

**GENETIC ANALYSIS OF BREAD MAKING QUALITY STABILITY IN
WHEAT
USING A HALBERD X LEN RECOMBINANT INBRED LINE POPULATION**

A Thesis

by

ASHIMA POUDEL

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

May 2012

Major Subject: Plant Breeding

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Recombinant Inbred Line Population

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ABSTRACT

Genetic Analysis of Bread Making Quality Stability in Wheat Using a Halberd X Len
Recombinant Inbred Line Population. (May 2012)

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Wheat grain quality has a complex genetic architecture heavily influenced by the growing environment. Consistency in wheat quality not only affects the efficiency of milling and baking but also the quality of end-use products. The objectives of this study were to 1) analyze the different wheat quality parameters in Recombinant Inbred Lines (RILs) grown under different environments, and 2) to identify Quantitative Trait Loci (QTLs) associated with quality stability in RILs grown under different environments. A set of 180 RILs derived from two spring wheat lines ‘Halberd’ and ‘Len’ were grown at Uvalde and College Station TX, in the 2009/2010 growing season and at Chillicothe and College Station TX, in 2010/2011 growing seasons. The experiment was laid out in Randomized Complete Block Design (RCBD) with four replications within each location. Each line was tested for multiple quality traits that included grain hardness, protein content, dough mixing properties and bread baking quality using Single Kernel Characterization System (SKCS), Near-Infrared Reflectance Spectrometry (NIRS) analysis, mixograph and the Sodium Dodecyl Sulfate Sedimentation (SDSS) test. Genetic linkage map construction was carried out with 116 single nucleotide

polymorphism (SNP) markers in the RILs. Then composite interval mapping was carried out to identify QTLs associated with quality traits.

The SDSS column height was positively correlated across four environments. Similarly, it was found to have significant positive correlation with mixing tolerance and peak time within and also across locations. However, the SDSS was negatively correlated with the hardness index. The protein percent was not significant with any of the quality traits within and across environments. We were able to detect many QTLs for different quality traits but most of them were site specific. Only a few QTLs were consistent across environments. Most of the QTLs for quality traits i.e., SDSS, peak time, mixing tolerance and hardness index were identified on chromosome 1B. We were able to detect overlapped QTLs for SDSS column height and mixing tolerance on chromosome 1B. Furthermore, overlapping QTLs for mixing tolerance and peak time were detected on an unknown chromosome. We also detected overlapping QTLs for hardness index on chromosome 1B. We identified one stable QTL for SDSS column height on chromosome 4B. This QTL was detected based on the coefficient of variation (CV) for SDSS in four different environments.

DEDICATION

To my parents: Mr. Binod K. Upadhyay and Mrs. Kalpana Poudel

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NOMENCLATURE

ANOVA	Analysis of Variance
CV	Coefficient of Variation
DIAM	Kernel Diameter
HI	Hardness Index
HMWG	High Molecular Weight Glutenin
KWT	Kernel Weight
LMWG	Low Molecular Weight Glutenin
LOD	Logarithm of Odd
NIRS	Near Infra-red Reflectance Spectrometry
PROT	Protein Percent
PT	Peak Time
QTL	Quantitative Trait Loci
RCBD	Randomized Complete Block Design
RILs	Recombinant Inbred Lines
SDSS	Sodium Dodecyl Sulfate Sedimentation
SKCS	Single Kernel Characterization System
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats
TOL	Mixing Tolerance

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1. Background

Wheat is one of the most important food crops in terms of its production acreage and human consumption worldwide. One of the reasons for its popularity and importance in world agriculture and the economy is the unique properties of its protein, which make it useful for dough that can be processed into different kinds of food products like bread, biscuits, noodles, breakfast cereals, cakes etc. (Neacșu et al., 2009). Due to significant changes in wheat consumption and end-use pattern, wheat quality has been one of the primary breeding targets in national and international breeding programs (Meng et al., 2009). Being a polygenic trait, bread making quality should be explored as a function of multiple small-effect genetic loci and the environmental factors under which particular genotypes are grown. Thus, in order to achieve genetic gain of wheat quality traits, both genetic as well as environmental components should be explored and characterized. The unknown genetic components affecting wheat quality traits such as protein content, grain hardness, gluten strength etc., can be identified and characterized using linkage based quantitative trait loci (QTL) analysis. Though several QTL analysis studies have already documented for many wheat quality traits, the usefulness of identified QTLs to develop successful cultivars is still uncertain. This is because the identified QTLs may not be reliable for all environments where a cultivar is grown.

This thesis follows the style of Crop Science.

Every year unpredictable fluctuation in growing conditions occurs due to changes in temperature and rainfall. This has a negative impact on wheat quality. Mixing and baking quality is especially influenced by environmental stress (Peterson et al., 1998). The milling and baking industries require consistent end-use quality to meet the demands for modern high speed processing facilities (Peterson et al., 1998). The goal of this study was to define QTL that regulate end-use quality stability across different environmental conditions. Identification of stable QTLs in wheat chromosomes can be used for marker assisted breeding. *The hypothesis of this study is that the genetic loci regulating quality stability overlaps with the loci regulating yield stability in wheat. We further hypothesize that those important quality stability and their genetic loci can be used for selection irrespective of environment under which the breeding lines are grown.*

2. Objectives

There were two working objectives of this study:

- 1. Analyze different wheat quality parameters in Recombinant Inbred Lines (RILs) grown under different environmental conditions*
- 2. Define the genetic loci regulating end-use quality stability for lines grown under different environments*

This work will identify relevant wheat quality parameters and evaluate their interrelationship within and between different growing environments. Identification and evaluation of highly heritable quality traits will be useful to locate corresponding QTLs in wheat chromosomes which can be used for marker assisted breeding. Detection of

significant QTLs regulating end-use quality stability will be a milestone achievement in breeding programs that are targeting improving end-use quality. The availability of reliable markers for quality traits could also enhance marker aided breeding focused on yield components.

3. Review of literature

3.1. Wheat grain quality and its genetic control

Wheat can be grown in varied environmental condition, except in some warm tropical areas (Bushuk, 1998). Significant research has and continues to be conducted on wheat crop and grain characteristics because of its importance in food processing and human nutrition (Shewry et al., 2003). Wheat cultivars possess quality differences which are very important in grain trading due to social trends and global economy (Pena, 2002). The seed storage protein, which accounts for more than 50% of the total protein in the mature cereal grain, is very important for human and livestock because of its high nutritional value (Shewry et al., 2003). Along with the nutritional value of wheat protein, its functional properties are also equally important. End-use quality of wheat is determined by number of intrinsic compositional and functionality factors such as kernel texture, seed coat color, milling characteristics, enzyme activity, flour protein contents, gluten protein composition and dough characteristic (Ross and Bettge, 2009).

The end-use properties of the grain is affected by its protein, which in cereal grain generally ranges from 10-15% of the grain dry weight (Shewry and Halford, 2002). The proteins of cereal grains other than wheat do not have dough forming properties.

Wheat gluten protein, a major storage protein of wheat, is responsible for the functional properties of dough in its various end-uses (Shewry et al., 1995). The gluten protein is divided into two groups; monomeric gliadin and polymeric glutenin. Further, gliadin is divided into four groups i.e., α , β , γ and ω gliadins (Gianibelli et al., 2001). Whereas, glutenin consists of High Molecular Weight Glutenin (HMWG) subunits and Low Molecular Weight Glutenin (LMWG) subunits. The wheat storage protein contains approximately 50% gliadin, 10% high molecular weight glutenin subunits and 40% low molecular weight glutenin subunits (Payne et al., 1984). The genes of wheat storage protein occur at nine different complex loci in the genome (Payne et al., 1984). In the short arms of chromosome 1A, 1B, and 1D the genes coding for the low molecular weight glutenin subunits can be found at the locus Glu-A3, Glu-B3, and Glu-D3 respectively (Gianibelli et al., 2001). The HMW-GS are located on the long arm of homeologous group 1 chromosomes (Payne et al., 1981; 1987), coded by the genes at three loci, Glu-A1, Glu-B1, and Glu-D1 on the chromosomes 1A, 1B and 1D respectively (Payne et al., 1984).

The physical properties of dough which exhibit both elasticity and extensibility should be in balance for good bread making quality (Shewry et al., 1995). In bread dough, glutenin imparts elasticity, whereas, gliadin is viscous and gives extensibility (Payne et al., 1984). Though gliadins are not directly related to wheat quality in terms of dough strength, they contribute to the viscosity and extensibility of gluten (Gianibelli et al., 2001). But, strong dough is desirable for good bread making and its strength is determined by its elasticity (Shewry et al., 1995). Insufficient elasticity cause poor

dough strength (Payne et al., 1987). Uthayakumaran et al. (1999) mentioned that flour with the stronger dough is achieved with the increase in glutenin to gliadin ratio at some desired protein level. Glutenin is the most important protein for the quality parameters of the flour (Gupta et al., 1992). High as well as low molecular weight glutenin subunits are very important for dough properties. The viscoelastic properties of the dough are governed by both LMW-GS and HMW-GS (Masci et al., 1998). Gupta et al. (1995) reported that, as the subunit controlled by the loci Glu-1 or Glu-3 is removed there is diminish elasticity in the gluten protein. The Glu-1 and Glu-3 subunits together can interact and form larger polymers which are very essential in dough properties instead of the presence of single locus subunits polymer (Gupta et al., 1995).

Among the different components of gluten protein, the high molecular weight glutenin subunits, are quantitatively minor but functionally important for overall end-use quality (Shewry et al., 1992). They determine the end use quality and dough properties of flour and the specific alleles of high molecular weight glutenin subunits can be very contributing to predict the good bread making quality of wheat (Anjum et al., 2007). The bread wheat contains six different high molecular weight glutenin subunits but the one subunit is always silent (Anjum et al., 2007; Gianibelli et al., 2001), thus the bread wheat contain at least three to five subunits of HMWG (Payne et al., 1981). The bread making quality of different cultivars varies because of the allelic variation in each locus (Payne et al., 1984). The 1Dx5+ 1Dy10 is thought to the most important allelic subunit pair in terms of quality, after this 1Ax subunits 1 and 2, and the 1B subunit pairs 1Bx17+1By18 and 1Bx7 + 1By8 (Shewry et al., 1992). Gupta et al. (1992) reported that the ratio of

HMW to LMW glutenin subunits is very important in determining the flour quality, as this ratio increases for fixed glutenin content the quality of the flour increases.

3.2. Wheat grain quality measurements

3.2.1. Single kernel characterization system (SKCS)

The single kernel characterization system is a widely used instrument for determining kernel hardness, kernel weight, moisture content and kernel diameter. In SKCS 300 kernels are analyzed individually for each measurement, that includes the average and standard deviation of kernel hardness index, moisture content, kernel diameter and kernel weight.

3.2.2. SDS sedimentation test

SDS sedimentation test is used to predict gluten strength and baking quality (Carter et al., 1999). It measures the sedimentation volume of a suspension of flour in diluted lactic acid, where glutenin swells and gliadins dissolved completely (Eckert et al., 1993). This test alone gives a better prediction of bread making potential of wheat flour (Moonen et al., 1982). Higher sedimentation volume indicates superior bread baking quality (Dick & Quick, 1983; Dexter et al., 1980; Eckert et al., 1993) and stronger dough (Dexter et al., 1980). Many researchers have reported, high correlation between SDS sedimentation and loaf volume (Preston et al., 1982; Dexter et al., 1980; Barnard et al., 2002; Moonen et al., 1982) therefore this test can be used as a reliable predictor of loaf volume.

3.2.3. Protein content and quality

The wheat flour moisture and protein content is best determined using an (Near Infrared reflectance spectrometry) NIRS instrument which is very rapid and inexpensive. It is based on absorption of NIRS energy at specific wave lengths by peptide linkages between amino acid and at reference weave lengths.

The protein content of wheat is important however the protein quality is more important for baking performance. Protein content of grain can be increased by environmental stress i.e. heat or drought condition. But this type of high protein does not always improve the baking performance. The gluten quality can be predicted by mixograph, a device that mixes flour and water together to form gluten protein in the dough. The mixograph is used to determine the dough mixing properties of flour. It requires a limited amount of flour. The mixograph curve can be used to determine mixing tolerance and peak time or mixing time. High water absorption, moderate mixing times (3-6 minutes), strong gluten strength and good dough mixing tolerance are desirable for good bread flour with adequate protein content.

3.3. Effect of heat stress in end-use quality

High temperatures has been shown to increase of grain nitrogen percentage (Stone and Nicolas, 1994; 1995; Viswanathan and Khanna-Chopra, 2001). Because of faster movement of nitrogen from the vegetative parts of plant to the grain, heat stress during early and mid grain filling stage negatively affects protein accumulation in developing grains (Corbellini et al., 1997; Troccoli et al., 2000). Protein deposition is

less affected than starch deposition in the grain which results in the high protein concentration of flour during heat stress (Corbellini et al., 1997; Spiertz et al., 2006; Blumenthal et al., 1993; Jenner, 1994). However, the increase in protein concentration because of high temperature does not improve the gluten protein quality, thus causing a negative overall affect on end-use quality (Panozzo and Eagles, 2000; Corbellini et al., 1997).

