IDENTIFICATION OF QUANTITATIVE TRAIT LOCI (QTLs) ASSOCIATED WITH MAINTENANCE OF BREAD MAKING QUALITY UNDER HEAT STRESS IN WHEAT (*Triticum aestivum*)

A Thesis

by

FRANCIS WARD BEECHER

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

MASTER OF SCIENCE

August 2009

Major Subject: Molecular and Environmental Plan Sciences

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ABSTRACT

Identification of Quantitative Trait Loci (QTLs) Associated with Maintenance of Bread Making Quality under Heat Stress in Wheat (*Triticum aestivum*). (August 2009)

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Co-Chairs of Advisory Committee: Dr. Dirk B. Hays
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The aim of this study was to identify QTLs associated with the maintenance of grain quality following post-anthesis heat stress. A population of 64 F_6 Halberd X Cutter recombinant inbred lines (RILs) was exposed to either heat stress or control conditions in the greenhouse, starting ten days after anthesis. Grain quality was determined using the sodium dodecyl sulfate (SDS) sedimentation test, a significant predictor of bread baking quality. The percent change in SDS sedimentation test scores between the heat and control populations was used to identify QTLs associated with quality stability. Four QTLs were identified, located one each on chromosomes 1B, 1D, 4A, and 7A. Three of the QTLs, those on 1B, 1D, and 4A, were associated with variations in SDS sedimentation level. The QTL on chromosome 7A was associated with the percent change in SDS sedimentation scores between heat-stressed and control conditions. This indicated a relationship between the identified QTL and quality stability. To confirm the detected QTLs, eighty advanced lines grown at three Texas nurseries were genotyped and tested for relationships between QTL-associated markers, quality traits, and stability of the quality traits. Quality trait stability was estimated using the coefficient of variability (CV%) of quality traits between

growing sites. Quality characters analyzed in the advanced lines included kernel hardness, mixograph peak time, kernel weight, flour yield, SDS sedimentation, and grain yield.

The analysis showed support for the effect of the QTLs on chromosomes 1B, 1D, and 4A. Further analysis will be needed to confirm the QTL on 7A, in particular. The mapping of additional markers will be necessary. However, the potential importance of this QTL and the abundance of other QTLs detected in this region make it worth investigating.

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NOMENCLATURE

CV%	Coefficient of variability
SDS	Sodium dodecyl sulfate
QTL	Quantitative trait locus
RIL	Recombinant inbred line
MAS	Marker assisted selection

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CHAPTER I

INTRODUCTION

Wheat (*Triticum aestivum*) is one of the world's major cereal crops as the unique molecular makeup of its grain allows its use as a primary structural ingredient of breads, pastas, tortillas, and other products worldwide. To achieve the food production levels needed to supply worldwide demand, plant breeders have focused on the development of agricultural varieties possessing two characters: high yield potential and high end-use quality. Identification of genetic markers associated with these traits has been used to simplify line development in a process called Marker Assisted Selection (MAS) (Collard et al. 2005). However, achievement of high yield and quality levels alone should not be the end goal of breeding programs. Developed lines must also possess stability, the ability to maintain the yield and quality characteristics despite the environmental stressors which afflict field grown crops. Variation in wheat grain quality poses a significant problem for end-users such as millers, who require quality consistency for efficient industrial flour production. Variations in grain quality, whether due to environment or to other stress factors, therefore cause significant yearly monetary losses. This project focuses on the development of genetic markers associated with the maintenance of grain quality levels under heat stress conditions. Identification of such markers will allow for their use in MAS and in the development of lines possessing the trait.

This thesis follows the style of Plant Molecular Biology.

Grain quality and the effect of heat stress

Bread making quality is controlled primarily by the content of gluten, a class of proteins, in the grain (Anjum et al. 2007; Belton 1999; Don et al. 2003; Uthayakumaran et al. 1999; Weegels et al. 1996). Wheat possesses two types of glutens, classified by their solubility in alcohol-water solutions; namely the soluble gliadins and insoluble glutenins (Wieser 2007). Gliadins are small monomeric proteins. Glutenins are polymers composed of high molecular weight glutenin subunits (HMWGSs) and low molecular weight glutenin subunits (LMWGSs) bound together by disulphide bonds (Don et al. 2005). The subunits themselves are soluble in alcohol-water solutions after the disulphide bonds have been broken (Wieser 2007). Glutenins and gliadins both possess a high glutamine and proline content as well as a low, but important, cysteine content. The cysteine residues are responsible for the formation of intra- and inter-chain bonds which hold together gluten polymers. The extent to which glutens are able to polymerize (aggregate) via the aforementioned bonds is a key factor in the formation of glutenin macropolymer (GMP) by HMWGSs and LMWGSs. GMP is one of the largest of the gluten polymers and can be used as a predictor of quality due to its direct relation to glutenin content and the ability of the flour's components to form chemical bonds (Don et al. 2003; Spiertz et al. 2006).

Heat stress has been shown to greatly affect the bread making quality of wheat grain. This reduction in quality is hypothesized to be due more to changes in gene expression within the plant than to damage caused by the increased temperature. The overall effect of heat stress on wheat plants is to cause early maturation and senescence. In the grain, this has the effect of causing the synthesis of gliadins and glutenin subunits to both begin early and end early. The maximum level of gene expression has been shown to

be similar in both cases, although protein content may be increased by heat stress (Blumenthal 1995). The starch to protein ratio in heat-stressed grain is lower than in non-stressed grain, indicating that elevated temperatures are tied to increased protein content, a reduction in starch content, or both (Dupont et al. 2006; Kolderup 1975; Viswanathan and Khanna-Chopra 2008). Protein content may be the causative factor, since it has been shown to increase under heat stress (Kolderup et al. 1975). Although increased protein content is a character typically associated with increased bread making quality, the increase in protein content induced by heat stress has been associated with a decrease in bread making quality levels (Randall and Moss 1990). This discrepancy is due to an alteration in the type of proteins being produced and their ratio to one another. Namely, heat stress is associated with increased gliadin levels and decreased levels of large glutenin polymers.

Based on the above, it has been hypothesized that the poor end-use quality of heat-stressed wheat grain is due to a disruption in the formation of GMP and the other complex protein aggregates necessary for dough strength (Don et al. 2005). The decrease in the number of GMP particles in flour from heat- stressed wheat plants may stem from the effect of heat on the production of the glutenin subunits which make up the GMP. Heat stress' effect on protein synthesis has been shown to cause a decrease in the concentration of HMWGSs relative to LMWGSs (Don et al. 2005). Since LMWGSs are unable to form glutenin particles on their own, the decrease in the HMWGSs:LMWGSs ratio causes an increase in the overall size of GMP molecules, although the total number of particles is decreased (Don et al. 2005). The number of HMWGS particles present in grain are therefore hypothesized to be highly correlated to the structure and properties of GMP and

relate directly to dough characteristics. Furthermore, the concentration of HMWGSs in dough has been shown to have a positive correlation with dough strength (Don et al. 2003).

Another effect of heat stress on protein synthesis is a decrease in the ratio of glutenins to gliadins (Blumenthal et al. 1990). It is hypothesized that this may be due to an increase in gliadin synthesis during heat stress (Jarvis et al. 2008). An increase in the amount of gliadins present in flour causes a reduction in the bread making quality of the grain (Castro et al. 2007; Jarvis et al. 2008). This decrease in quality is attributed to the chemical structure of some gliadins, namely their possession of a single disulfide bond forming cysteine residue, since a single residue allows these gliadins to attach to expanding gluten chains only as chain terminators. In contrast other gliadins and LMWGSs have multiple cysteine residues which allow them to make larger complexes because they interconnect, chaining multiple HMWGSs together. In fairness it must be noted again that the literature is not consistent as some studies have shown no increase in the monomeric (gliadin) content of dough due to heat stress or have shown a decrease in gliadin content (Castro et al. 2007; Ciaffi et al. 1996); however, the hypothesized increase in gliadin synthesis after heat shock is supported by the detection of heat shock elements within the sequence of some gliadin proteins (Blumenthal et al. 1990), thus, the upregulation of gliadin synthesis after heat stress may occur as part of a heat shock response.

Heat stress also affects the synthesis of non-storage proteins responsible for grain development. Banowetz et al. (1999) found that wheat plants kept at post-anthesis temperatures of 35/25° for 12 days suffered a reduction in kernel weight of 23%. This weight reduction was hypothesized to be related to a decrease in the synthesis of proteins

related to starch production; most notable among these being ribulose 1, 5-bisphosphate carboxylase (RUBISCO). Since a reduction in RUBISCO synthesis would lead directly to a decrease in the rate of photosynthesis, the reduction in grain weight may be related to a decrease in starch and to a lesser extent protein content (Majoul et al. 2004). A reduction in starch content is another possible explanation for heat stress induced reduction in grain weight. Dupont et al. noted that the starch accumulation rate increased under heat stress but that the final concentration of starch in heat stressed grain was less than in unstressed grain (Dupont et al. 2006). Additionally, the weight loss in heat stressed grain may be tied to endosperm cell proliferation, a process which plays a large role in determining grain weight and which is thermosensitive (Banowetz et al. 1999). Endosperm cell proliferation is hypothesized to be effected by a heat induced decrease in the amount of cytokinins in the grain. A reduction in cytokinin levels is hypothesized to be due to a decrease in cytokinin transport following heat stress (Banowetz et al. 1999). The reduction in grain weight induced by heat stress has not been shown to affect grain germination rates (Banowetz et al. 1999).

Mapping of QTLs

Traits in crop species are typically divided into two types: qualitative and quantitative. Qualitative traits are those controlled by one or a few genes such as seed color. The presence or absence of qualitative traits is simple to determine because they are often tied to a single marker or gene. In contrast, quantitative traits are controlled by numerous genes which often have complex interactions between them. In most cases, individual genes affecting a quantitative trait may only have a small effect on the trait and

their influence may vary due to epistasis or environmental interactions. Indeed, being strongly affected by the environment is a characteristic of quantitative traits. Additionally, quantitative traits do not follow a Mendelian model of inheritance, instead the pattern of expression for most quantitative traits is a continuous variable with the population variation ideally forming a normal distribution. For these reasons, the inheritance of quantitative traits is referred to as polygenic inheritance. Many traits of interest in wheat and other crops are quantitative traits, typically including yield, quality, tolerance to environmental stress, and others. Selection for quantitative traits during breeding is complicated by the strong effect of the growth environment on the expression of the trait. To determine a cultivar's genetic potential for a trait, it is typically necessary to perform trials in several environments so as to control for environmental effect. By analyzing the genotype's character scores across environments, specific chromosomal elements can be linked with the expression of those traits. Trait-linked chromosomal elements linked to a quantitative trait are useful tools in plant breeding, simplifying the identification of lines possessing favorable alleles, and therefore aiding line selection.

