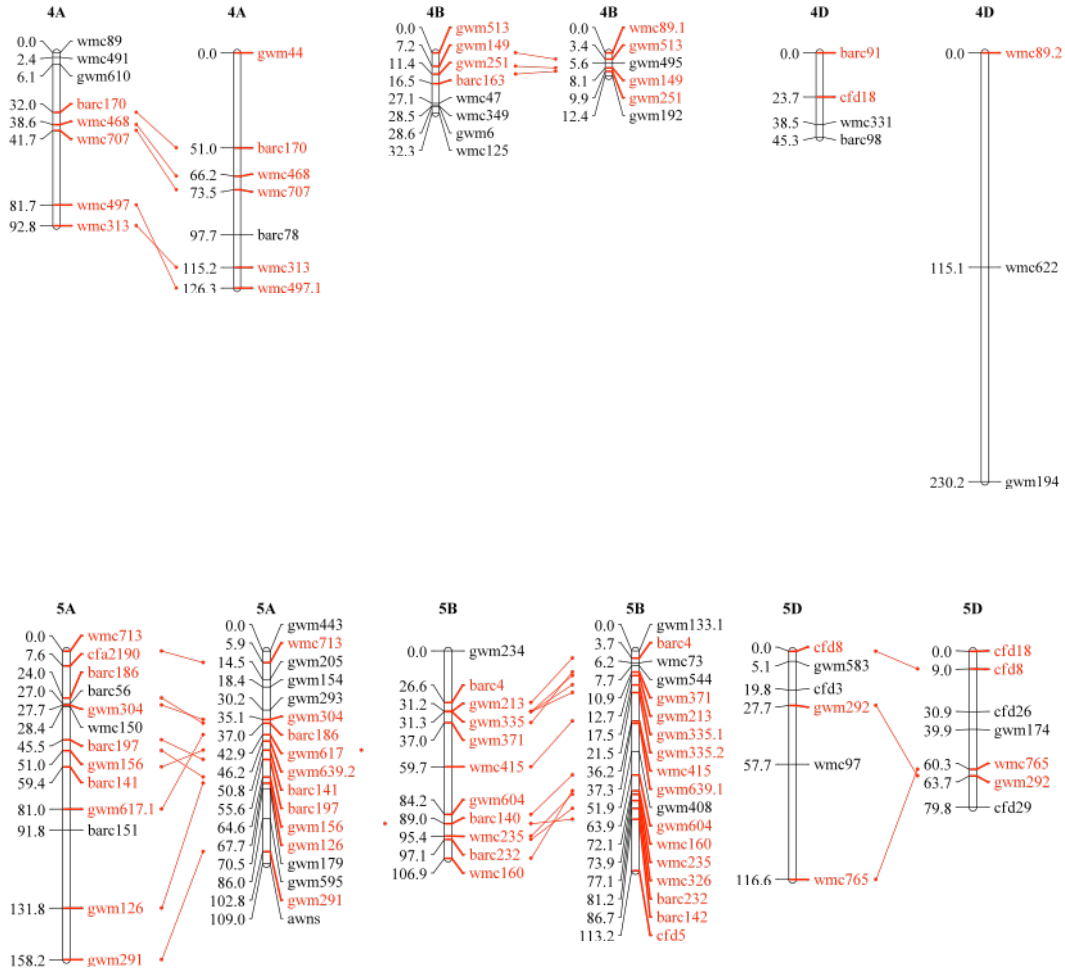

Fig. A.1 Continued

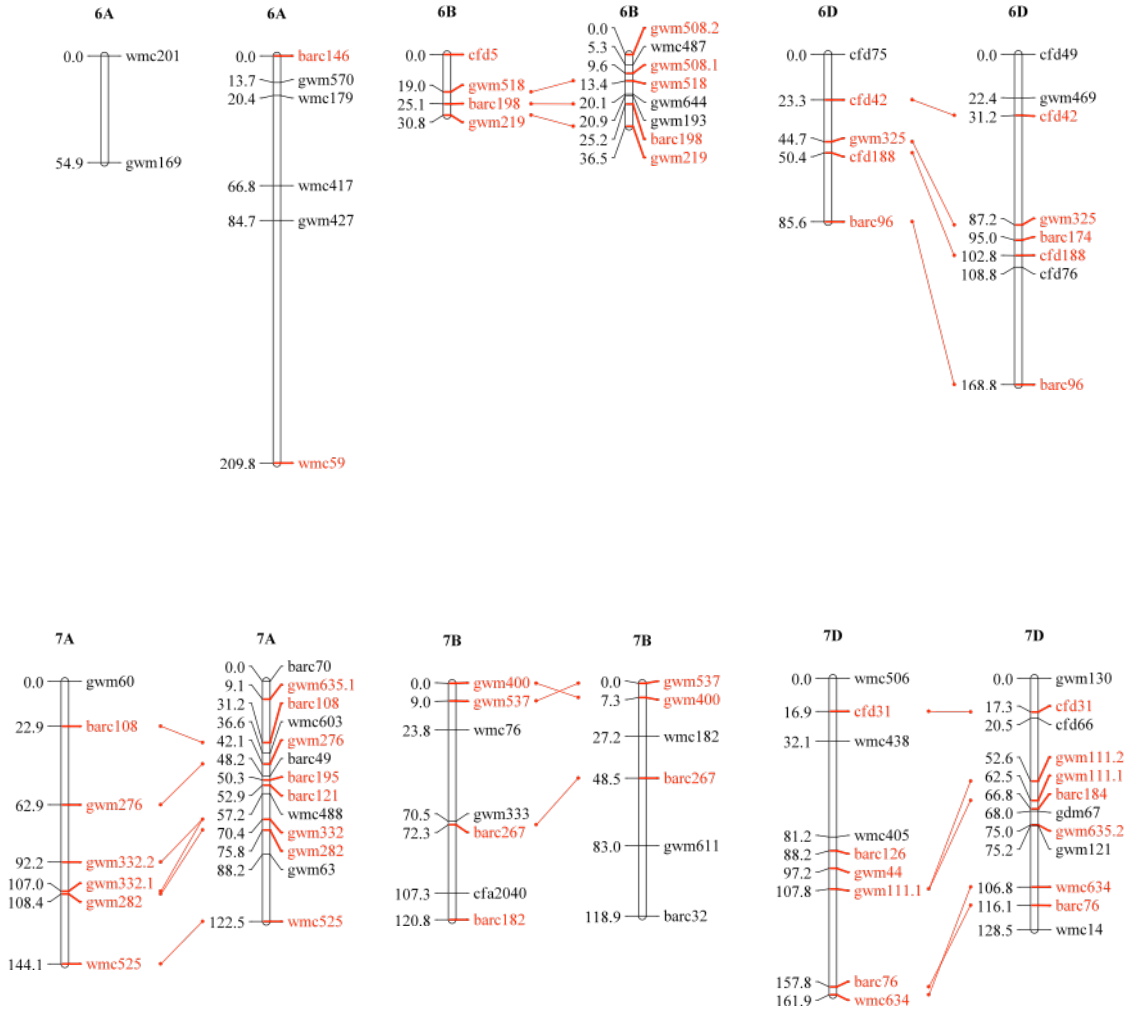

Fig. A.1 Continued

VITA

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