The temperature during the grain filling period of wheat is also very crucial for overall quality (Randall and Moss, 1990). In spite of having similar protein content, different growing locations bread making quality of wheat is significantly effected (Jarvis et al., 2008). Heat stress during late grain filling condition may not reduce grain yield and protein concentration significantly yet will affect dough strength, reducing the commercial value of products (Corbellini et al., 1997). Heat stress at all the stages of grain filling is detrimental for the rheological properties (Corbellini et al., 1997). However, temperature above 30 degree only for few days causes weakening of dough (Blumenthal et al., 1993; Randall and Moss, 1990).

Dough strength is associated with protein quality and not with protein content (Panozzo and Eagles, 2000). Wheat grown at different location has different protein quality because of variation in temperature at growing season (Randall and Moss, 1990). In similar protein content sample, dough strength decreases because of heat stress (Ciaffi et al., 1996). Increase in temperature ($>35^{\circ}\text{C}$) during grain filling results in the increase of gliadin synthesis and thus decrease the dough strength (Blumenthal et al., 1990). In contrast, Stone and Nicolas (1994), found that during high temperature synthesis of

gliadin is not always high, but different cultivars vary in their response to gliadin synthesis. This results in weakening of dough strength (Blumenthal et al., 1995). High temperature i.e., above 35⁰C has negative effect on the complex protein aggregates causing reduced dough mixing properties, where mixing tolerance of dough can be reduced by 40-60% because after only 5 days of heat shock (Corbellini et al., 1997). Dough stability is lowered because of decrease in GMP (Don et al., 2005).

The quality of wheat is affected by the growing environment or different temperature regimes by differentially affecting synthesis of unique proteins of gluten (Ciaffi et al., 1996). An increase in temperature (>35⁰C) during grain filling results in less inhibition in gliadin synthesis versus HMW glutenins, primarily, omega and gamma gliadins (Blumenthal et al., 1990; Ciaffi et al., 1996). Though, synthesis of some low molecular weight glutenin is not affected by heat stress, but high molecular weight glutenin synthesis is significantly reduced (Ciaffi et al., 1996). This result seems obvious as negative correlation exists between glutenin and gliadin protein in flour because high proportion of total protein accounted by these two classes (Panozzo and Eagles, 2000). Glutenin Macro Polymer (GMP) is more influenced by growing conditions than the amount of protein content (Don et al., 2005). As the heat stress increases, concentration of soluble polymeric protein (SPP) becomes higher, whereas, the amount of insoluble polymeric protein (IPP) goes down (Corbellini et al., 1997). However, the proportion of total polymeric protein (TPP) is not affected even though IPP/TPP ratio is lowered during heat stress (Ciaffi et al., 1996). Similarly, reduced synthesis of HMW-GS affects the formation of larger aggregates (Ciaffi et al., 1996). Heat stress slows down the

biosynthesis of glutenin (DuPont and Altenbach, 2003), but meanwhile it causes hyper aggregation of glutenin particles which results in low GMP and larger particles (Don et al., 2005). Temperatures above 35⁰C during grain filling affects the formation of large aggregates or composition of polymeric fraction (soluble/insoluble polymers protein ratio) without influencing their synthesis (Ciaffi et al., 1996).

Different cultivars have the ability to respond differently to high temperature or heat shock (Blumenthal et al., 1993). Skylas et al. (2002) reported that the heat shock treatment decrease the dough strength of heat susceptible cultivar, whereas heat tolerant cultivar showed an increase in dough strength because of a stronger and more diverse heat shock response. During heat stress condition many genotypes differ in their ability to synthesize HMW-GS and heat shock protein (HSP) (Blumenthal et al., 1998). The extensibility of dough is increased because of heat stress (Blumenthal et al., 1993). Heat stress in the growing condition cause the weakening of dough which results in smaller loaf volume (Blumenthal et al., 1993).

3.4. Heat stress and grain yield

Wheat can be grown over a wide range of elevations, climatic conditions and soil fertility (Bushuk, 1998). The most productive agricultural regions also face short period of abiotic stresses during maturation and ripening of cereals within almost any year (Barnabas et al., 2008). The reproductive development process can be adversely affected by long period of stress condition during vegetative growth (Barnabas et al., 2008). Heat stress reduces both grain growth duration and grain growth rate (Viswanathan and

Khanna-Chopra, 2001), hence, affecting the final grain weight, which is a determinant of the total yield in cereals (Barnabas et al., 2008). Spiertz et al. (2006) reported that high growth temperatures reduced the grain dry mass because of limited supply of assimilates. Hot weather not only reduces the size and number of starch granules per endosperm (Tester et al., 1995), but also significantly reduces the formation of high molecular starch and rate of carbon deposition in the grain (Spiertz et al., 2006). In heat stress condition the availability of assimilates is not the major factor limiting starch synthesis, but the conversion of sucrose to starch is the major limitation in temperature above 35⁰C (Jenner, 1994). Soluble starch synthase is the key enzyme in starch synthesis, and its activity is lost during high temperature which causes yield loss of crops (Wardlaw and Wrigley, 1994). High temperature during grain filling condition reduces starch accumulation which cause large yield losses in cereals (Barnabas et al., 2008). During high temperature the supply of assimilates, especially carbohydrate, is reduced due to senescence of leaf which accounts for the reduced grain growth (Jenner, 1994).

The adverse effect of extreme temperature or heat shock conditions (>32⁰C) for only a few days can inhibit kernel development, cause premature senescence, and alter the starch and protein composition during grain filling (Wardlaw and Wrigley, 1994). High temperature enhances the rate of senescence, reducing photosynthesis which affects the carbohydrate accumulation more than the nitrogen accumulation (Troccoli et al., 2000). Grain nitrogen percentage and individual kernel mass has strong negative relationship (Stone and Nicolas, 1994). Increased temperature causes a decrease in kernel mass resulting into early physiological maturity (Jenner, 1994). The major cause

of reduction in kernel weight is the decrease of kernel growth duration. Slight increases in kernel growth rate can not compensate for the loss in dry matter accumulation due to the reduced grain filling period under heat stress condition (Fokar et al., 1998).

3.5. Quantitative trait loci (QTL) mapping for wheat quality traits

A quantitative trait locus (QTL) is the region within genomes which is associated with expressed genes related to the particular quantitative trait (Collard et al., 2005). As we do not know the position of QTL, genetic markers are used to identify their putative location in the chromosome. Different types of DNA markers, such as Restriction Fragment Length Polymorphism (RFLPs), Microsatellites or Simple Sequence Repeats (SSRs), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphisms (AFLPs), Single Nucleotide Polymorphism (SNP) etc. are available for genetic map construction (Semagn et al., 2006). Data from DNA markers are then used to construct a linkage map, which provides information about the position and relative genetic distance between markers along chromosomes (Collard et al., 2005). The process of determining the relative position and distances between markers along chromosomes is known as genetic mapping (Semagn et al., 2006). The relative distance between two DNA markers is called the genetic distance which is measured as a function of recombination frequency (i.e. mapping function). Different kinds of population such as recombinant inbred lines (RILs), double haploid (DHs), backcross (BC), near isogenic lines (NILs) and F2 population can be used for mapping. Backcross (BC) and F2 are simple type of population which require less time to produce and are highly

heterozygote, whereas, NILs, RILs and DH are homozygous population which can be multiplied without genetic change (Semagn et al., 2006). The most important consideration in the construction of linkage map is that there should be sufficient genetic polymorphisms for the markers between parents, so that the markers will be inherited from either parents (homozygous) or both parents (heterozygote) to progenies (Semagn et al., 2006). The principle of QTL mapping is that during cross-over or genetic recombination in meiosis, the genes and markers segregate, this allows the analysis in the progeny (Collard et al., 2005). Different kinds of software are used for QTL mapping. The detected QTLs in one environment may not be equally useful in another environment to predict the phenotype (Tanksley et al., 1993). But the QTL with larger effect in one environment can be important in another environment (Tanksley et al., 1993).

The study conducted by Rousset et al. (2001) in the population of Recombinant substitution lines developed by substitution of chromosome 1A, 1B and 1D from high quality wheat Cheyenne into low quality wheat Chinese Spring revealed the strong effect of the Glu-1 locus on the long arm of chromosome 1A and short arm of chromosome 1B on SDS sedimentation and bread loaf volume. They also concluded that bread making quality is associated with a complex genetic architecture, where Glu-1 is a component of the whole system (Rousset et al., 2001). The SDS sedimentation volume, protein content etc. are related to bread making quality of wheat, but using these characters as the sole predictor of quality is not reliable because the genetic system which control them only partially overlap (Rousset et al., 2001).

In a study of a 185 line DH population conducted in three locations in two different years, Huang et al. (2006), found a significant difference among genotypes and environments for all the quality traits. They observed a very high environmental influence on quality traits such as SDS volume, mixing development time, peak height and protein content. In QTL analysis, three QTLs for mixing development time (MDT) (on chromosome 1B, 1D and 3B), three QTLs for SDS sedimentation volume (1B, 2D and 5D), and two QTLs for flour protein content (on 2D and 4DS) were identified.

The study conducted by Pshenichnikova et al. (2008) in a set of 63 RILs grown in two different environments, detected 22 QTLs on 10 chromosomes for grain quality related to milling and physical properties of dough. They detected 3 QTLs for grain hardness on chromosomes 5DS, 6AL and 3AL, (Pshenichnikova et al., 2008). Similarly, Li et al. (2009) have reported 19 QTLs for grain hardness including three minor effect QTLs on chromosome 3B, 4B and 5D which were observed in both location. They also detected 12 QTLs for SDS sedimentation volume, among which 6 QTLs were stable across environments. Zhang et al. (2009) have reported a major QTL for grain hardness on chromosome 5D. Similarly, they found significant QTLs on chromosome 5D and 3A for protein content, and on chromosome 1A, 1B and 1D for mixograph peak time respectively. Among all the QTLs, one was observed to be associated with Glu-D1 locus.

The study conducted by Campbell et al. (2001) in RILs derived from the cross between soft and hard wheat found that bread baking quality of wheat is highly influenced by the glutenin loci. The QTL for mixograph tolerance and mixograph peak

height were found on chromosome 1AL and 1BL, whereas, QTL on 1D and 4AL were associated with mixograph peak time. However, they found the flour protein content QTL on chromosome 2B and 1A. McCartney et al. (2006) have reported five QTLs for flour protein content which is mapped to the major QTL cluster on chromosome 4D, while three QTLs were detected for flour yield. At the same time 16 QTLs were identified in clusters on chromosomes 1B, 4D and 7D for different mixographic properties of wheat flour.

In the study of 79 F7 recombinant inbred lines (RILs) developed from the cross of 'Marius' a soft grained French winter wheat and 'Cajeme71' hard spring wheat, Kerfal et al. (2010), found a total of 20 QTLs for quality traits. For the sedimentation volume three QTLs were detected on chromosome 7AS, 5BS and 6DS, whereas the mixograph QTLs for mixing time were found on chromosome 3BS and 1DL which is near the Glu D1 loci of storage protein. Similarly, the mixing tolerance QTLs were found on chromosomes 2AS and 7AS. Zanetti et al. (2001) identified nine QTLs for Zeleney sedimentation volume, in which four of them were located on 1B, 2A, 5A and 5D had big effect on the traits ($R^2 > 15\%$). The QTL on 1B and 5A were detected in all four environments. Similarly, the large effect ($R^2 > 15\%$) and stable QTL for protein content and kernel hardness were found on chromosome 5A and 2A respectively.

Arbelbide and Bernardo (2006) detected two QTLs for kernel hardness on chromosomes 1A and 5D by using Mixed-model QTL mapping. Similarly, Perretant et al. (2000), reported three QTLs for kernel hardness, one having the major effect on chromosome 5DS and two with minor effect on chromosomes 1A and 6D respectively,

in a DH population derived from Chinese Spring and Courtot. In the same study Perretant et al. (2000), observed transgressive segregation for kernel hardness and kernel protein content. They also found two main QTLs for kernel protein content on chromosomes 1B and 6A.

The study conducted by Kuchel et al. (2006), in a set of 182 double haploid (DH) population developed from the cross between 'Trident' and 'Molineux' which differed for high molecular weight and low molecular weight glutenin loci (Glu-B1, Glu-A3, Glu-B3), found that the environmental effect was very strong in the quality traits because the correlation assessed in 1996 and 2003 were relatively poor. For flour protein content QTL on chromosome 7D and 7A were detected in 1996 and 2003 growing season respectively, whereas, QTLs on 1B, 6A and 6D were found in both the years.

Environmental factor had significant influence in quality traits, thus having a direct effect on detected QTLs (Li et al., 2009). The study conducted by Mann et al. (2009) in the DH population reported that there was inconsistent genetic control of protein content because they could not detect the QTL for protein content in all the five location. However, only the QTLs on loci 3A and 7A were detected for three sites. In the next chapters we will try to detect the stable QTLs for different wheat quality traits which could be expressed in different growing environments and compare with the previously reported results.