Genetic mapping of quantitative traits is typically done through the establishment of quantitative trait loci (QTLs). A QTL is a genetic marker the alleles of which have statistically significant effects on the quantitative trait in question. Several methods have been developed for the identification of QTLs. The most basic is the use of analysis of variance to compare the score of the quantitative trait between each marker allele (Soller et al. 1976; Zeng 1994). Although easy to carry out, this method has several severe drawbacks: it requires a high population of samples, it cannot distinguish between multiple

QTLs on the same chromosome or marker, and it cannot describe the probable position of the QTL on the chromosome.

A more powerful method for QTL detection is interval mapping. Interval mapping uses multiple markers to assign a likelihood profile to each region of the chromosome. The individual regions are defined as intervals between two markers, with the strength of the correlation between each set of markers and the score of the quantitative trait being used to assign the likelihood profile. Regions with a likelihood profile greater than a pre-assigned threshold are designated as QTL. This technique is superior to analysis of variance as it gives the probable location of the QTL and requires fewer samples. However, it has a similar degree of difficulty in distinguishing between multiple QTLs on the same chromosome (Lander and Botstein 1989; Zeng 1994). To ameliorate this problem a technique called Composite Interval Mapping (CIM) is may be used. Using CIM it is possible to distinguish between separate QTLs on the same chromosome by assigning groups of markers as a proxy for the already established QTL. The effect of these QTL proxy markers is then taken into account when calculating likelihood profiles for other regions on the chromosome (Zeng 1994). Composite interval mapping aids in distinguishing between multiple QTLs on the same chromosome but is dependent on researcher's accuracy in designating the markers to serve as QTL proxies.

Before determining a mapping strategy to use, a marker map must be established. Genetic regions suitable for use as markers are those which can be reliably amplified from genomic DNA and differ between populations or individuals. Early mapping of traits to populations was done with Restriction Fragment Length Polymorphisms (RFLPs) (Rafalski and Tingey 1993). These are genetic sequences formed by digestion of extracted

genomic DNA with restriction enzymes. The presence of RFLP alleles would be determined using Southern blot analysis. Due to the amount of DNA required and the difficulty of the testing procedure, RFLPs have been largely replaced by PCR based genetic markers.

Random Amplification of Polymorphic DNA (RAPD) is another technique used to identify DNA markers. This technique involves the creation of random DNA primers and their use to amplify regions on the genome. The amplified region serves as the genetic marker. RAPD markers in the genome are always dominant, there are no alleles; the RAPD is either present or not present. Those present together with the trait are "coupled"; otherwise the RAPD marker is in "repulsion" to the trait (Rafalski and Tingey 1993). For use with quantitative traits, a threshold value is used to decide whether the trait is present or not in a sample. To rapidly identify RAPDs associated with a specific trait or genetic region, a method called Bulked Segregant Analysis (BSA) has been found useful (Michelmore et al. 1991). In BSA, the DNA from individual specimens is bulked into two groups based on the presence or absence of the trait. RAPDs or RFLPs are then identified which are polymorphic between the two groups. Since the two groups are heterozygous for all but the target trait, the identified markers will be linked to it. A major strength of RAPD markers is that their use does not require prior knowledge of the organism's DNA sequence (Tingey and del Tufo 1993). This makes them valuable tools for plant breeders working on crops such as wheat or wild rice where the genome has not yet been entirely sequenced.

Simple Sequence Repeats (SSRs), also known as microsatellites, are repeating sequences of nucleotides, such as $(AC)_n$. They have been found in abundance on the

majority of eukaryotic chromosomes and are often highly polymorphic (Rafalski and Tingey 1993). It is hypothesized that the highly repetitive nature of SSRs makes slippage during replication a common event, leading to the high levels of polymorphism found between populations (Tautz et al. 1986). SSRs can be used as genetic markers by amplifying a short fragment containing the repeating sequence. The products are then resolved by agarose or acrylamide gel electrophoresis. These markers provide the advantage of multiple alleles and require only small amounts of DNA to detect. Markers with multiple alleles provide co-dominance, the ability to distinguish homozygotes from heterozygotes.

Amplified Fragment Length Polymorphisms (AFLP) are genetic markers created by amplifying fragments from restriction digested chromosomal DNA. The primers used in amplification are based on the restriction site for the enzyme used to digest the DNA. Amplification by this method can result in numerous (50-100) fragments, which are then resolved by acrylamide gel electrophoresis (Vos et al. 1995).

Roder et al. (1995) analyzed the *T. aestivum* genome and predicted the existence of enough microsatellites that the average distance between them would be 271kb. This indicates that SSRs would provide excellent coverage of the wheat genome and indeed, SSR markers in wheat have been shown to possess more alleles than do RFLPs. SSR markers have been found to be highly polymorphic in crop species such as barley, although Bohn, Utz et al. (1999) found low levels of polymorphism in wheat (Powell et al. 1996; Russel et al. 1997). Bryan et al. (1997) however found high levels of polymorphism with SSRs in wheat but reported trouble with inaccuracies in marker amplification. Gupta, Rustgi et al. (2003) found that SSRs derived from wheat expressed sequence tags provided

a suitable level of polymorphism for analysis. Due to their abundance and polymorphism, SSRs have been favored over RAPDs when establishing genetic markers in wheat (Roder et al. 1995). Due to their high number of alleles (heterozygosity) and ease of use, SSRs have been widely used for genetic mapping in both plants and animals.

T. aestivum is a hexaploid organism possessing a diploid chromosome number of 42 chromosomes and a gametic number (haploid) of 21 chromosomes. The 21 chromosomes of wheat gametes can be divided into groups based on homology and genome of origin. There are seven homologous groups of chromosomes and three genomes (A, B, and D). The A genome is theorized to have been derived from *T. boeoticum,* a wild wheat species domesticated as *T. monococcum*. The D genome was donated by *Aegilops squarrosa* (also called *Aegilops tauschii*) a species of goatgrass. The origin of the B genome is unknown (Sears 1969). Chromosomes in each homologous group tend to possess genes for similar traits. Certain wheat varieties contain a translocation of the rye chromosome on the long arm of wheat chromosomes 1B and 1A. This translocation provides improved yield and disease tolerance, but results in reduced grain quality (Graybosch 2001).

Work to identify QTLs can be carried out in populations with simplified genetic structures, such as Recombinant Inbred Lines (RILs), Near Isogenic Lines (NIL), or double haploid lines. RIL are developed by crossing two inbred parent lines which differ in a specific phenotype. The progeny are allowed to self-fertilize until homozygosity is achieved and the lines are then used to identify QTLs related to the phenotypic differences between the parents. However, since the development of new cultivars relies on the introduction of diverse germplasm which may differ greatly from the lines used in QTL

mapping. An individual QTL is therefore only useful inasmuch as it is transferrable to other populations of the same or related crop species (Collard et al. 2005).

In any population of cross progeny, the observed phenotypic variability will be due partly to genetics and partly to the environment. In plant breeding, it is desirable for a high degree of the variability to be due to genetics because it makes selection for that phenotypic trait more reliable. Heritability is the name given to the percentage of variability which is due to genetics. It is calculated by dividing the genetic variability by the total phenotypic variability. The general heritability of a trait gives an idea of how successful selection for that trait will be. Analysis of heritability of the SDS sedimentation column height trait has been mixed. Barnard et al. (2002) reported low heritability for the SDS sedimentation trait. Other studies have found a moderate to high heritability for the trait (Clarke et al. 1993; Clarke et al. 2000; Fischer et al. 1989; O'Brien and Ronalds 1987; Sarrafi et al. 1989).

Genetic basis of grain quality

Numerous QTLs which correlate with parameters of bread-making quality have been detected on the wheat genome, and include loci on most wheat chromosomes. (Groos et al. 2007, Mansur et al. 1990). Among these, many have low heritability, or are strongly affected by genotype, or environment. Those loci with the most significant effects, particularly those associated with glutenin subunits or gliadins, are probably the best characterized. Blanco et al. (1998) detected a putative QTL for SDS ($\alpha = .01$) associated with a *Glu-A1* allele and another associated with the *Gli-B1/Glu-B3* allele. Moonen, Scheepstra et al. (1982) reported a significant relationship between variation in glutenin

subunit alleles on the long arm of chromosome 1D and variation in SDS sedimentation column height. Li et al. (2009) reported a QTL associated with SDS sedimentation score on the short arm of chromosome 1B, near the *Gli-B1/Glu-B3* locus. The *Gli-1, Gli-2,* and *Gli-3* loci are associated with the production of the gliadins (α, β, ω, γ) (Payne et al. 1982). The low molecular weight glutenins are controlled by *Glu3* loci which are usually positioned adjacent to the *Gli1* loci. Additional QTLs have been mapped which are associated with these loci.

Although it has been theorized that all chromosomes possess loci associated with grain quality, the majority of those identified thus far are on chromosomes 1A, 1B, and 1D (Blanco et al. 2002; MacRitchie and Lafiandra 2001). On the short arms of these chromosomes are found the complex *Gli-1/Glu-3* loci coding for LMWGSs and ω and γ gliadins. The long arms of chromosomes 1A, 1B, and 1D each house a *Glu-1* locus, associated with the HMWGSs. (MacRitchie and Lafiandra 2001; Payne et al. 1982; Payne et al. 1987). The α and β gliadin loci, *Gli-A2, Gli-B2,* and *Gli-D2,* are located on the short arm of chromosomes 6A, 6B, and 6D. The HMWGS *Glu-1* loci are each made up of two genes, each encoding a different HMWGS, however, it is not unusual for one to be silenced. Out of a total of six, this leaves from three to five alleles active in the plant (Anjum et al. 2007). Each locus has numerous alleles of different strengths for end-use quality (Li et al. 2006). Allelic variation at these loci plays a large role in determining wheat end-use quality (Luo et al. 2001). By comparing lines differing in allelic composition, the effect of specific HMWGS allelic pairs at the *Glu-1* loci on bread making characteristics has been established (MacRitchie and Lafiandra 2001; Payne et al. 1982; Payne et al. 1987). The *Glu-A1* locus has 2 functional subunits (“1” and “2”) and a null.

The 1 and 2 loci possess a similar quality score, which is higher than that of the null (Payne et al. 1987). The *Glu-B1* locus, on chromosome 1B, has five known allelic pairs: 17+18, 7+8, 7+9, 7, 6+8, and 20x+20y. The allelic pairs are listed here in order of decreasing quality. Chromosome 1D, on the *Glu-D1* locus, has four possible allelic pairs 5+10, 2+12, 3+12, and 4+12 (Payne et al. 1987).

The HMWGSs are strongly correlated with bread making characters and are often associated with QTLs for bread making traits. QTLs for SDS sedimentation have been detected which have a significant correlation with some of the HMWGS or gliadin/LMWGS loci (Blanco et al. 1998; Moonen et al. 1982; Rousset et al. 2001). Additionally, bread mixing and loaf volume traits have been found to associate significantly with the *Glu-1* loci. However, the same studies have shown that grain quality is a complex trait and that the *Glu-1* loci interact with other loci to manifest it (Rousset et al. 2001).

