LINKING QTLS THAT REGULATE THE DISTINCT EPICUTICULAR LAYERS IN THE SPIKE GLUME, AND ITS VARIABLE COMPOSITION TO IMPROVE REPRODUCTIVE STAGE HEAT TOLERANCE IN WHEAT

A Dissertation

by

AHMED ABDELFATTAH ELSAYED

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

DOCTOR OF PHILOSOPHY

Chair of Committee,	Dirk B. Hays
Committee Members,	Amir M.H. Ibrahim
	Hongbin Zhang
	Russell Jessup
Intercollegiate Faculty Chair,	Dirk Hays

May 2016

Major Subject: Molecular and Environmental Plant Sciences

ABSTRACT

Global climate experiments project an average increase of ambient temperatures of 0.2°C per decade. Such prediction emphasizes the importance of crop varieties that have high heat tolerance. Wheat is significantly affected by high temperature. Optimizing heat and drought tolerance in wheat is one way to improve breeding efficiency.

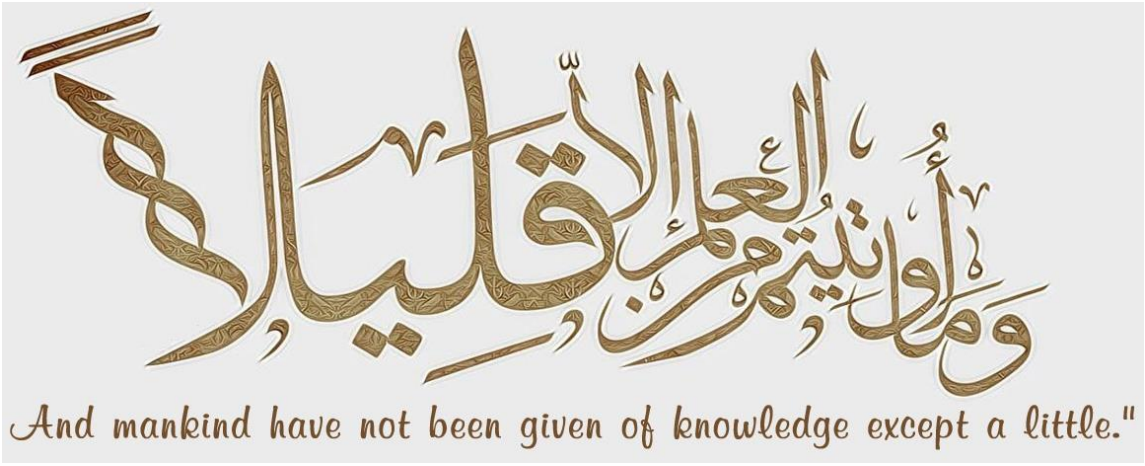
Previous studies on wheat leaf epicuticular wax (EW) have shown a strong association between wax load and high temperature stress tolerance. This study aimed to investigate the relationship between EW on wheat glume and high temperature tolerance. This study also compared the effect of glume EW to the effect of leaf EW on the plant agronomic productivity. A recombinant inbred line (RIL) population derived from the heat tolerant Australian cultivar 'Halberd' which we have previously identified as having a unique genetic loci regulating spike cooling, was used in this experiment. The RIL mapping panel contains 180 lines derived from Halberd and a heat susceptible cultivar, Len. The population was grown at multiple field locations at Collage Station, Texas, Uvalde, Texas, and Obregon, Mexico for the growing seasons of 2013 and 2014. The EW of leaves and glumes were extracted using published methods (Richardson et al, 2007). An alpha lattice design with 180 recombinants and 2 replications was used in four different environments over 2 years. The EW samples were collected at 10DAP and leaf/spike temperatures were recorded at the same time. Spectral canopy reflectance was measured between 350–1100 nm range. Yield components were estimated after harvest. Spike temperature depression was measured. The 180 RIL and their parents were mapped using 90K SNPs markers to identify linkage groups or QTL for EW.