CHAPTER II

ANALYSIS OF DIFFERENT WHEAT QUALITY PARAMETERS IN RILS GROWN UNDER DIFFERENT ENVIRONMENTS

1. Introduction

Wheat (*Triticum spp.* L.) is one of the most important staple crops which constitutes 29-30% of total global cereal production, and is the largest supplier of calorie (19% per capita/day) and protein (21% per capita/day) to humans (FAOSTAT, 2011). Basically, two major types of wheat, bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* L.), are grown and traded internationally (Williams et al., 2008). However, hexaploid bread wheat makes up to 95% of total global wheat production, whereas, durum wheat occupies the remaining 5% (Shewry, 2009). In some regions where common hexaploid wheat has lower yield potential, tetraploid durum wheat are grown and used for bread making process (Boggini et al., 1995). However, durum wheat is not used for making bread commercially as its protein quality or gluten strength is lower than that of bread wheat (Ammar et al., 2000). Durum wheat is generally used for making pasta products.

In the past years, wheat breeding programs have focused on breeding cultivars with resistance to biotic and abiotic stresses and high grain yields (Kerfal et al., 2010). This can have a direct impact on the quality of wheat because cereal crops with high yield generally contain low protein. Wheat quality can be defined as the ability to produce different kinds of products suitable for the end user (Panozzo and Eagles, 2000).

Wheat grain protein has great effect on the functional properties for food processing (Shewry and Halford, 2002). There should be the minimum levels of protein content and protein quality in flour for bread making process (Payne et al., 1984). Generally wheat protein ranges from 9 to 16 % of dry weight (Payne et al., 1984).

Bread making properties are governed mainly by seed storage proteins, called gluten proteins. In wheat, only the gluten protein has the ability to form dough which are viscoelastic in nature and appropriate for making bread of different kinds. The gluten protein is divided into two groups i.e., glutenin and gliadin. Gliadins are monomeric prolamins which primarily determine a dough's extensibility; whereas, glutenins are polymeric glutes responsible for dough elasticity (Payne et al., 1984). Gluten coding genes have been identified at nine complex loci on six different chromosomes (Payne et al., 1984). As the gluten content of wheat accounts for only 30-60% of the variation of total bread making quality, a substantial amount of variation is determined by non gluten factors (Li et al., 2009). The non gluten factors may be either unknown polygenic loci or environmental factors.

Quality fluctuation is one of the major problems in wheat cultivar. Large variation in quality exists not only between different varieties but also between different growing conditions of the same cultivar. Wheat quality can be influenced by different factors i.e., genetic factors which influence protein quality, grain hardness, seed coat color etc., and environmental factors which influence protein content, moisture content, grain infestation, soundness, maturity etc. Also the seeding rate, time of seeding, nitrogen application etc. can have significant influence on wheat grain quality. Spiertz et

al. (2006) reported that the quality of wheat genotype is strongly affected by weather condition during the grain filling stage. Wheat breeding has the common target of improving quality as well as yield (Spiertz et al., 2006). Changes in environmental conditions during grain filling stage affect both on the yield and quality of wheat (Pierre et al., 2007). Even the short period of extreme high temperature i.e., heat shock has adverse effect on quality and yield of many cultivars in the US and Australia (Wardlaw and Wrigley, 1994). Wheat grain yield as well as quality is affected by heat stress during the grain filling stage (Corbellini et al., 1997).

A successful breeding program always intends to develop stable wheat genotypes which can maintain yield and quality irrespective of the growing conditions. In this regard, selecting appropriate quality traits and understanding their interrelationship is very important. Thus, the objective of this experiment was to analyze different wheat quality parameters in wheat genotypes grown under different environmental conditions. A set of 180 Recombinant Inbred Lines (RILs) were used to understand the quantitative behavior of different quality traits within and among different growing environments.

2. Materials and methods

2.1 Plant material

In this study, a population comprised of 180 recombinant inbred lines (RILs) developed by crossing two spring wheat lines ‘Halberd’ and ‘Len’ were used. The parent Halberd is a heat tolerant cultivar, whereas, the other parent Len is known for its good agronomic characteristics. The RILs were developed by advancing the F₁ progeny through single seed descent method to the F₆ generation.

2.2 Growing environment

The RILs were grown in the field at two locations, College Station (CS10) and Uvalde (UVL10), TX in the 2009/10 growing season, and College Station (CS11) and Chillicothe (CH11) TX, in 2010/2011. These experiment locations possessed difference in growing conditions in terms of monthly average temperatures and precipitation (Fig.1). The field experiment was set in randomized complete block design (RCBD) with four replications in each location. The same field management practices, which were followed by the small grain breeding program for field experiments, were used in our study at all the locations. At maturity, the grains were harvested from each plot for measurements on yield and quality traits.

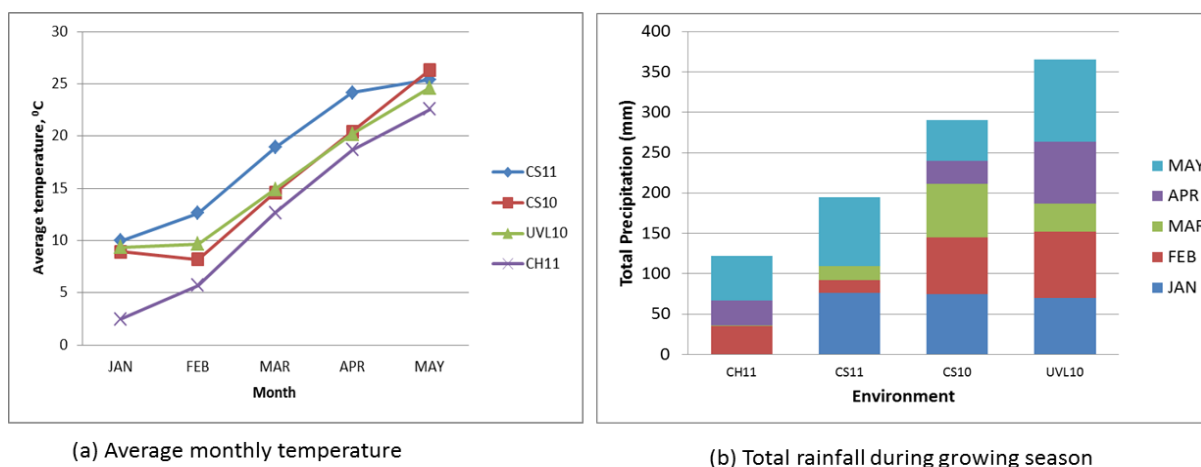


Fig.1. Average monthly temperature pattern (a), and total precipitation (b) in four test environments during wheat growing season at Texas

2.3 Measurement of quality and yield parameters

From each line, a sample of about 110 gram of wheat grain was taken from all experimental locations. The wheat sample of 80 gram was divided into two sub sample of 30 and 50 gram each. The 50 gram sample was milled in Brabender Quadramat Junior (Brabender® GmbH & Co. KG, Kulturstr Duisburg Germany) after tempering to 14% moisture level. Then the flour was kept in airtight plastic bags and used later to determine different quality parameters. The remaining 30 gram sample of wheat kernel was used to determine kernel characteristics.

2.3.1 Kernel characteristics

Different types of kernel characteristics i.e., kernel hardness index (HI), kernel diameter (DIAM) and kernel weight (KWT) were determined in a sample of 300 individual kernels by using Perten Model SKCS (Single Kernel Characterization

System) 4100 (Perten Instruments North America Inc.). After removing broken kernels and foreign material, were analyzed for SKSC which analyzed 300 kernels individually. The results include the average and standard deviation of kernel weight, diameter, moisture content and hardness index measured in milligram (mg), millimeter (mm), moisture percentage, and hardness index (in a numeric scale of -20 to 120).

2.3.2. Near-infrared reflectance spectrometry (NIRS) test

From each RIL, protein (PROT) and moisture content of flour was determined using a near-infrared reflectance spectrometry (NIRS). Spectra were recorded by filling a standard cup with the sample and scanning in the NIRS machine.

2.3.3. Mixograph test

To determine the mixing properties of dough, Mixograph system (Mixograph National Manufacturing CO, Lincoln, NE, USA) was used. From each RIL, 10 gram of flour was weighed and put in mixogram bowl. Then water was added according to the protein content of sample and run for 8 minutes. After dough was developed, a mixograph curve was produced. The curve was used to determine the mixing tolerance (TOL) and peak time (PT).

2.3.4. Sodium dodecyl sulfate sedimentation (SDSS) test

To determine gluten strength and baking quality, the sodium dodecyl sulfate sedimentation (SDSS) test was performed (Moonen et al. 1982). At first, the stock

solution was prepared by using lactic acid and water. A fresh working solution was prepared daily which contained 1:48 ratio of 85% lactic acid-water (1:8, v/v) and sodium dodecyl sulfate (2% solution). This test was performed in a batch of 10 samples at a time. Standard clear glass test tubes of size 16 x 150 mm were placed in a metal rack. One gram of wheat flour was weighed and put in a test tube. Then, 4 ml of distilled water was added in each test tube and each content were mixed properly in a high speed vortex for 4 second and allowed it to sit for 4 min and 54 sec. Again, after 5 minutes the mixture was vortexed and allowed to sit for 5 minutes. Then 12 ml of SDS-lactic acid reagent (1L 95% SDS, 20ml USP 85% lactic acid) was added to each sample and all the tubes were covered with a foam plug and inverted ten times, and placed in an upright vertical position. The mixture was allowed to sit for 15 min, and then the height of sediment column was measured using a millimeter (mm) scale. The same sample was repeated 3 times and the mean was taken as the final reading.

2.3.5. Yield and yield components

At the physiological maturity stage, 50 heads from each plot were randomly harvested. The harvested heads were threshed by using combine thresher and the weight measured. Similarly, all the plots were combine harvested. The yield from each plots were determined for all locations.

2.4. Statistical analyses

Statistical analyses of all phenotypic traits were performed using JMP 7 (JMP Version 7, SAS Institute Inc., Cary, NC). Distributions of all the quality traits were determined and tested for normality. Pearson's correlation coefficient for all the quality traits and yield within and across environment were calculated. Similarly, principal component analysis was used among different mixographic traits and SDSS volume to visualize the interrelationship between these traits and their growing environments.

3. Results

3.1. Phenotypic distributions

All the kernel characteristics and quality traits showed a continuous distribution pattern indicating that these are quantitatively inherited traits. Though most of the quality traits were normally distributed (Shapiro-Wilk 'W' test), some of them were deviated from normality. Histograms showing the phenotypic distribution pattern of some of the traits are given in fig.2.

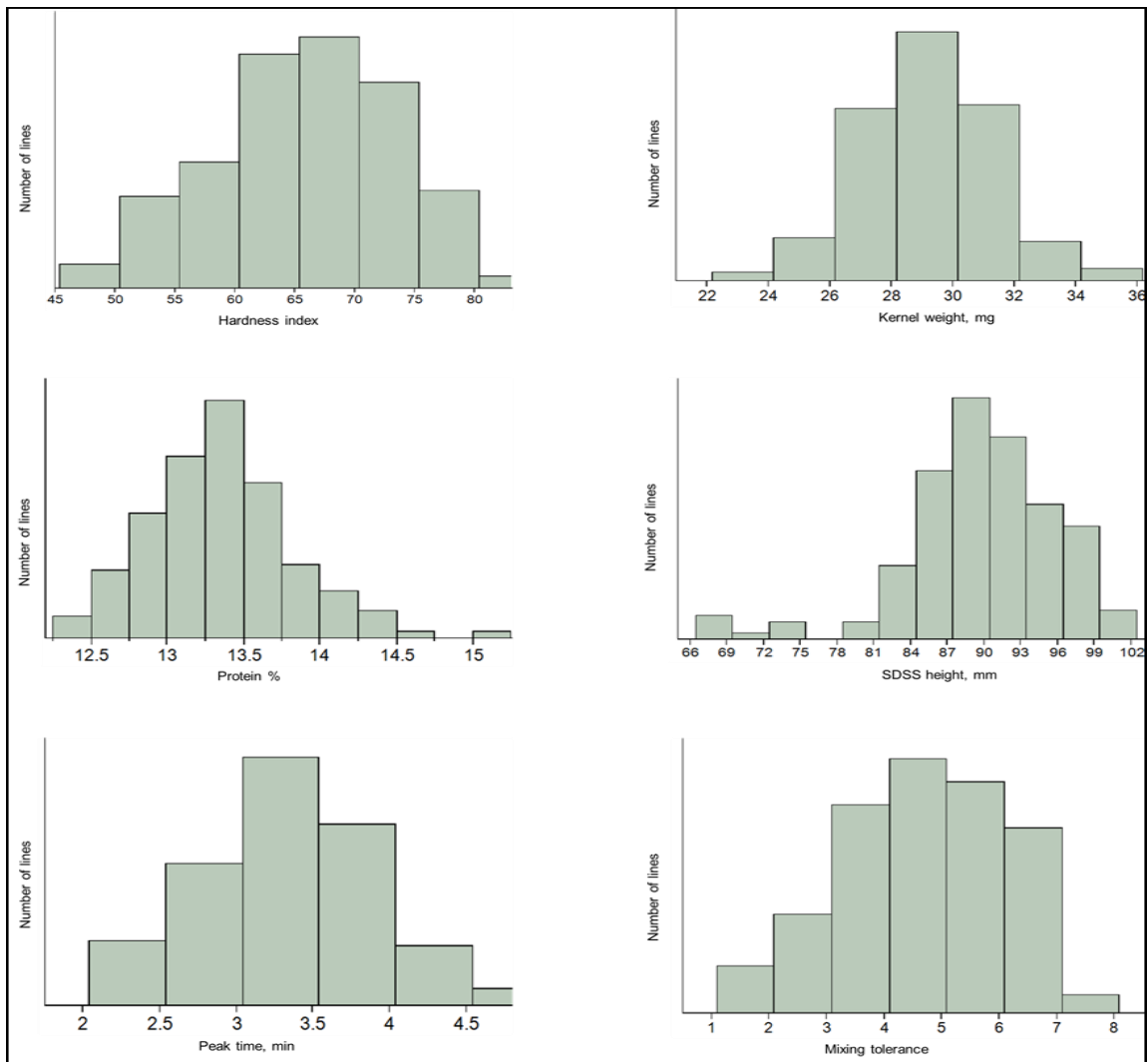


Fig.2. Phenotypic distribution of some of the quality traits in RILs.