Mapping of QTLs requires careful scoring of the phenotype(s) of interest before the phenotype can be correlated to genetic elements on the chromosome. When testing for QTLs associated with grain quality, the measurement of phenotype(s) may use several different techniques, including analysis by mixograph, farinograph, and SDS sedimentation. SDS sedimentation is a test commonly used by plant breeders since the procedure is simple, and the quantity of flour required is small. The technique measures the height of an aggregated column of insoluble flour “gelprotein” (Figure 1). This “gelprotein” is constituted primarily by glutenin proteins and the height of the column it forms serves as a predictor of grain quality. The results of the SDS sedimentation test have

been shown to correlate significantly with the results of mixograph analysis for grain quality (Barnard 2002).

CHAPTER II

IDENTIFICATION OF GRAIN QUALITY AND QUALITY STABILITY QTLs IN A RECOMBINANT INBRED LINE POPULATION

Introduction

Wheat is a major crop for human consumption. Its importance hinges upon unique rheological properties of wheat flour which allow for the production baked goods. In recent years, wheat production has been increasing rapidly enough to keep pace with population growth, and is predicted to continue increasing at an average yearly rate of 1.9%, rising from 609 million tons in 1997 to a projected 641 million tons in 2011 (Chiran et al. 2008; FAO 2009). In order to meet the demands of future populations, we will need to develop new methods not only for increasing wheat yield, but also for increasing the utility and reliability of the resultant grain. The utility of wheat grain for bread making can vary greatly in field conditions due to the effect of environmental stress. This project focuses on one of the primary environmental stresses affecting wheat: heat.

Heat stress is a common problem in many wheat-growing regions worldwide. It causes a shortening of the crop's reproductive cycle, damaging both the quantity and quality of the resultant grain. Heat stress is particularly harmful in the developing world where it is estimated to affect 57.3% of wheat-producing land (Kosina et al. 2007). Heat-tolerant wheat varieties are typically described as such due to an ability to maintain high grain yield despite high-temperature conditions. Due to their polygenic nature, such varieties may maintain yield, but may lose grain quality upon exposure to heat stress. This loss of quality poses a particular problem for end-users such as bakers and millers whose

industrial machinery relies on consistent quality levels. To address these problems, heat-tolerant varieties which maintain their quality levels after exposure to heat are needed.

Current line selection in breeding programs focuses on the development of plants possessing high yield and high grain quality. In *Triticum aestivum,* numerous quantitative trait loci (QTL) associated with these traits have been identified and varieties have been designed which possess both to a high degree. However, the ideal yield and quality achieved by plant breeders often cannot be maintained during exposure to the heat and other environmental stresses commonly affecting field grown crops. An important goal in future breeding projects will be the development of varieties possessing stability traits allowing the maintenance of both yield and quality levels despite environmental stress. Some recent research has focused on this problem as it relates to the maintenance of yield but little has been done to develop wheat varieties possessing grain quality stability (Barnabas et al. 2008; Rane et al. 2007).

Quality in bread wheat is determined primarily by the protein makeup of the grain, a trait called “protein quality”. As discussed previously, the proteins most strongly associated with quality characters are the glutens: gliadins and glutenins. They interact to form polymers, the properties of which are responsible for the elasticity and extensibility of dough. Variations in the ratio of gliadins to glutenins affect the formation of the gluten polymers, and by extension, the grain’s bread making potential. Additionally, glutenin is itself a polymer, made up of high molecular weight glutenin subunits (HMWGSs) and low molecular weight glutenin subunits (LMWGSs) (Blumenthal et al. 1990; Don et al. 2005). The relative concentration of these subunits in wheat grain plays a role in determining the flour’s bread-making properties. In particular, the HMWGS content has been shown to

relate closely to both dough strength, and to gluten macropolymer (GMP) content, a predictor of bread making quality (Don et al. 2003). The effect of heat stress on bread-making characters is therefore hypothesized to be related to its effect on protein synthesis, and may be due to a disruption of the delicate protein balance necessary for bread-making quality.

The importance of protein quality to bread-making quality is underscored by the number of quality associated QTLs which have been identified in association with loci for the glutenin subunits and gliadins. Allelic variation at these loci, which are located primarily on chromosomes 1A, 1B, and 1D, have been significantly associated with changes in bread making quality (Blanco et al. 2002; Li et al. 2006; Luo et al. 2001; MacRitchie and Lafiandra 2001; Payne et al. 1987). However, the potential quality level of wheat grain is only meaningful if it is realized; lines possessing alleles associated with high quality levels may still be susceptible to environmental stress induced alterations in quality. To develop lines suitable for use in areas where heat stress is prevalent, QTLs must be identified which are significantly correlated not with quality characteristics but with the *maintenance* of those characteristics under heat stress conditions.

This project focuses on the identification of QTLs associated with maintenance of quality characteristics after post-anthesis heat stress. A population of RILs grown under both heat and control conditions will be tested for grain quality using the SDS sedimentation test. This test has been shown to be significantly associated with HMWGS content and to predict flour bread making characteristics (Moonen et al. 1982). We have used the percent change in SDS sedimentation values between the heat and control groups to identify QTLs regulating the maintenance of quality.

Methodology

Plant material

T. aestivum lines Halberd and Cutter were crossed to develop the RILs used in this study. The parent line Halberd was selected as a source for heat tolerance. The parent line Cutter was selected due to its high score in yield and other agronomically important traits. The F_1 progeny of these parents were advanced through single seed descent to the F_4 generation. The F_4 seed was harvested using bulk selection to yield F_5 lines, sixty-four of which were selected for advancement to F_6. Initial growth conditions were ~20°C (daytime) and ~18°C (nighttime) in an air conditioned greenhouse with 600mmol m^{-2} s^{-1} PAR supplemented natural light. Ten days after pollination ten plants from each RIL were placed under either heat (38°C daytime and 20°C nighttime) or control (20°C daytime and 15° night) conditions for a three-day period. Grain was harvested from both the primary and secondary heads and counted.

Measurement of quality parameters

Grain from the primary heads was ground using a genogrinder (SPEX Genogrinder 2000). The resultant grain meal was analyzed using the sodium dodecyl sulfate test, an accepted predictor of grain quality (Moonen et al. 1982; Figure 1). A grain meal sample for each line was weighed out to 1g, within 0.01g. Each sample was placed in a test tube and 4ml of water were added. The tube was vortexed for 4s and allowed to sit for 4 min and 54 s. Then 12ml of SDS-lactic reagent (1L 95% SDS, 20ml USP 85% lactic acid) was added to each sample and the tubes were inverted ten times. The resulting mixture was allowed to sit for 15 min at which point the gelprotein column height was measured. A set of 3 results

were obtained for each treatment of each RIL. The mean of the three SDS scores for each treatment was used to represent an RIL during data analysis. A one-way ANOVA between the results from each test tube rack used in this test did not find a significant difference, indicating that there was no artificial variation in results due to rack used (data not shown).

Statistical analyses

Statistical analyses were performed using SPSS (SPSS 16.0 SPSS Inc.). A paired samples T-test was used to compare the overall mean SDS sedimentation scores of heat and control to test for a difference. Percent change was calculated to indicate the stability of each line between heat-stressed and control conditions. Percent change was calculated as the percent by which the mean control SDS score deviated from the mean heat stress SDS score. The mean heat and control values for each line were the mean of the three measurements taken.

$$(\text{mean}_{\text{control}} - \text{mean}_{\text{heat}})/\text{mean}_{\text{control}} = \%\ \text{change}$$

Mapping of QTLs

The phenotypic quality data obtained through SDS sedimentation was analyzed for correlation to a previously developed SSR map of this RIL population (Mason et al, in review). A genetic linkage map was developed by screening the RIL population with seven-hundred wheat SSR primers. These were analyzed for polymorphism using 10uL PCR reactions visualized by agarose gel electrophoresis. Rates of polymorphism among the identified marker QTLs were analyzed by the program Mapmaker/Exp v3 to form the linkage map. The program QTL cartographer was used to analyzed phenotypic traits (SDS

sedimentation) and use correlations between trait variability and polymorphism to identify QTLs. The program analyzed the correlation between phenotype and marker polymorphism with single marker analysis. Multiple interval mapping was used in the determination of the position of QTLs. Likelihood of ODds (LOD) thresholds for QTL identification were determined using one-thousand permutations (Table 2; Figure 3).

Figure 1: Image of the SDS sedimentation test. The visible columns consist of insoluble gel protein. A measurement of their height serves as the SDS score. Height of the column increases as gluten content, and bread making quality, increase.

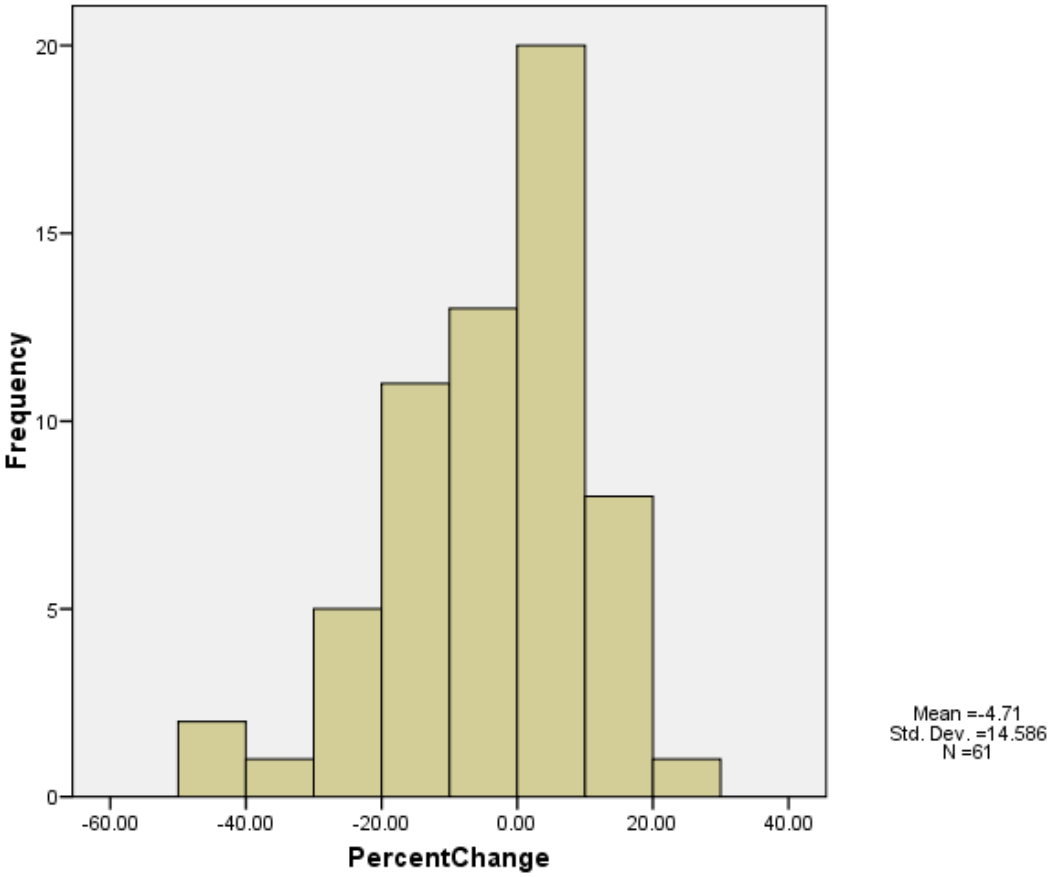

Figure 2: Histogram showing distribution of percent change scores between the mean SDS sedimentation values of heat stressed and control treated RIL lines from a cross between heat tolerant cultivar Halberd and the cultivar Cutter.