A strong correlation was found between mean wax load for leaf and that for glume as function of mean high temperature recorded for 10DAP across the environments with R^2 = 0.6719 and R^2 = 0.8483 respectively . The maximum mean of leaf EW at OBR14 being 5.37 and that of CS13 was 2.51 mg/dm^2, while the glume EW mean at OBR14 was 5.97 mg/dm^2 and CS13 was 2.38 mg/dm^2. The EW mean was higher in glumes as compared to that of leaf for all locations except for CS13, which was considered a more optimum climate for wheat. A strong correlation between the two wax loads for UVL13 was observed with R^2 = 0.8285 and r=0.9195 significant at $p \leq 0.001$. A significant correlation also was observed for the two wax loads for OBR14 with R^2 = 0.0304 and r=0.1744 significant at $p \leq 0.05$. All yield and yield components data showed significant variation between the different growing locations.

Correlation between WI was significant at $p \leq 0.05$ for most water status indices were associated with the glume wax with R^2 ranging from 0.274 to 0.2198, whereas there was no correlation between WI and leaf wax. The thermal index had negative correlation and was only significant with glume wax content with r = -0.5943 and significance level of $P \leq 0.001$. Spike temperature had a positive correlation with both leaf and glume wax content with an R^2 values of 0.078 and 0.1952 respectively.

Two significant QTL for EW were detected on chromosome 5B. Leaf EW, QLWax.tam-5B, was on position 104.584 and explained 6.8% of the variation. Glume EW, QGWax.tam-5B, was located on position 102.098 and explained 6.6% of the variation.

DEDICATION



This work is dedicated to my father Dr. Abdelfattah Elsayed who inspired, supported, and encouraged me to pursue my PhD.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my major advisor, Dr. Dirk Hays, for his excellent guidance, support, patience, and encouragement throughout my research studies. I would like to thank Dr. Amir Ibrahim, who gave unlimited support and shared valuable knowledge to improve this work. I thank my committee members Dr. Hongbin Zhang, and Dr. Russell Jessup, for their advice and support. To my lab mates and colleagues, especially Mr. Alfredo Delgado, Dr. Suheb Mohammed, and Dr. Trevis Huggins for being there when needed.

I extend my gratitude to Dr. Suchismita Mondal, Dr. Mariano Cossani, Dr. Maria Tatars, and Dr. Ravi Singh for their collaboration in CIMMYT. I also acknowledge Monsanto's Beachell-Borlaug International Scholars Program for supporting this research along with Dr. Edward Runge, the director of the program, who was like a guardian angel through this process. I am deeply grateful to Dr. Samy Sabry and ARC Egypt for their collaboration and support.

I owe my sincere appreciation to my parents whom I am grateful to them for their inspiration, love, and encouragement.

Finally, I would like to thank my beloved wife and the joy of my heart for her continuous support and endurance.

NOMENCLATURE

CT	canopy temperature
CTD	canopy temperature depression
DAP	days after pollination
EW	epicuticular wax
LOD	likelihood of odds
LSD	least significant deference
MSHW	mean single head weight
NDVI	normalized difference vegetation index
NDWI	normalized difference water index
NIR	near infrared
NWIs	normalized water indices
PRI	photo reflective index
PAR	photothynstic active region
QTL	quantitative trait loci
RILs	recombined inbred lines
RWC	relative water content
SKW	single kern weight
SNP	single nucleotide polymorphism
SRI	spectral reflectance indices
SR	simple ratio index
TI	thermal index

TABLE OF CONTENTS

Page

Page

Page

LIST OF FIGURES

Page

Page

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Model simulation experiments on global climates projects an average increase of ambient temperatures of 0.2°C per decade for the 2000 to 2100 period. This translates into an increase of about 1.7 and 5.8°C of overall global temperature by the end of this century (IPPC, 2007). Such prediction emphasizes the importance of crop varieties that have high heat tolerance. Wheat is one of the very important crops because it is a staple food for the world population. Heat and drought are major factors limiting wheat yields worldwide, especially in regions where 60% of global land area is classified as arid or semiarid. Controlling heat and drought tolerance in wheat is one way to improve breeding efficiency.

Generally speaking, most rain-fed farmers are limited in resources, own small land holdings, and have minimal capacity to adopt high input technologies. Consequently, heat and drought tolerant wheat varieties are appropriate solution because they are farmer friendly and are based on seed technology that is easy to disseminate. Although steady progress has been made with up to date breeding work (e.g. Trethowan et al., 2002; Ammar et al., 2008), overall performance of cereals still shows considerable grain yield loss to high temperatures (Wardlaw et al., 1989; Reynolds et al., 1994).