3.2. Single kernel characterization system results

By using SKCS we obtained the DIAM, KWT, HI and moisture percent. The HI ranged from 46.3 to 81.5 in CS10; whereas in UVL10, it ranged from 44.5 to 84.8. The average HI of lines grown in CS10 and UVL10 were 65.47 and 69.6 respectively. The HI of Halberd was 54.1 whereas; Len's HI was 73.8 and both are categorized as hard. In CS10, nine lines were categorized as mixed which had the HI of less than 53; whereas, in UVL10 eight lines were categorized as mixed. None of the lines were in the soft category or below an HI of 50.

KWT of Halberd was slightly higher (33 mg) than that of Len (31.5 mg). The grain weight of RILs grown in CS10 ranged from 22 to 35.3mg; whereas it ranged from 23.3 to 34.8 mg in UVL10. The average grain weight of CS10 and UVL10 was 28.90 mg and 28.44 mg respectively.

The DIAM was equal for both the location. The average of DIAM was 2.7 mm was observed in both the locations. The DIAM was same i.e., 2.8 mm for both the parents. The DIAM ranged from 2.5 to 2.9 mm for both the locations. There was not demarcated difference in DIAM in both the locations.

3.3. Near infra red spectrometry (NIRS) results

Flour protein and moisture percent was determined by using NIRS. Len was slightly higher (14.23%) than that of Halberd (12.53%). In comparisons to both locations, CS10 had higher protein than UVL10. In CS10 grown lines, the protein ranged from 15.2% to 12.26%. In UVL10 grown lines, the maximum protein was

14.88% and the minimum was 11.75%. For the RILs, average protein was 13.48% in UVL10 and 13.34% in CS10.

3.4. Mixograph results

We used mixograph to determine the mixing properties of dough for each line. From mixogram we calculated peak time and mixing tolerance. In general, a higher the peak time is associated with higher gluten strength. The lines grown in CS10 showed a slightly higher peak time. The peak time ranged from 2.3 to 6 minutes. Whereas, for the lines grown in UVL10, the peak time ranges from 2 to 4.5 minutes. In average, the CS10 and UVL10 had peak time of 3.65 and 3.13 minutes respectively. The peak times of the parents were 3 and 2.8 minutes for Halberd and Len respectively.

The mixing tolerance indicates the tolerance of the dough to over-mixing. The tolerance score for Halberd was 2 and Len was 4. The performance of lines ranged from 2 to 7 in CS10 and from 1 to 7 in UVL10. The average tolerance score for CS10 and UVL10 were 5.12 and 4.10 respectively.

3.5. SDS sedimentation test results

SDS Sedimentation volume test predicts the gluten strength and baking quality of wheat flour (Fig.3). The SDS sedimentation height ranged from 67 to 97 mm in RILs for CS10, whereas, for UVL10 it ranged from 56.67 to 92.33 mm. The average SDSS height was 87.95mm and 81.27mm in CS10 and UVL10 respectively. The parents Halberd and Len showed SDSS height of 84 and 87 mm respectively. In 2011 CS grown lines, the

average SDSS height was 55.63 mm, whereas 2011 Chillicothe, had average height of 57.15 mm. However the range was 30 to 91 mm in CS11 and 31 to 92 mm in CH11. The parent Halberd had higher SDSS height (65mm) than the parent Len (41mm) in CH11. But in CS11, Len had higher SDSS height (51 mm) than Halberd (48 mm).

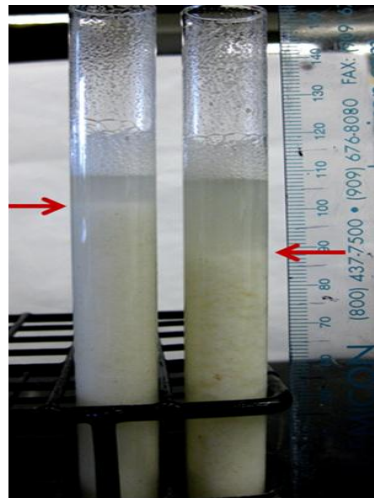


Fig.3. Two lines with marked difference in SDSS height

3.6. Yield and yield components

Halberd had higher yield in all the locations compared to Len (Fig.4). While comparing the three locations, Halberd's performance was highest in CS10 followed by CS11 and CH11. Whereas, the Len's performance was highest in CS11 followed by CS10 and CH11. The average yield was higher in CS10 followed by CS11, UVL10 and CH11. In CS10 the yield ranged from 1.63 to 5.90 t/ha, whereas in CS11, it ranged from 1.56 to 5.97 t/ha. Similarly in UVL10 it ranged from 1.05 to 4.35 t/ha, and in CH11 the range was 1.12 to 2.95 t/ha.

The 100 seed weight of Halberd was higher than Len in all locations. Halberd performed better in CS10 followed by CS11 and CH11. Whereas, Len was better in CH11 followed by CS11 and CS10.

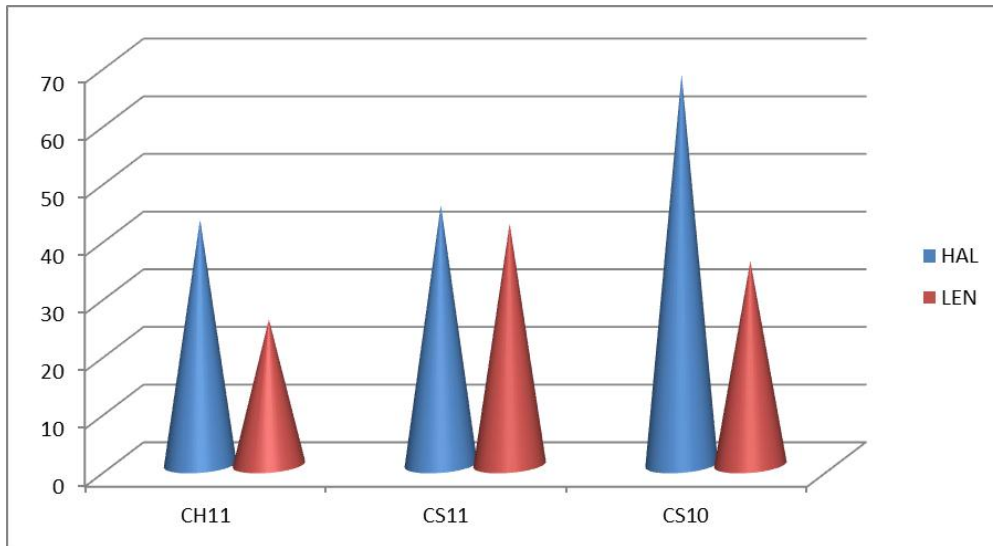


Fig.4. Grain yield comparison of two parents Halberd (Hal) and Len in three different environments, Chillicothe 2011 (CH11), College Station 2011 (CS11), College Station 2010 (CS10).

3.7. Correlations between different quality traits

The Pearson correlation coefficients among different quality traits in two environments are given in table 1. The protein percent of UVL10 was significantly positively correlated with the SDSS volume and protein percent of CS10. Similarly, the peak time and mixing tolerance of UVL10 was significantly positively correlated with the SDSS volume, peak time, mixing tolerance and hardness index of CS10. However, the mixing tolerance of UVL10 was negatively correlated with protein CS10. HI of

UVL10 was negatively correlated with SDSS_CS10 but positively with peak time and tolerance of Uvalde. Kernel weight and HI was negatively correlated.

Table 1. Correlation coefficients of different quality traits across environments (CS10 and UVL10)

	PROT_CS	SDSS_CS	PT_CS	TOL_CS	HI_CS	KWT_CS
PROT_UVL	0.64**	0.16*	0.08	0.04	-0.01	0.06
SDSS_UVL	-0.03	0.53**	0.28**	0.46**	-0.21**	-0.01
PT_UVL	-0.09	0.31**	0.40**	0.57**	0.22**	-0.07
TOL_UVL	-0.21**	0.36**	0.47**	0.70**	0.27**	-0.03
HI_UVL	-0.03	-0.21**	0.19*	0.18*	0.79**	-0.10
KWT_UVL	0.06	0.05	-0.13	-0.06	-0.15*	0.44**

* Significant at 0.05

** Significant at 0.01

Trait abbreviations: PROT= Protein, SDSS=Sodium dodecyl sulfate sedimentation, PT=Peak time, TOL=Mixing tolerance, HI=Hardness index, KWT=Kernel weight, CS=College Station 2010, UVL=Uvalde 2010

Similarly, the correlation coefficients among quality traits within CS10 are given in table 2. The SDSS volume was positively correlated with the peak time, mixing tolerance and negatively with the kernel hardness index. Similarly, the peak time was positively correlated with the mixing tolerance and kernel hardness index. And the mixing tolerance was positively correlated with the kernel hardness index. However, kernel weight was significantly negatively correlated with kernel hardness index and protein percent.

Table 2. Correlations of different quality traits within College Station

	PROT_CS	SDSS_CS	PT_CS	TOL_CS	HI_CS
SDSS_CS	0.04				
PT_CS	0.03	0.29**			
TOL_CS	-0.13	0.42**	0.55**		
HI_CS	0.10	-0.18*	0.17*	0.21**	
KWT_CS	-0.15*	0.04	-0.11	-0.13	-0.15*

* Significant at 0.05, ** Significant at 0.01

Within UVL10, the SDSS volume was positively correlated with the protein percent, peak time and mixing tolerance, whereas it was negatively correlated with the hardness index (Table 3). And also the peak time was positively correlated with the mixing tolerance, hardness index but negatively with the kernel weight. Similarly the mixing tolerance was positively correlated with the hardness index. Hardness index and kernel weight were negatively correlated.

Table 3. Correlations of different quality traits within Uvalde

	PROT_UVL	SDSS_UVL	PT_UVL	TOL_UVL	HI_UVL
SDSS_UVL	0.25**				
PT_UVL	0.14	0.54**			
TOL_UVL	0.01	0.59**	0.83**		
HI_UVL	-0.10	-0.25**	0.23**	0.25**	
KWT_UVL	0.01	0.03	-0.21**	-0.11	-0.28**

* Significant at 0.05, ** Significant at 0.01

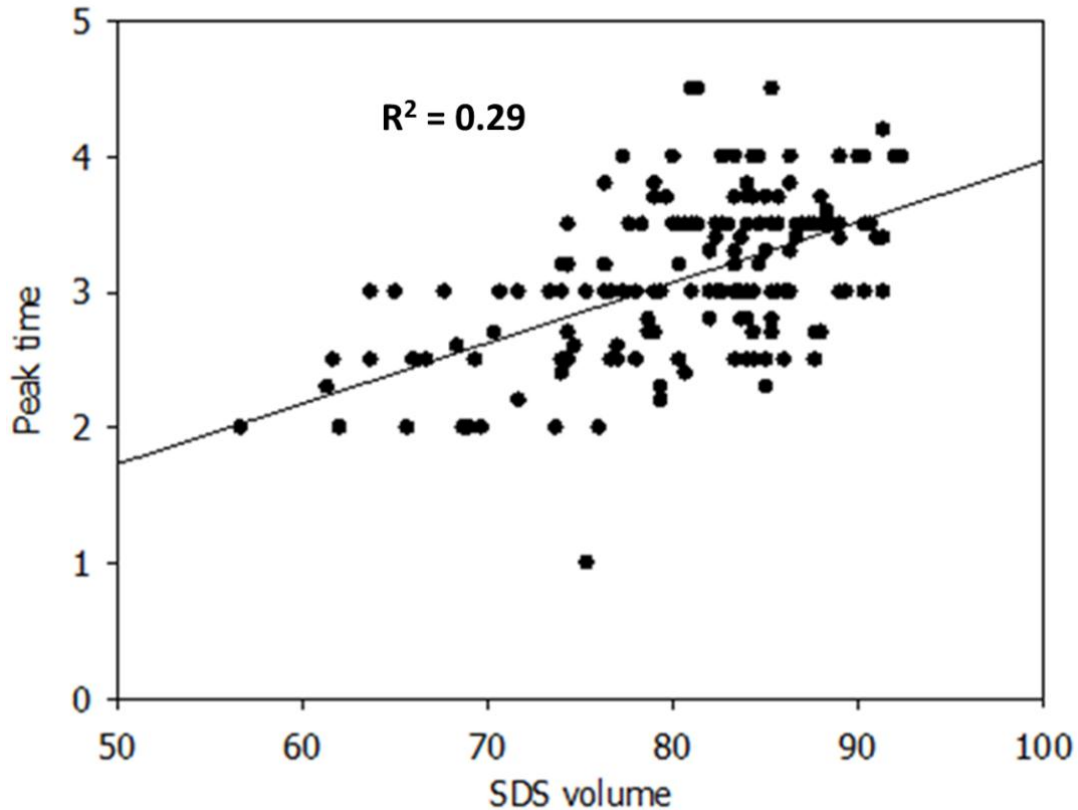


Fig.5. Regression plot showing the linear relationship between peak time and SDSS height of RILs grown in Uvalde 2010.