Table 1: QTLs associated with SDS sedimentation in a Halberd X Cutter recombinant inbred line

QTL name	Linkage group	Marker(s)	Trait	LOD	Effect[¥]	R^2
Qsdscon.tam-1B	1B	*barc71* *barc8* *barc137*	SDS control	4.3	0.47	19
Qsdsheat.tam-1D	1D	*barc119* *gwm773*	SDS heat	4.0	-0.67	22
Qsdscon.tam-4A	4A	*barc170* *wmc468* *wmc707*	SDS control	6.1	0.8	30
Qsdssta.tam-7A	7A	*gwm60* *barc108*	SDS stability	3.2	6.7	18

[¥]Effects given are for the Halberd allele

Table 2: Contrast analysis comparing mean percent change values by allele for QTL associated chromosomal markers

Marker	Chromosome	Genotype	Control	Heat	% Change
barc71	1B	0	6.8***	7.0***	4.9
		1	7.7***	8.0***	4.5
barc8	1B	0	6.8***	7.0***	4.0
		1	7.7***	7.9***	3.9
barc137	1B	0	6.9***	7.1**	4.7
		1	7.7***	7.8**	2.9
barc170	4A	0	7.0**	7.5	9.3**
		1	7.7**	7.6	-0.2**
wmc468	4A	0	7.2	7.5	6.1
		1	7.5	7.3	-1.6
wmc707	4A	0	7.4	7.7	6.1
		1	7.3	7.3	1.6
gwm337	1D	0	7.2	7.4	4.6
		1	7.4	7.6	3.9
barc119	1D	0	7.2*	7.1***	-0.2***
		1	7.8*	8.3***	8.3***
gwm60	7A	0	7.2	7.8	9.4**
		1	7.5	7.3	-2.8**
barc108	7A	0	7.1	7.4	5.6
		1	7.4	7.5	3.3

Statistical comparisons are between the Halberd (0) and Cutter (1) alleles of each marker.
Key - Significance: * = 0.1, ** = 0.05, *** = 0.01

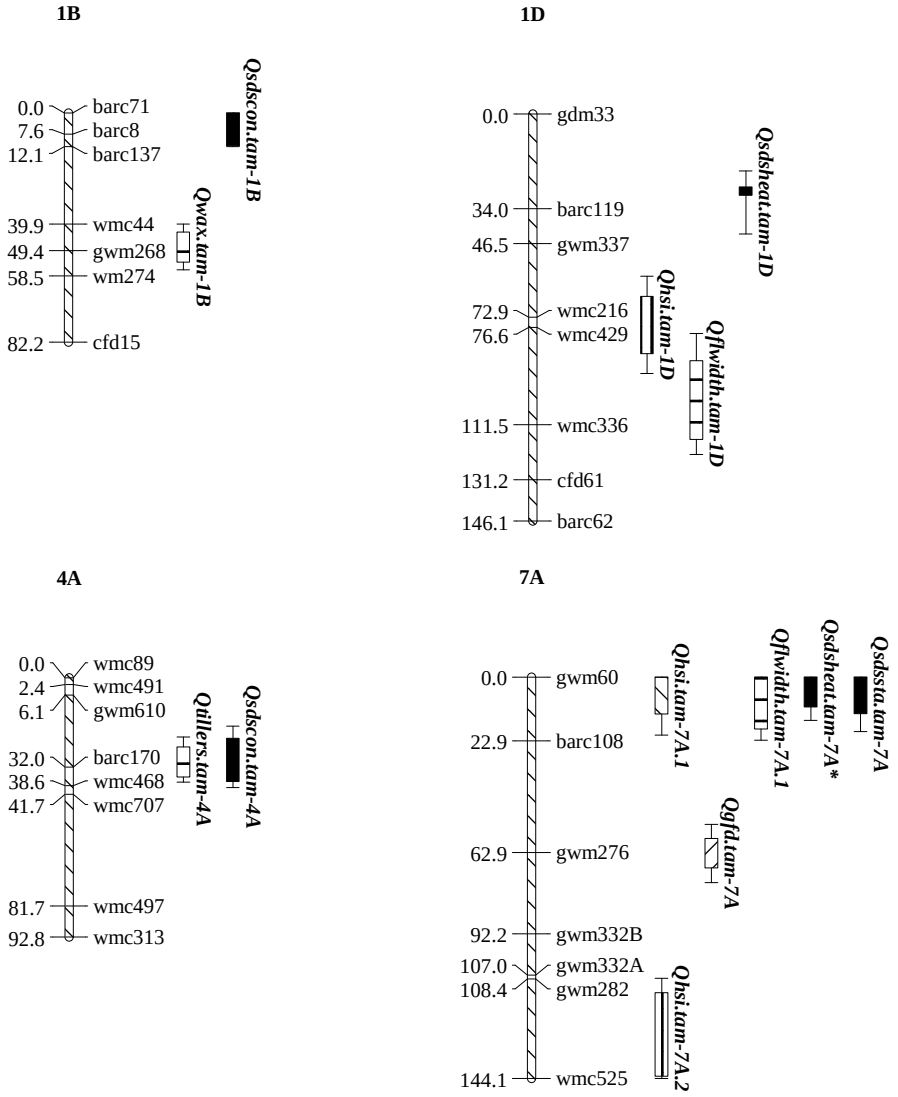

Figure 3: Locations of identified SDS sedimentation and SDS percent change QTL on the wheat genome. The black bars indicate QTLs identified in this study as being associated with SDS sedimentation column height and the maintenance of SDS sedimentation height between heat stress and control conditions. The other bars indicate QTL associated with other traits which have been detected in a previous analysis of this RIL population (Mason et al. In review).

Results

SDS sedimentation

SDS sedimentation scores were obtained for 63 lines. The mean SDS sedimentation column height of the control treated lines was significantly lower than that of the heat treated lines (7.2 and 7.4, respectively; α=0.1). Percent change between the heat and control lines was calculated for use in further statistical analyses as a measure of quality stability (Figure 2).

Detection of QTLs

Analysis detected four QTLs associated with the phenotypic SDS sedimentation data and the RIL population SSR markers (Figure 3). Two were associated with SDS score under control treatment, one with SDS score under heat treatment, and one with percent change. A second heat treatment SDS associated peak was included in the analysis as a putative QTL due to its proximity to the threshold value.

The QTLs associated with SDS scores under control conditions were located on chromosomes 1B and 4A. The chromosome 1B QTL, on the short arm, was related to SSR markers *barc8*, *barc71*, and *barc137* (Figure 3). This QTL displayed an R^2 of 0.19 (19%). The Halberd allele at all three of these markers associated significantly with slightly lower SDS sedimentation scores than the Cutter allele under both heat stress and control conditions. Heat stress caused the average SDS sedimentation score of both alleles to increase slightly (Table 1).

The other SDS control associated QTL was located on chromosome 4A, in the centromeric region but extending onto the short arm. This QTL was flanked by SSR

markers *barc170, wmc707,* and *wmc468.* QTL Cartographer reported an R^2 value of 0.30 (30%; Table 2). Contrast analysis showed that the Cutter allele at this locus was significantly associated with increased SDS sedimentation levels under control conditions and with a low percent change in response to heat stress (Table 1).

The putative QTL associated with SDS score under heat treatment was located on the short arm of chromosome 7A. This putative QTL was related with SSR markers *gwm60* and *barc108* (Figure 3). It was predicted to be in approximately the same position as the QTL associated with percent change. The R^2 for this QTL was 0.14 (14%; Table 2). Contrast analysis did not find a significant difference between the mean heat stress SDS scores of the parental alleles. Allelic effects of this QTL can therefore not be hypothesized.

The QTL associated with heat treatment SDS score was located on the short arm of chromosome 1D, flanked by markers *barc119* and *gwm337* (Figure 3). The Halberd allele of the *barc119* marker was significantly associated with a lower percent change than the Cutter allele as well as with lower SDS sedimentation scores under both heat and control conditions (Table 1). QTL Cartographer reported that the Halberd allele of the QTL associated with an additive effect of -0.67 and an R^2 of 0.22 (Table 2).

A QTL associated with percent change was identified on the short arm of chromosome 7A. This QTL associated with SSR markers *gwm60* and *barc108*. QTL Cartographer reported an additive effect of 6.7 associated with the Halberd allele (Table 2). Contrast analysis supported this result, finding that the Cutter allele of the *gwm60* marker was significantly associated with a much lower percent change than the Halberd allele (Table 1). This QTL was projected to be in approximately the same position as the

putative chromosome 7A QTL associated with SDS sedimentation levels under heat stress (Figure 3).

Discussion

The goal of this study was to delineate the relationship between heat stress QTLs associated with grain quality, and those associated with the maintenance under heat stress of grain quality and yield maintenance. Evidence for such a relationship would primarily be seen in the location of QTLs associated with the two traits. Overlapping QTLs for quality maintenance and yield maintenance could indicate a locus with a pleiotropic effect.

The percent change associated QTL on chromosome 7A is the most promising candidate for a pleiotropic effect between yield maintenance loci and quality maintenance loci. The chromosomal region in which it resides contains several other QTLs: one for SDS sedimentation under heat treatment, one for flag leaf width, and one for yield under heat treatment (Mason et al. in review). The existence of a gene cluster for a family of genes associated with these traits may explain the number of heat tolerance and grain quality QTLs identified in this region. Alternately a single locus could be responsible for two or more of the observed QTLs. In either case, if the loci underlying the observed QTLs have a low recombination rate then they could be useful for Marker Assisted Selection (MAS). Selection for flanking markers would ensure both traits in the selected lines. Additionally, selection for the SDS sedimentation maintenance QTL could be useful even without a pleiotropic effect on yield. Where the SDS sedimentation associated QTLs associate with variations in the gluten quantity, this percent change associated QTL correlates not with a certain gluten level but with the degree of variation in gluten level.

Selection for maintenance in gluten levels between heat stressed and control conditions could yield grain useful for bakers. Consistency in baking characteristics is vital for use in large industrial processes without requiring fine tuning to account for variations in dough quality.

The presence of the putative QTL for SDS sedimentation under heat treatment, also on 7A, seems understandable: the presence of a locus allowing for high SDS sedimentation scores under heat tolerance could be expected to mitigate the effect of quality loss from heat stress. The percent change and heat treatment QTLs could be hypothesized to be the result of the same or related genes. Increased mapping of genetic markers in this region will allow us to determine whether this QTL and the percent change QTL are the same.

The flag leaf width QTL on 7A is more puzzling. Flag leaf width does not appear to have a correlation with increases in either grain quality or yield. Indeed, it has been found to be negatively correlated with photosynthetic rate and to have no correlation with yield or grain protein content (Austin et al. 1982; Blake et al. 2007). Grain protein content has been shown to correlate significantly to some end-use quality characters and can therefore be used as a general quality predictor (Moonen et al. 1982). The importance of the presence of this QTL, since the related trait seems to lack a correlation with end-use quality characters, is therefore questionable.

The presence of a yield heat tolerance QTL sharing what seems to be the same locus could be an important find (Mason et al In review). Having a yield and quality maintenance QTL in the same region raises the possibility of a single gene or gene cluster which affects both traits. A locus pleiotropic for both of these traits would be a powerful selection tool as it would provide a way to simultaneously select for stability in quality and

in yield.

However, more work needs to be done before the utility of the percent change QTL on chromosome 7A can be determined. In particular the size of the region makes it difficult to assess any relationship between the individual QTLs identified in this region. The percent change QTL is projected to lie in the 22.9cM between markers *barc108* and the *gwm60*. The other three loci (putative heat treatment SDS score, flag leaf width, and yield heat tolerance) are projected to lie in this same area. The size of the region leaves open the possibility that the various identified QTLs are from unassociated loci. To resolve this question, more markers in the area will need to be identified and the various QTLs mapped more specifically.

The SDS control QTL on chromosome 1B may be related to the *Glu-B3* and *Gli-B1* loci, which have been mapped to the same region of the chromosome (KOMUGI 2002; Somers et al. 2004). These gluten loci are within ~23cM of two of the markers associated with this QTL (*barc8* and *barc137*). The functionality of these loci could explain the presence of the QTL. Allelic variation at the *Glu-B3* locus, which codes for a LMWGS, is known to affect bread making quality (Wang et al. 2009). It is possible that variation in the alleles of the *Glu-B3* locus between the Halberd and Cutter varieties is responsible for the detected QTL. Allelic variation at the *Gli-B1* locus, which codes for a gliadin, is also associated with differences in bread making quality (Nieto-Taladriz et al. 1994). Additionally, certain alleles of the *Gli-B1* locus are associated with human allergic reactions to wheat consumption (Denery-Papini et al. 2007).

The QTL associated with SDS sedimentation score under heat stress on chromosome 1D is similarly close to genes *Glu-D3* and *Gli-D1*. Allelic variation in the

Gli-D1 and *Glu-D3* loci has been associated with variations in bread-making quality (Branlard et al. 2001; Wang et al. 2008).

The QTL located on the short arm of chromosome 4A is correlated with SDS sedimentation score under control conditions. This QTL is located in the same region as a QTL for tillering number (Mason et al in review). Tiller number has been associated with increased yield although Narasimhamoorthy et al. (2006) found little correlation between a chromosome 3B tillering allele and agronomic traits. The QTL had a significant relationship with kernel weight and biomass; but they predicted little utility for the QTL in plant breeding. Li et al. (2009) detected a QTL for SDS sedimentation score on the long arm of chromosome 4A. A QTL for disulfide reductase activity was identified by Emakova et al. (2008) on the long arm of chromosome 4A. This enzyme is associated with the decomposition of disulfide bonds, a process important in glutenin subunit polymerization. Variation in this trait may be affecting grain quality. Another QTL found on chromosome 4A which may be associated with variations in quality is a height (*Ht*) QTL (Borner et al. 2002). Height variations could have an effect on quality, making this locus a possible explanation for the detected SDS sedimentation stability QTL.

An interesting phenomenon was the increase in SDS sedimentation score seen in RIL lines exposed to heat stress. Irmak et al. (2008) observed a decrease in SDS sedimentation due to heat stress. They suggested that this result was due to an overall decrease in unextractable polymeric protein caused by heat's interference with the ability of glutenin subunits to polymerize. Tahir et al. (2009) found that SDS sedimentation scores increased with post-anthesis heat stress treatments in wheat. Stone and Nicolas (1998) also found that sudden heat shock increased the SDS sedimentation column height in both

wheat cultivars tested but found that gradual application of heat treatment did not have the same effect. Gradual heat stress did however lead to an increase in the percent protein content of the heat susceptible variety Oxley. The amount of monomeric proteins, presumably gliadins, did not increase in either variety (Stone and Nicolas 1998). Heat stress has been shown to cause increases in other associated quality measures, including mixograph peak height and mixograph descending slope (Tahir et al. 2006). Other measures of quality were shown to decrease (mixograph peak time, curve at two minutes past peak). It has been hypothesized that the differences in whether traits associated with quality increases or decreases are due to the level of temperature stress and to late sowing. The varying response of the SDS sedimentation test to heat stress raises questions about the test's reliability in measuring heat tolerance. Tahir et al. (2009) found a significant positive correlation between SDS sedimentation scores from heat stressed plants, protein content, insoluble protein content, and mixograph peak height. A significant negative correlation was found between SDS sedimentation and several other quality measurements: mixograph peak time, mixograph descending slope, and mixograph curve width two minutes past peak. Although they reported that SDS scores from heat treated plants are reliable for distinguishing between genotypes, they cautioned against using SDS sedimentation as the sole means of evaluating the effect of heat stress on quality (Tahir et al. 2009). Possible improvement of the test's accuracy in analyzing heat stressed lines could be achieved by controlling for protein content, a procedure which has been shown to improve the accuracy of SDS sedimentation (Carter, et al. 1999). This could be due to heat's effect on protein synthesis rates. The information desired from SDS sedimentation relates primarily to the quality, represented by the glutenin polymer forming ability, of the

flour. Controlling for heat's effect on the overall protein content may allow us to distinguish this quality factor in the SDS sedimentation score from noise generated by the other effects of heat stress.

Studies have shown an upregulation of heat shock proteins under heat stress, but a decrease in the overall synthesis of proteins. Heat shock proteins are therefore produced at the expense of other protein types. (Hendershot et al. 1992; Stone and Nicolas 1998a; Stone and Nicolas 1998b). However, gliadins have been identified as possessing heat shock elements and may be upregulated under heat stress (Blumenthal et al. 1990). Since increased gliadin levels are associated with a decrease in end use quality, their upregulation after heat stress could partially explain the heat stress associated loss of quality (Fido et al. 1997).

The identification of novel QTLs associated with the SDS sedimentation test and percent change provide an opportunity both in cultivar development and in crop genetics research. Improvements in cultivar development may be achieved through marker assisted selection if the QTLs are found to be reliable across different wheat breeding populations. An increased number of markers in those chromosomal regions will allow the QTLs to be narrowed down to more accurate positional estimates. Furthermore, fine mapping of the chromosomal regions containing the QTL may allow identification of the genes responsible for the observed effects. The QTL on chromosome 7A seems the most promising for further analysis due to its association with quality-maintenance and its close proximity to a yield-maintenance QTL. The proximity of these two QTLs suggests the possibility of a genetic region possessing a pleiotropic effect, improving both yield and

quality maintenance simultaneously. Such a genetic region, if identified, would be ideal for breeding plants with resistance to heat stress.

CHAPTER III

CONFIRMATION OF PUTATIVE END USE QUALITY QTLs IN ADVANCED LINES

Introduction

In wheat growing regions worldwide, heat stress induced loss of yield and quality is a common problem. This is particularly true in the developing world, where heat stress affects a large amount of the land used in wheat production (Kosina et al. 2007). To ensure grain production in these areas, cultivars must be developed possessing not only traits for high yield and high quality but also possessing the ability to maintain those traits in response to high temperature stress. To identify and select cultivars possessing the quality maintenance trait, trait-linked genetic elements can be identified. Selection for these will then yield plants possessing the desired character.

In this project, four QTLs have been identified based on the SDS column height of RILs grown in both heat stressed and control environments. Three of the identified QTLs are associated with the SDS sedimentation score and the fourth with percent change between SDS sedimentation score under heat stress and control trials. MAS based on these QTLs could assist in the development of lines resistant to heat stress. However, SDS sedimentation values, like most quality traits, are extremely variable and strongly affected by environmental influences. Using potentially unreliable phenotypic values as the foundation on which QTLs are identified includes a degree of uncertainty in the process, meaning that the QTLs themselves may be unreliable. Confirmation is therefore a vital process in the development of QTLs, and one which must be carried out before they can dependably be applied to breeding populations.

Of the numerous factors affecting QTL discovery, the one with the largest effect is population size. As population size increases, so does the statistical power of the analysis, making estimates of QTL strength and position more accurate. A large population greatly increases QTL detection and is a requisite for reliably detecting small QTLs. Indeed, although the size of the confidence interval estimating a QTL's position can be decreased with the addition of genetic markers, it will only improve to a point which is itself dependent on the population size (Darvasi et al. 1993). Population size is therefore the single most important determinant of the success of QTL identification. Beavis (1994) detailed the relation between population size and predicted QTL strength, nicknaming the inflation of a QTL's effects the "Beavis effect" (Beavis 1994). In this study it was shown that small (~100 individuals) mapping populations inflate the amount of variation explained by the QTL, and it was suggested that a group of >1000 individuals were necessary for accurate prediction. Furthermore, other studies have shown that small sample sizes lead to poor transferability of the detected QTLs to other populations (Melchinger et al. 1998).

A minor but still important factor affecting QTL detection is experimental error. In particular incorrect identification of marker alleles (genotyping) and mistakes in phenotype scoring can affect QTL accuracy. Replication of experiments and comparisons of genotyping between clones are procedures which can be used to reduce this type of error (Collard et al. 2005).

The potentially misleading effects of population size, experimental errors, and other factors underscore the importance of carrying out experimental confirmation of QTL detection before MAS is attempted. Confirmation should come not only from replications

of the experiment but also through QTL identification in independent populations. The use of independent populations allows for estimation of the widespread applicability of the QTL to populations of diverse genetic background. The process of testing the efficacy of markers in populations other than the original mapping population is called marker validation. Alternately, populations with simplified genetic structure such as RILs or NILs may be used to confirm QTLs. As stated above, the most important factor is population size. Experimental replications with a small number of samples may detect QTLs not found in the original population or may fail to confirm those identified in earlier populations. This is hypothesized to occur primarily due to the low chance of detecting QTLs with small effects or because of environmental effect (Melchinger et al. 1998).

In addition to increased testing for correlations with phenotype, QTL confirmation can also involve improvements in the genetic basis of the QTL. The identification of additional genetic markers on the mapped chromosomes, called fine mapping, may improve the estimation of a QTL's position. Moreover, statistical analyses which include the added markers may decrease the size of the QTL's confidence interval. Identification of markers closely linked to the QTL also reduces the chance of recombination which could otherwise separate the genetic marker from the trait. If this occurred, MAS results would become unreliable and misleading. Lastly, fine mapping aids in discrimination between multiple genes in the same region. However, as discussed above, to achieve the accuracy required to carry out fine mapping extremely large populations are needed (>1000 individuals).