We found positive linear relationship between mixograph peak time and SDSS sedimentation column height from both of the environment i.e. UVL10 and CS10. In fig.5 we can see the regression plot for lines grown in UVL10.

There was the significant positive correlation of SDSS height with peak time and mixing tolerance; whereas, significant negative correlation with kernel hardness index. Similarly, we found significant positive correlation between mixing tolerance and peak time. And also the kernel diameter was negatively correlated with peak time and mixing tolerance whereas, hardness index was positively correlated with the peak time and

mixing tolerance. Kernel weight was negatively correlated with the hardness index and peak time whereas; it was positively correlated with the kernel diameter (Table 4).

Table 4. Correlation coefficient of different quality traits averaged across experiments

	SDSS	PROT	PT	TOL	HI	DIAM
PROT	0.14					
PT	0.50**	0.05				
TOL	0.58**	-0.08	0.80**			
HI	-0.26**	-0.01	0.25**	0.26**		
DIAM	-0.06	0.08	-0.24**	-0.16*	-0.10	
KWT	0.03	0.00	-0.19*	-0.10	-0.21**	0.85**

* Significant at 0.05, ** Significant at 0.01

Kernel weight of CS10 was negatively correlated with kernel hardness index and positively correlated with kernel diameter (Table 5). Similar was the case in UVL10 i.e., kernel weight was negatively correlated with the hardness index and positively with the kernel diameter. We found significant positive correlation between HI_CS, DIM_CS, and KWT_CS with HI_UVL, DIM_UVL, and KWT_UVL respectively.

Table 5. Correlations among kernel characteristics across two environments

	HI_CS	DIM_CS	WT_CS	HI_UVL	DIM_UVL
DIM_CS	-0.03				
WT_CS	-0.15*	0.85**			
HI_UVL	0.79**	-0.04	-0.10		
DIM_UVL	-0.02	0.44**	0.39**	-0.14	
KWT_UVL	-0.15*	0.34**	0.44**	-0.28**	0.85**

* Significant at 0.05, ** Significant at 0.01

The correlation of SDSS volume across four different environment showed that there was significant positive correlation across all four environments i.e., CH11, UVL10, CS10 and CS11 (Table 6).

The two mixograph traits i.e., peak time and mixing tolerance, are positively correlated within location and also across locations (Table 7).

Table 6. Correlations of SDSS test across four environments

	CH2011	CS2011	CS2010
CS2011	0.20**		
CS2010	0.27**	0.22**	
UVL2010	0.42**	0.20*	0.53**

* Significant at 0.05, ** Significant at 0.01

Table 7. Correlations of Mixograph traits across environments

	PT_CS	TOL_CS	PT_UVL
TOL_CS	0.55**		
PT_UVL	0.40**	0.57**	
TOL_UVL	0.47**	0.70**	0.83**

* Significant at 0.05, ** Significant at 0.01

3.8. Correlation between yield and quality

In all the experiments, the grain yield and flour protein percentage was negatively correlated (Table 8). Protein percent of UVL10 was negatively correlated with the yield of UVL10 ($r=-0.21$), and also with yield of CS11 ($r=-0.17$). Similarly the protein percent of CS10 was negatively correlated with CS11 ($r=-0.16$), CH11 ($r=-0.19$) and CS10 ($r=-0.17$).

Table 8. Correlation between yield and protein percent

	PROT_UVL10	PROT_CS10
Yield_CS11	-0.17*	-0.16*
Yield_CH11	-0.07	-0.19*
Yield_CS10	-0.08	-0.18*
Yield_UVL10	-0.21**	-0.07

* Significant at 0.05, ** Significant at 0.01

There was no significant correlation between yield and SDSS volume CS10, CS11 and UVL10; whereas, we found significant positive correlation ($r=0.22$) for CH11. We did not find any significant correlation between yield and mixograph traits i.e.,

mixing tolerance and peak time in any of the locations. There was significant negative correlation ($r=-0.17$) between 100 seed weight of CS10 and SDSS volume of CS11, whereas, for other locations the 100 seed weight and SDSS volume were not significantly correlated.

Kernel hardness index and yield did not have significant correlation in all the locations. Protein percent and 50 heads weight of UVL10 were negatively correlated in both UVL10 and CS10 experiments. There were no significant correlations between 50 heads weight and SDSS volume for any of the location except for CS11 SDSS volume and CS10 50 heads weight ($r=-0.16$).

3.9. Principle component analysis of SDS sedimentation volume test

Principle component analysis of SDSS volume was performed on RILs grown in four environments i.e., CS10, UVL 2010, CS11 and CH11. We found two distinct clusters in principal component axis (PCA) biplot (Fig.6a). The first cluster was formed by these environments i.e., CS10, UVL10 and CH11 illustrating their comparable growing conditions that affect the SDSS volume of RILs; whereas, CS11 was found to be different from three other environments. In this model, the principle component axis first (PCA1) and principle component axis second (PCA2) explained 48.8 and 21.7% of total variation respectively.

Principal component analysis of SDSS volume and mixograph traits i.e., peak time and mixing tolerance is shown in (Fig.6b). In this model, the principle component axis first (PCA1) and principle component axis second (PCA2) explained 58.4% and

14.4% of total variation respectively. We found two different clusters in PCA biplot. The first cluster was formed by SDSS sedimentation of both environments, whereas another cluster was formed for both mixographic traits i.e., peak time and mixing tolerance of both environment UVL10 and CS10. This clearly showed that the two growing environments, College Station and Uvalde 2010, were not much different in their performance on quality traits.

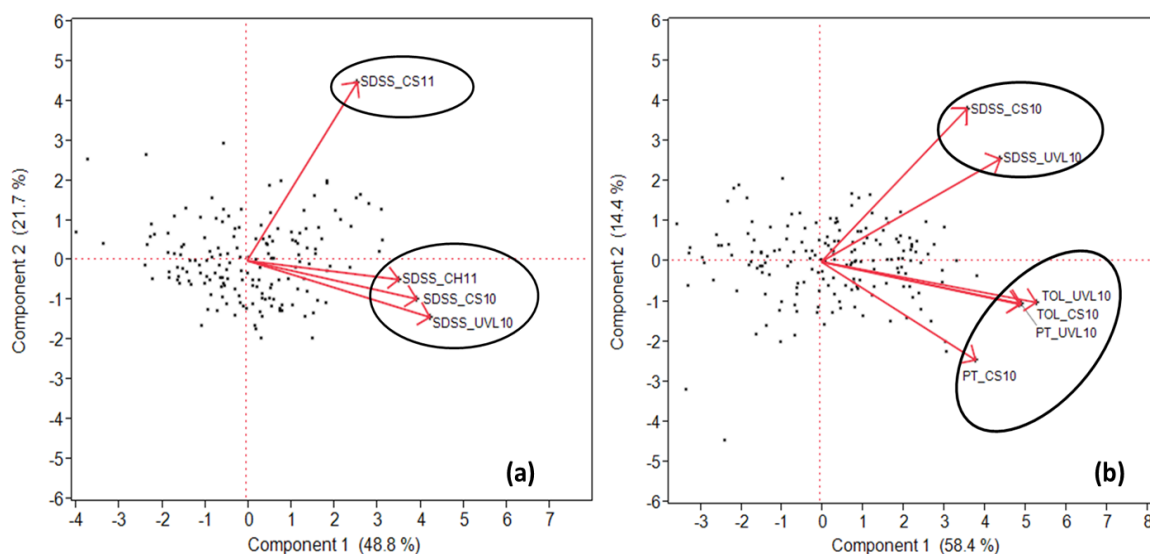


Fig.6. Principal Component Analysis of (a) SDSS volume test in four different environments, and (b) mixographic traits and SDSS test volume in two environments

3.10. Identification of most stable lines in terms of SDSS volume test

Stability ranking of each line was given based on coefficient of variation (CV) of SDSS volume of each line across four environments. The lines with higher CV are ranked as less stable lines and vice versa. Lines 88, 139, 95, 56, 11, 91, 60, 80, 157 and 100 were the most stable lines with lower CV, whereas, the lines 182, 94, 54, 79, 145, 102, 78, 148, 8 and 77 were the least stable lines. One of the parent Len falls under the

least stable lines with CV of 40.55%. Whereas, other parent Halberd was moderately stable. We also found that the least stable lines were poor performer because there average SDSS height was lower than the grand mean (71.06mm); whereas, the stable lines had higher average SDSS height than the grand mean. We found a perfect negative relationship between the mean SDSS height and coefficient of variation of RILs (Table 9).

Table 9. List of ten most stable and least stable lines based on coefficient of variation measurement on SDSS volume across four environments

Most stable lines				Least stable lines			
Line no.	CV%	Rank	Mean	Line no.	CV%	Rank	Mean
88	3.59	1	91.08	182	40.55	173	59.67
139	5.94	2	86.25	94	41.23	174	69.33
95	6.98	3	85.50	54	41.63	175	51.17
56	8.56	4	88.33	79	42.37	176	61.92
11	8.60	5	87.25	145	43.48	177	61.75
91	10.69	6	83.92	102	43.52	178	62.42
60	11.00	7	83.67	78	43.59	179	58.83
80	11.51	8	79.50	148	44.35	180	61.67
157	11.77	9	79.83	8	45.90	181	59.42
100	11.78	10	87.83	77	50.05	182	62.08

4. Discussion

Most of the quality traits i.e., hardness index, kernel weight, protein and peak time were continuously and normally distributed as was for a quantitative traits. But some traits like mixing tolerance, SDSS test, and kernel diameter were not normally distributed, they were continuously distributed.

We observed high environmental variation on the quality traits, Peak time, SDSS volume, protein percent, HI, and mixing tolerance. The RILs showed higher average SDSS volume, peak time and mixing tolerance in CS10 as compared to UVL10. This could be because of high average temperature in UVL10 than in CS10 during the wheat growing seasons. Tahir et al. (2006) also reported negative effect of high temperature on peak time and mixing tolerance, but found high SDSS volume under heat stress condition. However, Corbellini et al. (1997) found decrease in SDSS volume during heat stress conditions. In contrast, the average protein percent of RILs was higher in UVL10 than in CS10. Similar results of high protein concentration due to heat stress were reported by Stone and Nicolas (1998), Spiertz et al. (2006) and Corbellini et al. (1997).

We did not find significant correlation between SDSS volume and protein percent in CS10. Similarly, Kerfal et al. (2010) also did not find any significant correlation between protein concentration and SDSS volume. However, Huang et al. (2006) and Nishio et al. (2005) reported significant relationship between SDSS volume and protein percentage. We also found a significant correlation between SDSS volume and protein percent within Uvalde. And also protein percent of UVL10 was positively correlated with the SDSS volume of CS10. Zanetti et al. (2001) found a significant

positive correlation for protein percent, kernel hardness index, thousand kernel weight and Zeleny sedimentation volume test across environments. In our study, SDSS volume was positively correlated with the peak time and mixing tolerance similar to the results of Kerfal et al. (2010). Though we expected a positive correlation between SDSS volume and kernel hardness index similar to the findings by Nishio et al. (2005), we observed a significant negative correlation between SDSS volume and kernel hardness index. Carver (1994) has reported a similar negative correlation between HI and SDSS, whereas, Kerfal et al. (2010) did not find any significant correlation between HI and SDSS.

Huang et al. (2006) found a negative correlation between mixing development time and flour protein. However, we did not find a significant correlation between peak time and flour protein. Though flour protein percent and HI were significantly positively correlated in a study by Kuchel et al. (2006), we were unable to find any relationship between protein percent and HI.