This project has involved the identification of QTLs associated with quality scores and with the maintenance of those scores under heat stress. To confirm the detected QTLs,

a population of advanced wheat lines was phenotyped for grain quality and then genotyped with the previously identified trait linked SSR markers. The relationship between grain quality associated traits, including kernel hardness, kernel weight, mixograph development time (MDT), flour yield, grain yield, and SDS sedimentation score, and the mapped QTLs was determined. This allowed for the detection of possible pleiotropic effects of the QTLs across multiple traits. To test for a relationship between the tested markers and the stability of a trait across the three growing sites, the mean coefficient of variation (CV%) was compared between alleles. Since CV% is a measurement of variation within the group, alleles associating significantly with mean CV% would be showing a significant relationship with trait maintenance. The population of advanced lines used in this study is known as the South Texas Advanced (STA) population. These lines are derived from Halberd, the same line used as the source of heat tolerance in the recombinant inbred lines used to identify the QTLs.

Methodology

Line selection

Eighty of one hundred and fifty STA lines were tested for the presence of SSR markers associated with the previously detected quality and quality maintenance QTLs. The lines to be tested were selected based on their parentage; the majority of the selected advanced lines (87.5%) were descended from a cross with the heat tolerant line, Halberd. Selection of lines sharing this parent was done to encourage homology with the RIL population from which the quality and quality maintenance QTLs were originally derived. Fifteen parent cultivars were used: Halberd, TAM303, Lockett, Cutter, Jagger, TAM112,

TXCS8618, TXCS5009, TXCS8544, Pecos, Thunderbolt, TXCS8551, Trego, Seri82, and Ogallala. Seed for each line was collected from three nursing sites in Texas: College Station (CS), Castroville (CAS), and McGregor (MCG).

DNA extraction

DNA for each line was extracted from the tissue of petri dish sprouted seedlings. Until extraction, tissue was stored at -20ºC. DNA extraction followed the procedure from Pallotta et al. (2003). Briefly for each individual line, 500µl of heated (65ºC) extraction buffer (1L: 100mL 1M Tris-HCl PH 7.5, 100mL 0.5M EDTA pH 8.0, 125mL 10% SDS, and 675mL ddH_2O) was added to tubes containing the thawed, ground, seedling tissue. Tubes were shaken and incubated at 65ºC for 30 minutes, then 250µl of 6M NH_4OAc were added to the solution, and it was mixed then placed in a fridge for 15 minutes. Tubes were centrifuged for 15 min at 5000 RPM and the supernatant transferred to new tubes containing 360µl of isopropanol. DNA was allowed to precipitate overnight before being centrifuged at 5000 RPM and the supernatant removed. DNA pellets were washed in 70% ethanol and then dissolved in water and stored at -20 ºC.

Identification of SSR marker alleles present in selected advanced lines

The Qiagen QIAxcel system provided the level of resolution required and was used to genotype the lines. The genetic markers analyzed were *barc71*, *barc137*, *barc170*, *wmc468*, *wmc707*, *gwm337*, *barc119*, and *barc108*, and *gwm60*. These markers were amplified in 15µL PCR reactions containing; 15mM Tris-HCl, pH8.3, 75mM KCl, 2.5mM $MgCl_2$, 0.3mM dNTP, 37.5ng of each primer, ~36ng DNA, and 0.75U *Taq* polymerase. Alleles were identified based on comparison with parent lines.

Confirmation of the effect of the putative quality maintenance QTLs

The resultant SSR marker values were compared to quality results for each line. Statistical analyses were carried out in SPSS. A contrast analysis was used to test for a significant effect of parental alleles on seven of the QTLs associated genetic markers (*barc71*, barc137, *barc170*, *wmc468*, *wmc707*, *gwm337*, and *barc108*). CV% was used to compare lines for yield and quality stability (Table 3-4).

CV% = (standard deviation/mean)*100

Allele affects were analyzed by CV% of the following quality traits: SDS sedimentation, mixograph peak time, percent protein in flour, flour yield, kernel hardness, kernel weight, and grain yield. Additionally, a contrast analysis was performed comparing the mean allelic SDS sedimentation scores. This included specific comparison of parental alleles as well as a combination analysis comparing Halberd alleles with those of all other lines containing Halberd in the pedigree. Only marker alleles with three or more data points were analyzed. Quality scores between the various sites were compared using an analysis of variance.

Results

Tested lines were genotyped based on the nine tested markers (*barc71*, *barc137*, *barc170*, *wmc468*, *wmc707*, *gwm337*, *barc119*, and *barc108*). Only seven of the markers gave results suitable for further analysis. The results for the marker *gwm60* were poor and

could not be analyzed. The genotyping results for *barc119* could not be analyzed due to low polymorphism.

Analysis of variance (ANOVA) was used to test for significant differences between the quality scores of the three sites. Variances between SDS sedimentation scores from the three sites were found to be homogenous, but the data from one of the sites was not normally distributed. Statistical analysis found the other quality traits (seed weight, mixograph peak time, etc.) similarly lacking in normality. Due to these flaws, all data was transformed by square root prior to analysis. One data set, flour percent protein content, lacked homogenous variances and was not analyzed by ANOVA. ANOVA indicated significant differences between the average SDS sedimentation scores, flour yield, kernel hardness, kernel weight, and kernel diameter of the three growing sites (College Station, Castroville, and McGregor). Quality scores for these characters across the three growing sites displayed homogenous variances, as indicated by Levene's test.

Allelic variation at the test markers significantly affected the mean for the following quality traits: kernel hardness, mixograph peak time, kernel weight, grain yield, and SDS sedimentation column height. Additionally, polymorphism at marker alleles was found to have significant effects on the coefficient of variation (CV%) for the following quality traits: kernel hardness, SDS sedimentation, kernel weight, flour yield, grain yield, and kernel diameter (Table 3-4).

Mean kernel hardness was affected by allelic variation at markers *barc71* on 1B, flanking markers *wmc468* and *wmc707* on 4A, and *barc108* on 7A. At markers *barc71*, *wmc468*, and *barc108* the TXCS5009 allele differed significantly from the other alleles ($\alpha = 0.05$, $\alpha = 0.01$, and $\alpha = 0.05$, respectively). The allele was associated with increased

hardness at markers *wmc468* and *barc108* and with decreased hardness at *barc71*. At marker *wmc707*, the Halberd and TAM303 alleles were both significantly associated with low hardness levels ($\alpha = 0.05$; Table 3-4).

The alleles for cultivar TXCS5009 also had an effect on kernel hardness CV% at markers *barc170* and *wmc468* on 4A, and *barc108* on 7A ($\alpha=0.01$, $\alpha = 0.01$, and $\alpha = 0.05$, respectively; Table 3-4). In all three cases the TXCS5009 CV% was lower than that of the other markers, indicating an association with stability of the kernel hardness trait across growing sites (Table 3-4). The TXCS5009 allele for marker *barc71* of chromosome 1B was significantly associated with a higher yield result.

Mean mixograph peak time was significantly affected by allelic variation at the *gwm337* marker on chromosome 1D ($\alpha=0.05$). The Halberd allele at this site was associated with an increased mixograph peak time. None of the tested markers showed a significant association between allelic variation and mixograph peak time CV% (Table 3-4).

Table 3: A contrast analysis of quality data segregated by allele for genotypes grown in South Texas Advanced nursery trials in 2008. Cultivar Halberd used in RIL mapping is the common parent cross to the other elite cultivars listed

			Hardness		MDT		Kernel weight		Flour yield		SDS sedimentation			Grain yield	
QTL	Marker	Line	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%	Std Dev	Mean	CV%
qsdscon.tam-1B	*barc71*-1B	Halberd	52.9^{a**}	14.1^{a}	2.3^{a}	8.1^{a}	31.3^{b}	12.0^{b}	68.3^{a}	2.2^{a*}	5.5^{a**}	15.5^{b}	0.8^{a}	46.3^{b}	23.2^{a}
		TAM303									6.9^{b}	13.9^{b}	0.9^{a}		
		Cutter									6.2^{b}	36.2^{b}	2.1^{a}		
		TXCS5009	66.0^{c**}	11.9^{a}	2.9^{a}	6.5^{a}	28.7^{a**}	7.3^{a**}	71.0^{a}	1.6^{b}	6.6^{b}	21.8^{b}	1.4^{a}	51.7^{a*}	20.1^{a}
		Pecos	60.5^{b}	11.2^{a}	2.8^{a}	7.7^{a}	31.8^{b}	9.9^{b}	74.9^{a}	1.1^{b}	7.3^{b}	7.2^{b}	0.5^{a}	45.5^{b}	20.4^{a}
		Thunderbolt									7.9^{b}	9.8^{b}	0.7^{a}	45.3^{b}	28.1^{a}
		Trego									5.7^{b}	27.6^{a*}	1.5^{a}	46.1^{b}	27.0^{a}
		Seri82									6.5^{b}	0.0^{b}	0.0^{a}		
		Ogallala									6.0^{b}	14.4^{b}	0.8^{a}	46.5^{b}	26.9^{a}
	barc137-1B	Halberd	58.1^{a}	14.6^{a}	3.0^{a}	6.6^{a}	30.4^{a}	14.0^{a*}	72.6^{a}	2.6^{a}	6.7^{a*}	16.2^{a}	1.1^{a}	50.2^{a}	24.8^{a}
		TAM303	54.9^{a}	16.9^{a}	2.2^{a}	10.5^{a}	30.9^{a}	13.6^{b}	71.9^{a}	2.4^{a}	5.5^{b}	14.8^{a}	0.8^{a}	45.5^{a}	27.0^{a}
		TXCS5009	66.4^{a}	7.2^{a}	2.2^{a}	7.9^{a}	27.9^{a}	5.1^{b}	69.2^{a}	1.6^{a}	6.2^{b}	20.9^{a}	1.3^{a}	49.5^{a}	20.8^{a}
		TXCS8544	53.2^{a}	8.9^{a}	3.0^{a}	3.8^{a}	28.6^{a}	8.2^{b}	73.3^{a}	2.3^{a}	4.8^{b}	18.2^{a}	0.8^{a}	46.7^{a}	13.9^{a}
		Pecos	60.8^{a}	11.6^{a}	2.8^{a}	7.5^{a}	31.6^{a}	9.3^{b}	74.7^{a}	0.6^{a}	7.0^{c**}	5.7^{a}	0.4^{a}	45.9^{a}	20.5^{a}
qsdsheat.tam-1D	*gwm337*-1D	Halberd	57.7^{a}	14.9^{a}	2.7^{a*}	6.9^{a}	31.1^{a}	11.0^{a}	72.8^{a}	1.9^{a}	6.2^{a}	16.9^{b}	1.0^{b}	46.8^{a}	20.8^{b}
		TAM303	52.9^{a}	15.8^{a}	2.1^{b*}	9.8^{a}	31.4^{a}	15.1^{a}	63.8^{a}	3.0^{a}	5.8^{a}	13.4^{b}	0.8^{b}	47.4^{a}	27.8^{b}
		TXCS8618									5.6^{a}	7.8^{b}	0.4^{b}	45.5^{a}	24.5^{b}
		Thunderbolt									7.9^{a}	9.8^{b}	0.7^{b}	45.3^{a}	28.1^{b}
		TXCS8551									5.6^{a}	6.1^{b}	0.3^{b}	48.7^{a}	18.4^{b}
		Trego									5.6^{a}	37.8^{a**}	2.0^{a**}	42.0^{a}	37.8^{a*}
qsdssta.tam-7A	*barc108*-7A	Halberd	54.3^{b}	11.9^{b}	2.6^{a}	5.6^{a}	31.1^{b}	9.8^{a}	73.7^{a}	2.2^{a}	5.7^{b}	19.7^{a}	1.1^{b}	45.1^{a}	22.1^{a}
		TAM303	54.5^{b}	16.1^{b}	2.2^{a}	7.4^{a}	32.1^{b}	15.0^{a}	60.8^{a}	2.9^{a}	6.0^{b}	15.1^{a}	0.9^{b}	49.4^{a}	26.7^{a}
		TAM112	54.8^{b}	26.2^{b}	3.0^{a}	2.2^{a}	30.9^{b}	15.8^{a}	72.0^{a}	2.8^{a}	6.5^{b}	21.7^{a}	1.3^{b}	49.7^{a}	24.9^{a}
		TXCS8618									5.4^{b}	6.8^{a}	0.4^{b}	44.6^{a}	20.9^{a}
		TXCS5009	66.1^{a*}	8.1^{a*}	2.7^{a}	7.3^{a}	28.2^{a*}	7.5^{a}	70.6^{a}	1.9^{a}	6.6^{b}	22.8^{a}	1.5^{a*}	51.3^{a}	20.5^{a}
		TXCS8544									6.2^{b}	12.8^{a}	0.8^{b}	55.1^{a}	17.5^{a}
		Pecos	60.8^{b}	11.6^{b}	2.8^{a}	7.5^{a}	31.6^{b}	9.3^{a}	74.7^{a}	0.6^{a}	7.0^{b}	5.7^{a}	0.4^{b}	45.9^{a}	20.5^{a}
		Thunderbolt									7.5^{a*}	10.7^{a}	0.8^{b}	45.5^{a}	22.3^{a}
		Trego									5.6^{b}	20.5^{a}	1.1^{b}	44.9^{a}	30.1^{a}