We observed a positive correlation of mixing tolerance with peak time and HI, similar to the results reported by Kerfal et al (2010). However, Carver (1994) did not find a significant relationship of hardness score with mixing tolerance and mixing time but found significant positive effect on protein content. In our experiments, we did not find a significant relation of HI on protein content. Similarly, kernel weight was negatively correlated with hardness index and positively with the kernel diameter. Nishio et al. (2005) also found the negative correlation between kernel weight and HI and positive with diameter.

Huang et al. (2006) found positive significant correlation among grain yield, test weight and thousand kernel weights. Grain yield and flour protein content showed negative correlation but was not significant (Huang et al., 2006). We also found significant negative correlation between grain yield and protein percent in our experiment.

The quality traits like SDSS volume, peak time and mixing tolerance were affected by growing environment. The principal component analysis showed two clusters in biplot for SDSS volume performed for lines grown in four environments. We identified ten most stable and least stable lines based on the coefficient of variation (CV) estimates for SDSS volume. The stable lines showed higher average SDSS volume than the least stable lines. This higher performance of stable lines could be because of the combination of heat tolerant parent Halberd and parent Len with good agronomic characteristics.

CHAPTER III
IDENTIFICATION OF GENETIC LOCI REGULATING END-USE QUALITY
STABILITY

1. Introduction

In crop species, different kinds of traits are found, which are categorized as qualitative and quantitative in nature. Qualitative traits are simple and have less environmental influence. Whereas, the quantitative traits are complex i.e., controlled by many genes and have high environmental influence. But most of the important traits in crops are quantitative in nature, for example yield and quality. In wheat, both grain yield and grain quality are very important quantitative traits. Wheat grain quality can be determined by several variables such as grain size, protein percentage and composition, starch content and composition etc. (Panozzo and Eagles, 2000). These traits are highly influenced by the growing environment. Every year, unpredictable fluctuation in growing conditions occurs due to changes in temperature, rainfall etc. This has a negative impact on wheat quality. But the milling and baking industries requires consistent end-use quality for processing.

The growth and productivity of major crop species including cereals are reduced by abiotic stresses such as high temperature (Barnabas et al., 2008). However, it is very difficult to immediately and fully recover from the severe and short period of heat stress in terms of starch and protein synthesis (Stone and Nicolas, 1994). However, some cultivars have been identified that are not as affected by heat stress in terms of yield. The aim of breeding is to identify genotypes which maintain a conservative transpiration, gas

exchange yet maintain a lower canopy temperature as compared with other genotype in the same field condition. Unfortunately, it is hard to maintain quality during environmental stresses. This inconsistency in quality due to environmental variation can be a problem for end-users for their high-throughput processing.

The genetic components affecting wheat quality traits such as protein content, grain hardness, gluten strength etc., can be identified and characterized using linkage based quantitative trait loci (QTL) analysis. QTL analysis is the statistical analysis of finding specific genetic loci associated with the traits of interest, after merging genotypic and phenotypic data together (Somers and Humphreys, 2009). Generally in breeding program, end-use quality is selected in later generation due to less grain available for test and also due to the time and cost required for rheological and baking tests (Ross and Bettge, 2009). However it could be possible to select for good end-use quality by using molecular markers linked to favorable alleles during early generations..

Many studies have reported QTLs affecting quality traits. But these identified QTLs are mostly useful in similar environmental conditions where they were identified. And these are not able to show their effect in different growing conditions. Empirical evidences have shown that some cultivars have the ability to perform in the same manner in different environmental conditions (Shewry, 2009). So, the stable QTLs affecting quality traits can be identified in these cultivars. The objective of this study is to detect QTLs affecting end-use quality stability or genetic loci for marker assisted selection (MAS) that condition good quality stability across both favorable and unfavorable environments. Identifying the quantitative loci overlapping the yield and

quality in wheat can also be very useful in selecting cultivars possessing both the traits using marker-assisted selection. The line showing high yield and good quality performance across environments would be favored as a stable genotype. It can be used as either a cultivar or as donor parents to develop cultivar better suited in different environments.

2. Materials and methods

2.1. Plant material

In this study a population comprised of 180 recombinant inbred lines (RILs) developed by crossing two spring wheat lines ‘Halberd’ and ‘Len’ were used. The parent Halberd is heat tolerant, whereas Len is known for its good agronomic characteristics. The RILs were developed by advancing the F₁ progeny through single seed descent method to the F₆ generation.

2.2. Growing environment

The RILs and the two parents were grown in the field at two locations, College Station and Uvalde, TX in the 2009/10, and College Station and Chillicothe TX in 2010/2011 growing seasons. These experiment sites possessed demarcated difference in growing conditions in terms of monthly average temperatures and precipitation. The experiments were laid out as randomized complete block design (RCBD) with four replications in each location.

2.3. Phenotypic measurement of quality parameters

The quality measurements taken were single kernel hardness index, kernel weight and kernel diameter using a Single Kernel Characterization system (Perten Model SKCS 4100). Similarly, protein percent was determined with near-infrared reflectance (NIR) by using the Technichon Infranalyzer 300. Mixograph (Mixograph National Manufacturing CO, Lincoln, NE, USA) was used to determine the dough mixing properties, whereas with mixogram was used to determine peak time and mixing tolerance. Sodium dodecyl sulfate sedimentation test (Moonen 1989) was performed to determine the baking quality of flour.

2.4. Mapping of QTLs

After phenotypic assessment of wheat quality parameters in RILs, genotyping of the population and QTL mapping were performed. All the lines were planted in the greenhouse along with the parental lines. Ten to fifteen coleoptiles were randomly selected from each line and extracted for genomic DNA. The DNA samples were then sent to KBioscience genotyping services for 1100 Single Nucleotide Polymorphism (SNPs) genotyping.

Whole genome linkage map was constructed by using QTL ICI Mapping software (Li et al., 2008). Composite interval mapping was carried out to identify significant QTLs associated with the above mentioned quality traits. LOD score was set by using 1000 permutation test. To convert recombination fraction into map distance (cM) Kosambi mapping function was used. Significant QTLs were characterized based on

their positions in linkage groups and their contribution to the total phenotypic variation. Finally, stable QTLs which are consistently significant in both environments were identified for all the quality traits and were compared with previously reported QTLs.

3. Results

3.1. Linkage map construction

For linkage mapping, we used 116 polymorphic SNPs out of total 1100 markers. We removed all the missing and redundant markers for final analysis. For 21 chromosomes of hexaploid wheat, we identified 24 linkage groups. The final linkage map was used in QTL mapping for all the quality traits. QTL mapping was performed using QTL ICiMapping software. The 1000 permutation test to determine the LOD threshold for different quality traits. The LOD threshold value ranged from 2.5 to 3.5 for different traits.

3.2. QTL for SDSS column height

We were able to detect four significant QTLs for SDSS column height from two different locations i.e., UVL10 and CH11 (Table 10). Among these four QTLs, three were detected in UVL10 and one was detected in CH11. The first QTL (*Qsds.tam-1B*) in UVL10 was located on chromosome 1B, flanked by SNP markers BS00009848 and BS00005009. The R^2 value of this QTL was 7.24%. The Len allele of this QTL contributed higher SDSS column height. The other two QTLs (*Qsds.tam-NA.1* and *Qsds.tam-NA.2*) from UVL10 were detected in unknown chromosomes. These two

QTLs were flanked by markers BS00003452 and BS00012302, and BS00004120 and BS00004120 respectively. Both of these QTLs explained 6.49 and 6.93% of phenotypic variation respectively. For both of these QTLs, the Halberd allele contributed a higher SDSS column height.

We identified one QTL (*Qsds.tam-4B*) on chromosome 4B in CH11. This QTL was flanked by BS00003879 and BS00009373 markers. It explained 8.87% of the phenotypic variation. The higher SDSS column height was contributed by Halberd allele for this QTL. We also detected two minor effect QTLs for SDSS column height in two locations i.e., CS10 and CS11 (Table 11). The QTL (*Qsds.tam-1B*) for CS10 was detected on chromosome 1B, flanked by BS00005009 and BS00003892 SNP markers. This QTL explained 6.86% of phenotypic variation. However, other QTL (*Qsds.tam-5A*) for CS11 was detected on chromosome 5A, flanked by BS00003696 and BS00000645 SNP markers. This QTL explained 23.95% of phenotypic variation. For both of these QTLs the higher SDSS value was contributed by the Len allele.

We identified one stable QTL (*Qsds.tam-4B*) for SDSS column height on chromosome 4B (Fig.7). This QTL was detected based on the coefficient of variation (CV) for SDSS test in four different environments. This QTL was flanked by markers BS00003879 and BS00009373 and explained 5.29% of phenotypic variation. The higher SDSS value was contributed by Halberd allele.

3.3. *Mixograph QTLs*

We detected three significant QTLs for peak time in two different locations i.e., UVL10 and CS10. In UVL10, we identified one QTL (*Qpt.tam-1B*) on chromosome 1B flanked by BS00009848 and BS00005009 SNP markers. This QTL explained 7.44% of the phenotypic variation. However, another QTL (*Qpt.tam-NA.3*) from UVL10 was detected on unknown chromosome, flanked by wE2252_01 and BS00012392 markers. This QTL explained 10.38% of variation. For CS10, we identified one QTL (*Qpt.tam-NA.3*) on unknown chromosome, between wE2252_01 and BS00012392 markers. This QTL explained 11.34% of phenotypic variation. For all of these three QTLs for peak time the Len allele contributed for higher peak time than Halberd allele.

One minor effect QTL (*Qpt.tam-1B*) from UVL10 was associated with peak time on chromosome 1B. This QTL was flanked by BS00012452 and BS00020861 SNP markers. This QTL explained 5.65% of phenotypic variation. Here, Len allele contributed for high peak time.

We also detected three QTLs associated with mixing tolerance in two different environments i.e., UVL10 and CS10. From UVL10, we detected two QTLs, one on chromosome 1B and another on unknown chromosome. The QTL (*Qtol.tam-1B*) on chromosome 1B was flanked by BS00009848 and BS00005009 SNP markers, and explained 7.66% of phenotypic variation. However, on unknown chromosome QTL (*Qtol.tam-NA.3*) was flanked by wE2252_01 and BS00012392 markers and explained 10.23% of phenotypic variation. From CS10, we detected one QTL (*Qtol.tam-1B*) on chromosome 1B, flanked by BS00009848 and BS00005009 SNP markers. This QTL

explained 7.34% of phenotypic variation. For all of these mixing tolerance QTLs, Len allele contributed for high tolerance score than the Halberd allele.

One minor effect QTL (*Qtol.tam-1B*) from UVL10 was associated with mixing tolerance on chromosome 1B. This QTL was flanked by BS00012452 and BS00020861 SNP markers. This QTL explained 11.42% of phenotypic variation. Here, Len allele contributed for high tolerance score. Another minor effect QTL (*Qtol.tam-5B*) from UVL10 was detected on chromosome 5B. This QTL was flanked by BS00001817 and BS00003612 markers, which explained 13.18% of phenotypic variation. However, the QTL (*Qtol.tam-NA.3*) from CS10 was detected on unknown chromosome, flanked by wE2252_01 and BS00012392 markers. This explained 6.26% of phenotypic variation. The higher tolerance score was contributed by Len allele.

3.4. SKCS QTLs

Two QTLs were detected for kernel weight each in two locations i.e., UVL10 and CS10. In UVL10 a QTL (*Qkwt.tam-2B*) was found on chromosome 2B flanked by BS00009574 and BS00009290 SNP markers. This QTL explained 38.14% of the phenotypic variation. The higher kernel weight was contributed by Halberd allele. However, another QTL (*Qkwt.tam-4B*) for kernel weight was detected in CS10 on chromosome 4B, flanked by BS00009974 and BS00003781 markers. This QTL explained 17.62% of the phenotypic variation. The higher kernel weight was contributed by Len allele.

The two QTLs associated with kernel diameter were found from UVL10. The first QTL (*Qdiam.tam-4B*) was detected on chromosome 4B and flanked by BS00003879 and BS00009373 SNP markers. This QTL explained 11.99% of phenotypic variation. The higher kernel diameter was contributed by Len allele. Whereas, the QTL (*Qdiam.tam-5B*) was detected on 5B flanked by BS00009843 and BS00001314 markers. This QTL explained 20.08% of phenotypic variation. The higher kernel diameter was contributed by Halberd allele.

We also detected three minor QTLs for hardness index from two different locations i.e., UVL10 and CS10. One QTL (*Qhi.tam-1B*) was detected on chromosome 1B from UVL10. This was flanked by BS00009848 and BS00005009 SNP markers. This QTL explained 13.58% of phenotypic variation. Other two QTLs (*Qhi.tam-1B*) were identified on chromosome 1B on the same position from both locations i.e., CS10 and UVL10. Both of these QTLs were flanked by BS00003944 and BS00012743 SNP markers. These two QTLs explained 11.22 and 10.72% of phenotypic variation respectively. The higher HI was contributed by Len allele for both QTLs.