** = significant at $\alpha = 0.01$, * = significant at $\alpha = 0.05$ between allelic mean of quality values or between CV% of quality values

Table 3: Continued

			Hardness		MDT		Kernel Weight		Flour Yield		SDS Sedimentation			Grain yield	
QTL	Marker	Line	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%	Std Dev	Mean	CV%
qsdscon.tam-4A	*wmc468*-4A	Halberd	55.2^{b}	14.7^{b}	2.3^{a}	9.9^{a}	31.5^{b}	12.7^{a}	73.3^{a}	2.3^{a}	6.0^{b}	10.6^{a*}	0.6^{a*}	47.0^{b}	24.1^{a}
		TAM303	53.7^{b}	16.5^{b}	2.4^{a}	7.6^{a}	31.8^{a}*	13.8^{a}	72.4^{a}	2.7^{a}	5.7^{b}	13.1^{b}	0.8^{b}	45.8^{b}	22.9^{a}
		TAM112	54.8^{b}	26.2^{b}	3.0^{a}	2.2^{a}	30.9^{c**}	15.8^{a}	72.0^{a}	2.8^{a}	5.9^{b}	24.0^{b}	1.2^{c**}	55.5^{b}	23.8^{a}
		TXCS5009	65.4^{a**}	8.9^{a**}	2.9^{a}	6.5^{a}	28.3^{b}	8.6^{a}	71.2^{a}	1.8^{a}	6.7^{b}	22.4^{b}	1.5^{b}	51.7^{b}	20.1^{a}
		TXCS8544	55.6^{b}	10.7^{b}	3.4^{a}	3.7^{a}	28.7^{b}	8.3^{a}	71.9^{a}	2.3^{a}	5.9^{b}	9.6^{b}	0.6^{b}	51.2^{b}	18.6^{a}
		Thunderbolt									7.7^{a*}	14.8^{b}	1.1^{b}	41.8^{a}*	24.7^{a}
	wmc707-4A	Halberd	53.4^{a*}	14.3^{a}	2.1^{a}	9.9^{a}	31.5^{a}	13.4^{a}	62.9^{a}	2.1^{a}	5.4^{a**}	13.7^{b}	0.7^{b}	47.6^{b}	26.4^{a}
		TAM303	53.1^{b*}	16.3^{a}	2.5^{a}	7.4^{a}	31.8^{a}	12.0^{a}	72.8^{a}	2.3^{a}	5.9^{b}	12.2^{b}	0.7^{b}	44.9^{b}	22.3^{a}
		TAM112	54.8^{c}	26.2^{a}	3.0^{a}	2.2^{a}	30.9^{a}	15.8^{a}	72.0^{a}	2.8^{a}	5.9^{b}	24.1^{b}	1.2^{b}	55.5^{b}	23.8^{a}
		TXCS5009	66.4^{c}	7.2^{a}	2.2^{a}	7.9^{a}	27.9^{a}	5.2^{a}	69.2^{a}	1.6^{a}	6.2^{b}	23.3^{b}	1.4^{b}	50.9^{b}	20.7^{a}
		TXCS8544									5.4^{b}	11.7^{b}	0.6^{b}	45.1^{b}	20.3^{a}
		Pecos	60.1^{c}	11.7^{a}	2.6^{a}	7.3^{a}	31.6^{a}	9.0^{a}	74.6^{a}	1.4^{a}	7.4^{b}	9.1^{b}	0.7^{b}	46.8^{b}	19.6^{a}
		Thunderbolt									7.3^{c*}	13.8^{b}	1.0^{b}	39.9^{a*}	24.0^{a}
		Trego									5.5^{b}	29.9^{a*}	1.6^{a*}	43.4^{b}	26.9^{a}
		Seri82									6.0^{b}	5.8^{b}	0.3^{b}	48.0^{b}	33.8^{a}
		Ogallala									6.3^{b}	10.3^{b}	0.7^{b}		
	barc170-4A	Halberd	44.4^{a}	13.3^{b}	2.3^{a}	9.9^{a}	31.6^{b}	12.5^{a}	73.6^{a}	2.1^{a}	5.7^{a**}	14.8^{a}	0.8^{b}	46.0^{a}	25.6^{a}
		TAM303	53.2^{a}	15.3^{b}	2.3^{a}	8.9^{a}	31.6^{b}	13.6^{a}	72.5^{a}	1.9^{a}	5.9^{b}	12.9^{a}	0.8^{b}	46.3^{a}	20.7^{a}
		TAM112	54.8^{a}	26.2^{b}	3.0^{a}	2.2^{a}	30.9^{b}	15.8^{a}	72.0^{a}	2.8^{a}	6.5^{b}	21.7^{a}	1.3^{b}	49.7^{a}	24.9^{a}
		TXCS5009	65.4^{a}	8.9^{a**}	2.9^{a}	6.5^{a}	28.3^{a**}	8.6^{a}	71.2^{a}	1.8^{a}	6.8^{b}	23.1^{a}	1.6^{a*}	51.6^{a}	20.2^{a}
		TXCS8544	55.6^{a}	10.7^{b}	3.4^{a}	3.7^{a}	28.7^{b}	8.3^{a}	71.9^{a}	2.3^{a}	5.9^{b}	9.6^{a}	0.6^{b}	51.2^{a}	18.6^{a}
		Pecos	60.1^{b}	11.7^{b}	2.6^{a}	7.3^{a}	31.6^{b}	9.0^{a}	74.6^{a}	1.4^{a}	7.4^{b}	9.1^{a}	0.7^{b}	46.8^{a}	19.6^{a}
		Thunderbolt									7.7^{b}	14.8^{a}	1.1^{b}	41.8^{a}	24.7^{a}
		Trego									5.6^{b}	32.8^{a}	1.8^{b}		

** = significant at $\alpha = 0.01$, * = significant at $\alpha = 0.05$ between allelic mean of quality values or between CV% of quality values

Table 4: Comparison of allelic means between cultivar Halberd and all other lines

		Hardness		MDT		Kernel Weight		Flour Yield		SDS sedimentation			Grain yield	
	Line	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%	Std Dev	Mean	CV%
Barc71-1B	Halberd	52.9^{a**}	14.1^{a*}	2.3^{a*}	8.1^{a}	31.3^{a}	12.0^{a}	68.3^{a}	2.2^{a}	5.5^{b**}	15.5^{a}	0.8^{a}	46.3^{a}	23.2^{a}
	Other	62.8^{b**}	10.1^{b*}	3.1^{b*}	6.3^{a}	30.4^{a}	9.5^{a}	72.8^{a}	1.6^{a}	6.7^{a**}	20.1^{a}	1.1^{a}	48.1^{a}	22.0^{a}
Barc137-1B	Halberd	58.1^{a}	14.6^{a}	3.0^{a}	6.6^{a}	30.4^{a}	14.0^{a*}	72.6^{a}	2.6^{a}	6.7^{a*}	16.2^{a}	1.1^{a}	50.24^{a}	24.8^{a}
	Other	58.4^{a}	11.8^{a}	2.5^{a}	7.8^{a}	29.9^{a}	9.6^{b*}	72.2^{a}	1.8^{a}	5.7^{b*}	16.8^{a}	0.9^{a}	47.3^{a}	20.6^{a}
GWM337-1D	Halberd	57.7^{a}	14.9^{a}	2.7^{a*}	6.9^{a}	31.1^{a}	11.0^{a}	72.8^{a}	1.9^{a}	6.2^{a}	16.9^{a}	1.0^{a}	46.8^{a}	20.8^{a*}
	Other	52.9^{a}	15.8^{a}	2.1^{b*}	9.8^{a}	31.4^{a}	15.1^{a}	63.8^{a}	3.0^{a}	5.9^{a}	14.6^{a}	0.8^{a}	46.3^{a}	27.8^{b*}
Barc170-4A	Halberd	54.6^{a}	13.3^{a}	2.3^{a}	9.9^{a}	31.6^{a}	12.5^{a}	73.6^{a}	2.1^{a}	5.7^{a}	14.8^{a}	0.8^{a*}	46.0^{a}	25.6^{a}
	Other	57.9^{a}	13.5^{a}	2.9^{a}	6.1^{a}	30.4^{a}	10.8^{a}	72.3^{a}	2.3^{a}	6.3^{a}	17.8^{a}	1.1^{b*}	47.2^{a}	22.3^{a}
WMC468-4A	Halberd	55.2^{a}	14.7^{a}	2.3^{a}	9.9^{a}	31.5^{a}	12.7^{a}	73.3^{a}	2.3^{a}	6.0^{b}	10.6^{a*}	0.6^{a*}	47.0^{a}	24.1^{a}
		57.8^{a}	14.8^{a}	2.8^{a}	5.8^{a}	30.1^{a}	11.7^{a}	71.9^{a}	2.4^{a}	6.3^{b}	16.5^{b*}	1.0^{b*}	48.7^{a}	21.8^{a}
WMC707-4A	Halberd	53.3^{a}	14.3^{a}	2.11^{a}	9.9^{a}	31.5^{a}	13.4^{a}	62.9^{a}	2.1^{a}	5.4^{a*}	13.7^{a}	0.7^{b}	47.6^{a}	26.4^{a}
	Other	56.5^{a}	15.8^{a}	2.6^{a}	6.7^{a}	31.0^{a}	11.1^{a}	72.4^{a}	2.2^{a}	6.2^{b*}	15.4^{a}	0.9^{b}	46.3^{a}	22.4^{a}
Barc108-7A	Halberd	54.3^{a}	11.9^{a}	2.6^{a}	5.6^{a}	31.1^{a}	9.8^{a}	73.7^{a}	2.2^{a}	5.7^{a}	19.7^{a}	1.1^{a}	45.1^{a}	22.1^{a}
	Other	58.5^{a}	15.0^{a}	2.8^{a}	5.9^{a}	30.9^{a}	12.3^{a}	67.4^{a}	2.3^{a}	6.3^{a}	15.5^{a}	0.9^{a}	48.5^{a}	23.7^{a}

** = significant at $\alpha = 0.01$, * = significant at $\alpha = 0.05$ between allelic mean of quality values or between CV% of quality values
Only lines with halberd in the pedigree were included in this analysis

Mean kernel weight was affected by allelic variation at *barc170* and *wmc468* on chromosome 4A, as well as by allelic variation at marker *barc108* on chromosome 7A. The significant association with mean kernel weight at markers *barc170* and barc108 was due to variation at the TXCS5009 allele (α=0.01and α=0.05, respectively). At marker *wmc468*, both the TAM303 and TAM112 alleles were significantly associated with high mean kernel weights (α=0.05, and α=0.01, respectively; Table 3-4).