3.5. Protein percent QTL

One minor effect QTL (*Qpro.tam-4B*) for protein percent was detected on chromosome 4B from UVL10. This QTL was flanked by BS00009974 and BS00003781 SNP markers. It explained 5.88% of the phenotypic variation. The higher protein percent was contributed by the Len allele.

Table 10. Major QTL and their characteristics for quality traits detected in Uvalde 2010, College Station 2010 and Chillicothe 2011 experiments

Trait†	Chr‡	Design§	Position¶	L-Marker#	R-Marker#	LOD††	PVE‡‡	Add§§	parent¶¶
SDSS_UVL10	1B	<i>Qsds.tam-1B</i>	57	BS00009848	BS00005009	3.09	7.24	-1.83	Len
SDSS_UVL10	NA.1	<i>Qsds.tam-NA.1</i>	6	BS00003452	BS00012302	2.76	6.49	1.73	Hal
SDSS_UVL10	NA.2	<i>Qsds.tam-NA.2</i>	0	BS00004120	BS00004120	3.03	6.93	1.79	Hal
SDSS_CH11	4B	<i>Qsds.tam-4B</i>	0	BS00003879	BS00009373	3.35	8.87	4.31	Hal
PT_UVL10	1B	<i>Qpt.tam-1B</i>	57	BS00009848	BS00005009	3.36	7.44	-0.16	Len
PT_UVL10	NA.3	<i>Qpt.tam-NA.3</i>	0	wE2252_01	BS00012392	4.76	10.38	-0.19	Len
PT_CS10	NA.3	<i>Qpt.tam-NA.3</i>	4	wE2252_01	BS00012392	3.48	11.43	-0.17	Len
TOL_UVL10	1B	<i>Qtol.tam-1B</i>	57	BS00009848	BS00005009	3.67	7.66	-0.41	Len
TOL_CS10	1B	<i>Qtol.tam-1B</i>	57	BS00009848	BS00005009	2.96	7.34	-0.32	Len
TOL_UVL10	NA.3	<i>Qtol.tam-NA.3</i>	0	wE2252_01	BS00012392	4.97	10.23	-0.47	Len
KWT_UVL10	2B	<i>Qkwt.tam-2B</i>	75	BS00009574	BS00009290	4.33	38.14	1.47	Hal
KWT_CS10	4B	<i>Qkwt.tam-4B</i>	77	BS00009974	BS00003781	3.92	17.62	-0.93	Len
DIAM_UVL10	4B	<i>Qdiam.tam-4B</i>	1	BS00003879	BS00009373	4.70	11.99	-0.04	Len
DIAM_UVL10	5B	<i>Qdiam.tam-5B</i>	128	BS00009843	BS00001314	3.87	20.08	0.05	Hal

† Quality traits with corresponding experiment used in QTL analysis

‡ Chromosome in which significant QTL are detected. The “NA” designation represents linkage group with unknown chromosome

§ Temporary designation of significant QTL

¶ Location of QTL in corresponding chromosome (cM)

Left and right flanking marker of the QTL

†† Logarithm of Odd value obtained from likelihood test

‡‡ Percent variation explained by QTL (also called R^2 or heritability of QTL)

§§ Additive effect of QTL. In this analysis, -ve and +ve sign represent the higher phenotypic values is contributed by Len and Halberd parents respectively

¶¶ Source parental allele responsible for higher phenotypic values (HAL=Halberd)

Table 11. Minor QTLs and their characteristics for quality traits detected in different environments

Trait†	Chr‡	Design§	Position¶	L-Marker#	R-Marker#	LOD††	PVE‡‡	Add§§	parent¶¶
SDSS_CS11	5A	<i>Qsds.tam-5A</i>	71	BS00003696	BS00000645	2.21	23.95	-6.82	Len
SDSS_CS10	1B	<i>Qsds.tam-1B</i>	62	BS00005009	BS00003892	2.02	6.86	-1.65	Len
PT_UVL10	1B	<i>Qpt.tam-1B</i>	349	BS00012452	BS00020861	2.69	5.65	-0.14	Len
TOL_UVL10	1B	<i>Qtol.tam-1B</i>	339	BS00003944	BS00012743	3.28	11.42	-0.50	Len
TOL_UVL10	5B	<i>Qtol.tam-5B</i>	40	BS00001817	BS00003612	3.17	13.18	0.53	Hal
TOL_CS10	NA.3	<i>Qtol.tam-NA.3</i>	0	wE2252_01	BS00012392	2.61	6.26	-0.30	Len
PRO_UVL10	4B	<i>Qpro.tam-4B</i>	66	BS00009974	BS00003781	2.24	5.88	-0.12	Len
HI_UVL10	1B	<i>Qhi.tam-1B</i>	39	BS00009848	BS00005009	2.02	13.58	-3.16	Len
HI_UVL10	1B	<i>Qhi.tam-1B</i>	338	BS00003944	BS00012743	2.31	11.22	-2.88	Len
HI_CS10	1B	<i>Qhi.tam-1B</i>	338	BS00003944	BS00012743	2.40	10.72	-2.51	Len

† Quality traits with corresponding experiment used in QTL analysis

‡ Chromosome in which significant QTL are detected. The “NA” designation represents linkage group with unknown chromosome

§ Temporary designation of significant QTL

¶ Location of QTL in corresponding chromosome (cM)

Left and right flanking marker of the QTL

†† Logarithm of Odd value obtained from likelihood test

‡‡ Percent variation explained by QTL (also called R^2 or heritability of QTL)

§§ Additive effect of QTL. In this analysis, -ve and +ve sign represent the higher phenotypic values is contributed by Len and Halberd parents respectively

¶¶ Source parental allele responsible for higher phenotypic values (HAL=Halberd)

3.6. Effect of QTL combination

Based on the parental allele (denoted as H and L for Halberd and Len respectively) combination of the nearest marker for three SDSS QTLs (one on 1B and two on unknown chromosome), the RILs were divided into eight genotype groups (Fig.8). Then one way ANOVA was performed to see the effect of different genotype. The allele combination LHH gave the highest SDSS score, whereas HLL gave the lowest (Table 12). The performance of HHH and LLL combination were similar. For peak time and mixing tolerance QTLs detected in two different environments, the Len parental allele was contributing the highest score for both traits (Fig.9).

Table 12. Mean comparison of SDSS volume of UVL 2010 for different combination of parental allele for three major quantitative trait loci

QTL genotype†	SDSS Mean	Std. Err. ‡	t-grouping§
LHH	86.75	2.12	A
LLH	85.76	2.26	AB
LHL	84.74	1.99	AB
HHL	83.71	2.26	AB
HHH	81.61	1.80	AB
LLL	81.54	1.80	AB
HLH	80.69	1.41	B
HLL	74.00	1.89	C

† Parental allele combination (H=Halberd, L=Len) based on one closest marker of three SDSS QTL *Qsds.tam-1B*, *Qsds.tam-NA.1*, *Qsds.tam-NA.2* identified in Uvalde 2010 experiment.

‡ Standard error of the mean,

§ t-grouping of means based on critical t-value 1.99 at $P=0.05$. The groups connected with same alphabet are not significantly different

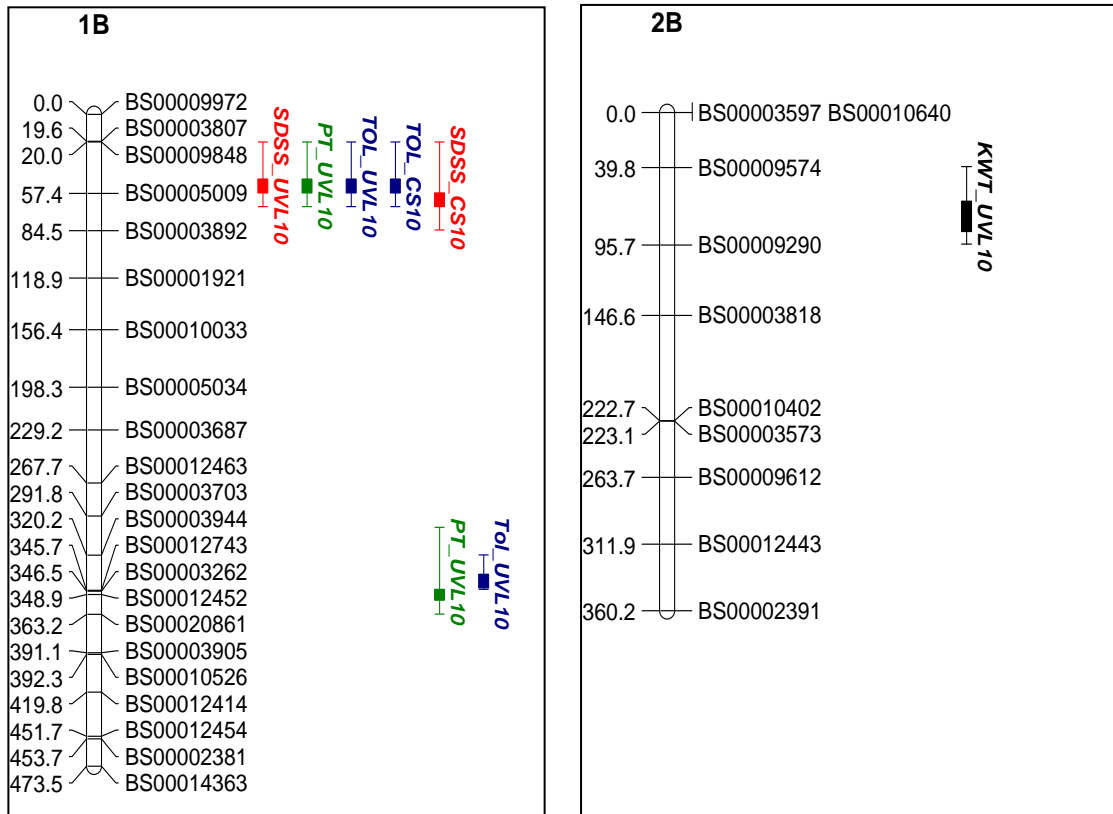


Fig. 7. Position of QTLs for different quality traits in different chromosomes

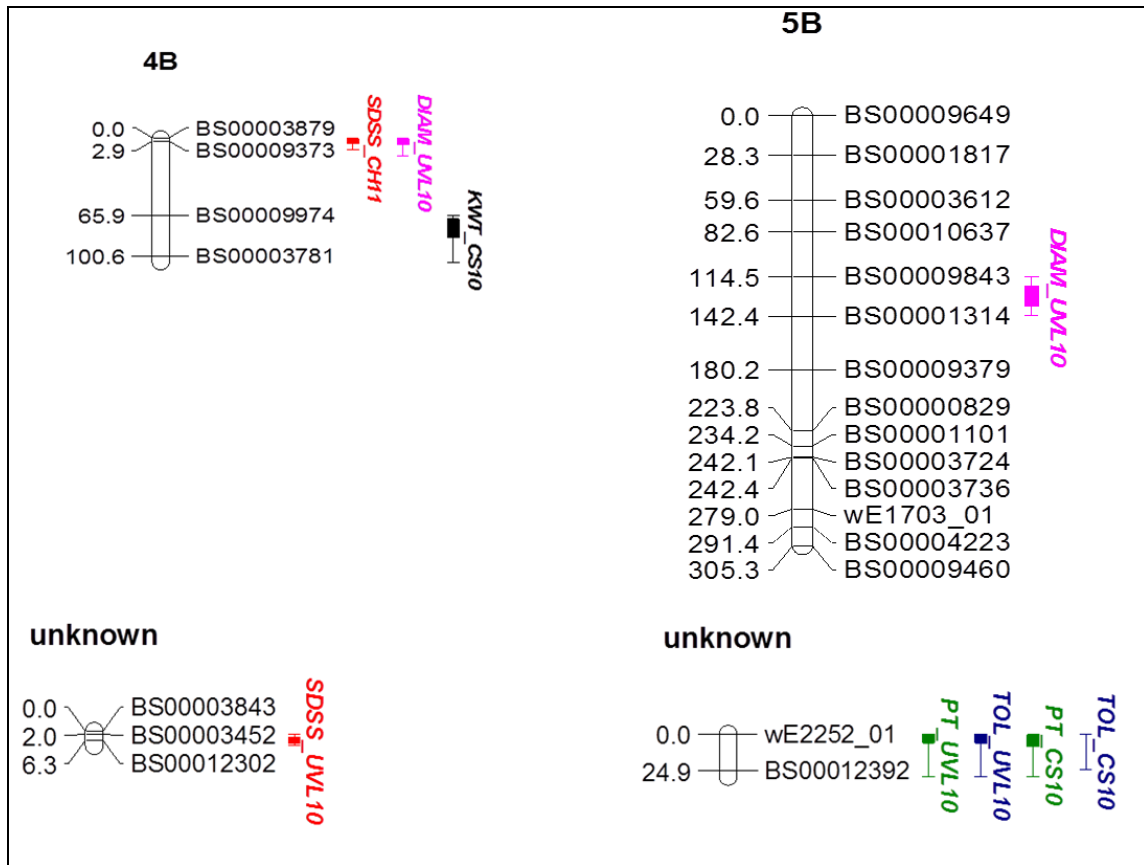


Fig. 7. Continued

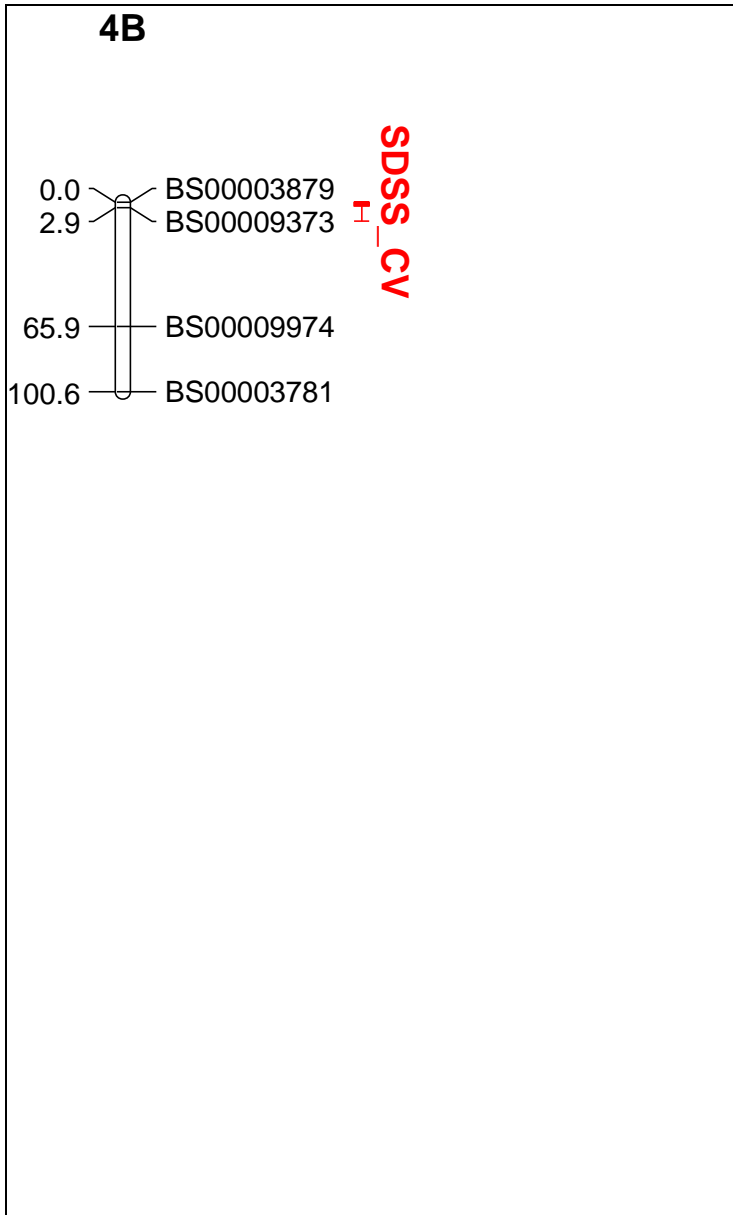


Fig. 7. Continued

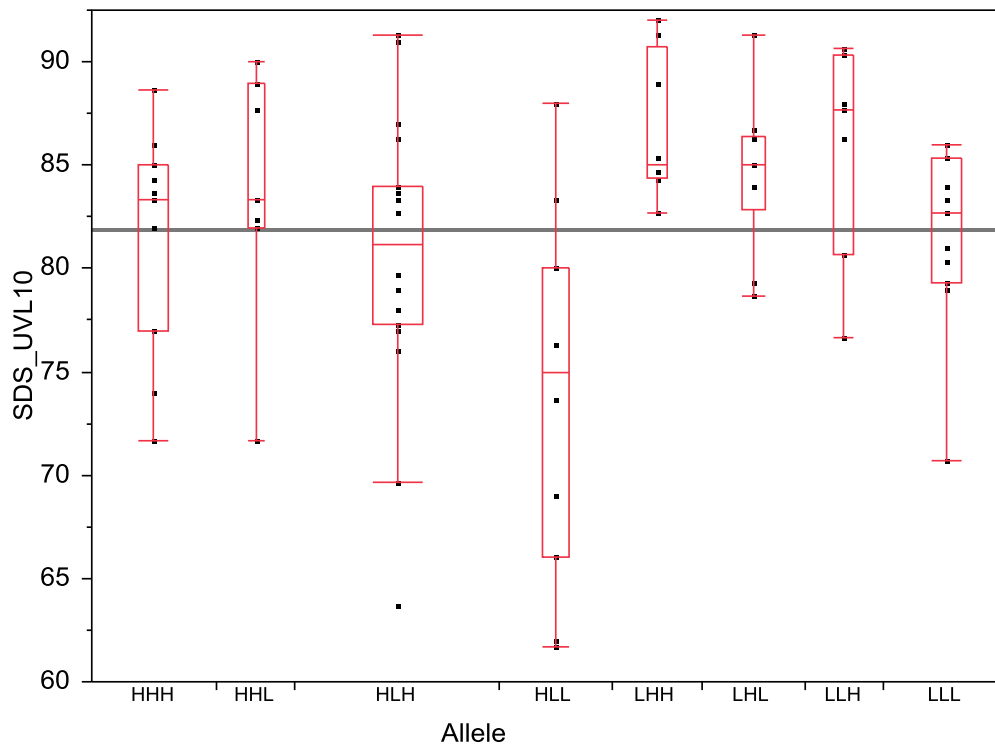


Fig. 8. Box plots of mean SDSS volume of 8 QTL genotypes based on parental allele combination of 3 QTL *Qsds.tam-1B*, *Qsds.tam-NA.1*, *Qsds.tam-NA.2* detected in UVL 2010. The three letter symbols for allele combination stands for either H (Halberd) or L (Len) allele of markers which are associated with aforementioned significant QTL

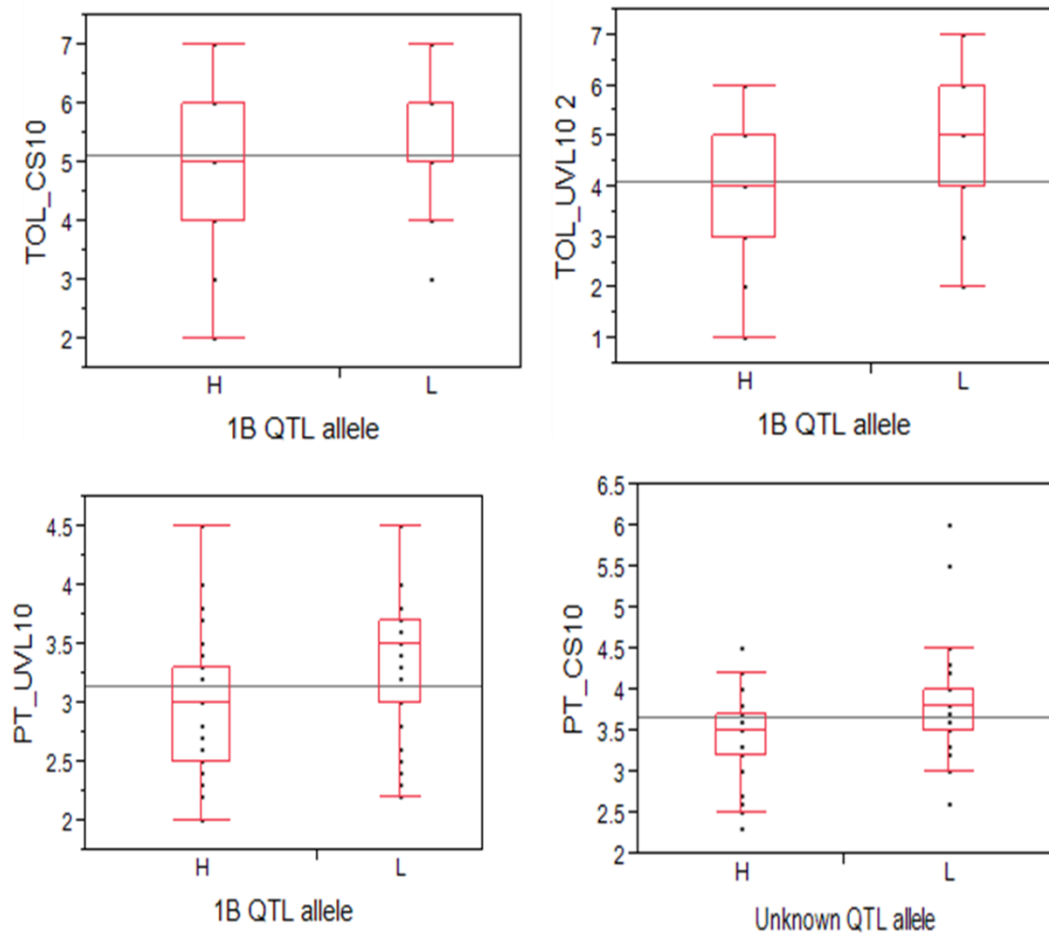


Fig. 9. Box plots showing the comparison of parental allele means for mixographic trait QTL detected in different experiments. H and L denote the RILs with Halberd and Len allele respectively

4. Discussion

The goal of this project was to identify the stable QTLs for quality traits, which could be expressed across the environments. We were able to detect many QTLs for different quality traits but most of them were site specific. Only few QTLs were detected in both the environment. These overlapping QTLs could be very useful for marker-assisted selection, yet these need verification.

Out of the total SNP makers screened in population, the frequency of polymorphic markers was very low (11.5%). So, the genome coverage by 116 polymorphic markers was relatively low. A total of 25 linkage groups were formed covering 17 chromosomes of hexaploid wheat. We did not find any polymorphic SNPs for chromosomes 3D, 4A, 4D and 7D. The total genetic distance covered by linkage map was 2746cM which was comparable to microsatellite consensus map of wheat by Somers et al. (2004).

We identified a QTL for SDSS column height on chromosome 1B. A similar QTL on 1B was reported in previous studies by Huang et al. (2006), Li et al. (2009) and Beecher (2009) for SDSS height. The QTL for SDSS score was identified on chromosome 1B in UVL10 and CS10 experiments were in map position of 57 and 62 cM respectively. One of the flanking markers BS00005009 was common. We also identified one stable QTL for SDSS height on chromosome 4B based on the coefficient of variation (CV). This QTL was flanked by markers BS00003879 and BS00009373 and explained 5.29% of phenotypic variation.

The QTL associated with peak time was detected on chromosome 1B. Similarly, Zhang et al. (2009) reported the QTL for peak time on chromosome 1B. Two other QTLs for peak time were identified in unknown chromosomal region. These two QTLs were from two different environments i.e., UVL10 and CS10. This QTL was flanked by wE2252_01 and BS00012392 markers.

Total of three QTLs for mixing tolerance were detected on chromosome 1B. Campbell et al. (2001) also found the mixing tolerance QTL on chromosome 1B. This QTL on chromosome 1B was detected in both the environment i.e., UVL10 and CS10. This QTL was detected on the same position on chromosome 1B (57cM), and flanked by the same markers BS00009848 and BS00005009. One more QTL for mixing tolerance was identified on unknown chromosomal region that also overlapped with the first one. This QTL was flanked by wE2252_01 and BS00012392.

We also detected three QTLs for hardness index; all of these QTLs were in chromosome 1B. Two of them were from different environments i.e., UVL10 and CS10. This overlapping QTL was in the same position i.e., 338cM and flanked by markers BS00003944 and BS00012743.

In spite of limited genome coverage of markers, we detected many QTLs associated with different quality traits. Most of the QTL peaks were significantly above the LOD threshold obtained by 1000 permutation tests. However, some were found slightly below the threshold. This is probably due to the low power of QTL detection imposed by low resolution linkage map. Most of the QTLs for quality traits i.e., SDSS, peak time, mixing tolerance and hardness index were identified on chromosome 1B with

potential overlap. As some genes for wheat storage protein (usually gluten) has been reported to reside on 1B chromosome (Payne et al., 1981; 1987), further study is necessary to confirm the novelty of our results.

CHAPTER IV

SUMMARY AND CONCLUSION

We identified fourteen major QTLs for different quality traits i.e. SDSS column height, peak time, mixing tolerance, kernel diameter and kernel weight in different environments. However, ten minor QTLs were detected for SDSS column height, peak time, mixing tolerance, protein percent and kernel hardness index. Most of the QTLs were identified on chromosome 1B. The majority of the detected QTLs were site specific although some were expressed in both locations.

In spite of high environmental influence, significant correlations were observed among growing environments for different quality traits such as peak time, mixing tolerance and SDSS, indicating stable expression of underlying genetic factors. This observation was further confirmed by the identification of stable QTLs for SDSS column height on chromosome 4B. The SNP markers closely linked with stable QTLs should be useful in screening breeding populations and advanced lines for better bread making quality in early generation selection.

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