Kernel weight CV% was significantly affected by allelic variation at marker *barc71* and *barc137*, both on chromosome 1B. The TXCS5009 allele at marker *barc71* was significantly associated with decreased kernel weight variation (α=0.01). The Halberd allele of marker *barc137*, on 1B, was significantly associated with a higher variability in kernel weight (α=0.05).

Variation in mean flour yield was not associated with allelic variation at any of the tested loci. However, allelic variation at marker *barc71*, on chromosome 1B, correlated significantly with flour yield CV%. The Halberd allele at this locus was associated with an increased variation (α=0.05).

Mean SDS sedimentation value showed a significant relationship to allelic variation at markers *barc71, barc137, barc170, wmc468, wmc707,* and *barc108* (Table 3-4). Only marker *gwm337* on chromosome 1D did not show a significant correlation with this trait. The Halberd allele of markers *barc71* and *barc137*, both on chromosome 1B, associated alternately with decreased and increased SDS values, respectively (Table 3-4). Additionally, the Pecos allele at the *barc137* locus correlated significantly with a high mean SDS sedimentation value (Table 3-4). The Halberd allele of markers *barc170*

and *wmc707*, on chromosome 4A, associated significantly with decreased mean SDS sedimentation values (α=0.01, both; Table 3-4). The Thunderbolt allele of markers *wmc468* and *wmc707*, also on chromosome 4A, was significantly associated with high mean SDS sedimentation values (α=0.05, both; Table 3-4). The Thunderbolt allele of marker *barc108* on chromosome 7A was also associated with a high mean SDS sedimentation value (α=0.05).

The CV% for SDS sedimentation was significantly associated with allelic variation at markers *barc71, gwm337, wmc468,* and *wmc707* (Table 3-4). The Trego allele on markers *barc71, gwm337,* and *wmc707* was associated with a high CV% for SDS sedimentation (α=0.05, α=0.01, and α=0.05, respectively; Table 3-4). On marker *wmc468*, chromosome 4A, the significant effect was associated with the Halberd allele and a low CV% (α=0.05).

Allelic variation at markers *barc71*, *wmc468,* and *wmc707* associated significantly with mean grain yield (α = 0.05 for each). Mean grain yield CV% was significantly affected by allelic variation at the *gwm337* locus (α=0.05). The Halberd allele in the lumped analysis was associated with lower grain yield while analysis by allele showed the Trego allele associating significantly with a high grain yield.

A contrast analysis of the trait flour percent protein content was run not assuming homogeneity of variances, as per the results of the ANOVA. The allele for cultivar Halberd at marker *gwm337* was significant (α=0.01) for the CV% of flour percent protein content. The average value was lower, with a contrast effect of ~-6.

Pearson correlation of SDS results indicated a significant ($\alpha = 0.01$) relationship between SDS sedimentation and mixograph peak time. Linear regression indicated a significant ($\alpha = 0.01$) positive correlation between the two variables (data not shown).

Discussion

Analysis of the STA lines was carried out in order to provide confirmation for the effect of the detected QTLs on bread making quality characters. Coefficient of variance (CV%) was used to estimate stability and the mean values were analyzed to determine the level of the trait associated with that QTLs. Additionally, the existence of mixograph scores for the STA lines allowed a comparison between mixograph and SDS results. Mixograph analysis is an accepted method for predicting the bread-making quality of flour. The significant correlation detected between SDS sedimentation and mixograph scores in this study indicates the suitability of SDS sedimentation as a bread-making quality predictor. QTLs associated with the SDS sedimentation column height trait and the stability of that trait could therefore be suitable for use in line selection.

Coefficient of variance measures of the variability present in the analyzed trait. When selecting for trait stability the allele associating significantly with the lower CV% is therefore the desired value. Although no specific environmental stress was imposed, heat stress is common in the three nursery locations. The low CV% values may therefore be taken to indicate a general tolerance to environmental stress, such as heat, across the three growing sites. Markers found to be associated with a low CV% may confirm the

association of the detected QTLs with improved quality stability as indicated by the SDS sedimentation test.

Only the Halberd allele of marker *wmc468* on chromosome 4A showed a significant correlation with a low CV% for SDS sedimentation. Allelic variation at this same marker significantly affected the traits of kernel hardness, kernel hardness CV%, kernel weight, and mean SDS sedimentation. The other markers, *barc170* (4A) and *wmc707* (4A), associated with hardness CV%, mean weight, and mean SDS sedimentation score; and mean kernel hardness, SDS mean, and SDS CV%, respectively. The association of all three chromosome 4A markers with SDS sedimentation may provide support for our detected QTL for SDS sedimentation level under control conditions. The effect on the additional characters may reflect pleiotropic effects of genes associated with these loci. Some of these, particularly the association of markers *wmc468* and *wmc707* with kernel hardness CV% could be of importance when breeding. The kernel hardness trait is important for the textural classification of wheat varieties and has been shown to be highly variable, and strongly influenced by environmental factors (Huebner 1992). The effects of the chromosome 4A markers on kernel hardness and other bread making traits may be associated with the presence of a tillering QTL contributed by Halberd that was detected in this region by Mason et al (In review). The Thunderbolt alleles of markers *wmc707* and *wmc468* on chromosome 4A were associated with significantly lower grain yield. This result may hint at a pleiotropic effect of the chromosome 4A QTL affecting both grain quality and grain yield.

The markers on chromosome 1B, *barc71* and *barc137,* associated significantly with the traits of kernel hardness, kernel weight CV%, flour yield CV%, mean SDS sedimentation, and SDS sedimentation CV%. Of these, only mean SDS sedimentation was significantly affected by both markers. The Halberd allele at these markers differed in its effect: on *barc71* it associated with low mean SDS sedimentation and on *barc137* it associated with high mean SDS sedimentation. This may indicative of differences in the recombination rate between the markers and the QTL or may indicate the presence of multiple QTLs associated with these loci. Overall though, the association between the chromosome 1B markers and mean SDS sedimentation level supports our identification of an SDS control QTL nearby. The effects of these markers may also be related to the presence of the *Glu-B3* and *Gli-B1* loci. These loci, identified to the short arm of chromosome 1B, have been demonstrated to have a significant effect on bread-making quality (Nieto-Taladriz et al. 1994; Wang et al. 2009).

The marker on 1D, *gwm337,* associated significantly only with SDS CV% and with mixograph peak time. The allele significant for SDS CV% was the Trego allele which may be an outlier. The Trego allele for three markers (*wmc707, gwm337,* and *barc71*) on different chromosomes associated significantly with high SDS CV% values and always caused an extreme increase in the SDS CV%. The Trego allele also showed a significant relationship with the grain yield trait. The only support for the purported grain quality QTL detected at this region is therefore the association with mixograph peak time, although it would be hoped that the SDS sedimentation scores would mirror

this result. More markers need to be mapped on this chromosome to provide confirmation of its effects.

The Halberd allele of the *gwm337* marker on chromosome 1D was significantly associated with a smaller percent protein content CV% than the Cutter allele. However, the growing site data for this trait showed non-homogenous variances, casting doubt on the reliability of this analysis. Protein content has an effect on both SDS sedimentation level and yield, and has been shown to be affected by heat as well as other forms of abiotic stress (Dupont et al. 2006; Randall and Moss 1990). These stressors have an additional effect on protein quality, which affects SDS sedimentation score and other quality measures. Additionally, an inverse relationship has been found between protein quality and yield (Blanco et al. 2002). It is interesting to note that there was not a significant relationship between SDS sedimentation scores and the Halberd allele of this marker. It was however, significantly associated with MDT, albeit with a higher value than the Cutter allele (Table 3-4). Variations in protein production due to environmental stress may therefore not completely explain the detected statistical relationship. Further analysis would need to be carried out to determine the reliability of this marker since its detection relied on the combination of data from groups lacking homogeneity of variances.

Chromosome 7A, which houses the detected SDS percent change QTL was represented in this analysis by marker *barc108.* Allelic variation at this marker associated significantly with mean hardness, hardness CV%, mean weight, and mean SDS sedimentation value. Of these, only the association with hardness CV% indicates a

possible relationship with the maintenance of quality traits. However, the other correlations indicate some relationship to quality parameters, but the high recombination rate between this marker and the QTL means that the QTL's effects may be poorly represented here. To confirm the presence of this QTL, it will be necessary to successfully genotype the other associated marker, *gwm60*, and/or to develop additional genetic markers in the same region.

The detected alleles with significant effects support the detected SDS sedimentation QTLs on chromosomes 1B, 1D, and 4A. Additionally, allelic variation on chromosome 4A may be associated with variations in kernel hardness, giving a possible pleiotropic effect of our detected QTL. The analysis of the chromosome 7A *barc108* marker showed a poor relationship with bread making characters and provided little support for the presence of the identified QTL. However, this marker is one of only two associated with the 7A quality maintenance QTL which is present in a large region lacking markers. The presence of several QTLs in this region (SDS percent change, flag leaf width, and yield maintenance (Mason et al, In review) implies that an effect may be found if more markers are established.

CHAPTER IV

SUMMARY AND CONCLUSION

In this project, four QTLs associated with SDS sedimentation levels were identified in a population of *T. aestivum* RILs. The association of the QTLs with SDS sedimentation levels as well as with other quality traits was confirmed through comparison of allelic and trait variability in a population of advanced lines. In addition, two of the markers associated with these quality QTLs demonstrated a significant association with mean grain yield and one showed a significant relationship to grain yield maintenance. Such findings indicate a possible pleiotropic effect between yield and grain quality. The identified QTLs may be valuable in MAS, allowing the simulataneous development of lines possessing both traits. Furthermore, fine mapping based on the identified genetic regions may provide insight into genes important for both quality and for yield.

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