Wheat is considered a temperate cereal, grown widely throughout the world in semi-arid regions of Central to West Asia and North Africa (CWANA). While it's normally well adapted to environment in these regions, it can be very sensitive to elevated temperature (Slafer and Satorre 1999). High temperatures induces heat stress in wheat, particularly during reproductive and grain-filling stages (Wollenweber et al. 2003). The ideal temperature

for wheat anthesis and grain filling is between 12 to 22℃, and temperatures above this reduce grain yield significantly (McDonald et al., 1983; Macas et al., 1999, 2000; Mullarkey and Jones, 2000; Tewolde et al., 2006). Heat stress during anthesis can cause increased embryo abortion, pollen sterility, tissue dehydration, lower CO_2 assimilation and increased photorespiration (Wardlaw and Wrigley 1994). It was found that many current Hard Red Winter Wheat (HRWW) cultivars grown in the Southern Great Plains are heat susceptible in terms of sterility, abortion and an early transition to the dry seed stage (Hays et al 2007a, and b).

In order for wheat to maintain growth and productivity, it must be adaptable to heat stress conditions via of specific tolerance mechanisms. Some of these mechanisms involve the alteration of various photosynthetic attributes and physiological traits under heat stress exposure. Changes after perception of heat stress signals also occur at the molecular level, altering the expression of genes and accumulation of transcripts as a stress tolerance strategy (Iba 2002). As an example of tolerance adaptation, germplasm from Australia, CIMMYT, and ICARDA were found to exhibit heat and drought tolerance while in active productive stage. These lines uphold photosynthesis and yield through production of high seed set, grain weight, and an extended grain filling under heat stress conditions.

The first line of defense against high temperatures in wheat is the epicuticular wax that covers the plants cuticle in various areas of the plant (figure 1.1). The presence of this waxy layer on the leaf and glume can reduce heat stress by epidermal transpiration and excess light energy (figure 1.2). Studies show that surface reflectance was reduced when the waxy layer from the leaf was removed with chloroform and reduction was observed for the abaxial surface and the adaxial surface (Uddin, M. Nizam et.al 1988). This indicates that

epicuticular wax is a major factor in leaf rolling mechanism and abaxial reflectance. As an adaxial and abaxial reflective surface to excess energy, epicuticular wax reduces transpirational cooling needs and stomatal conductance. The scattering of heat during high temperatures by the epicuticular layer help maintain a temperate cellular environment, minimizing water loss and optimizing metabolic function. Consequently, epicuticular wax can hinder induction of drought responses that reduce photosynthesis and promote seed abortion. Therefore, it is important to perform studies that focus on epicuticular wax to optimize structural content and chemical composition in crops such as wheat. Such studies could prove effective as a strategy in reducing economic disadvantages of irrigation, increasing yield, improving quality, and producing cultivars with high heat tolerance to elevated temperature environments.

The *long-term goal* of this project was to produce new wheat cultivars that have high heat tolerance by using a focused program of enhancing the structure or function of the leaf and glume epicuticular wax layer. The main objective of this study was to preform quantitative and qualitative analysis on the epicuticular wax layer in wheat glume tissue to define the importance of structure and function of this layer and correlate this analysis in the context of higher yield and quality stability during reproductive stage heat stress. The hypothesis is that the presence of higher epicuticular wax in the glume tissue confers to higher adaptation to heat stress tolerance in wheat. Furthermore, the epicuticular layer in the glume in particular is important in maintaining a cooler metabolically optimal sink environment for the developing grain. To maximize the impact of the study, recombinant inbred line (RIL) that has been developed which differs in glume wax content, glaucousness, and spike temperature depression (or spike cooling) have been used. The RILs have been

used to define the molecular and ecophysiological basis of improved adaptation to heat stress using physiological and quantitative trait loci mapping. Field evaluations also used to evaluate heat tolerance in the RIL population. Each RIL and panel line was planted in Uvalde, and College Station, Texas, and in Obregon, Mexico in an alpha lattice design. These locations consistently experiences high temperatures during mid-spring reproductive development. The study aimed to define the content and chemical composition in wax layers in glumes tissues specifically during reproductive stage heat stress. All obtained results were combined with our preexisting wheat breeding program to improve heat and drought tolerance. The following specific objectives were used to test the hypothesis:

Objective1. Define the correlation between high glume wax, cooler canopies, and increased yield and yield stability during reproductive stage heat stress.

Objective2. Identify the link between QTLs regulating the epicuticular wax in the spike glume during reproductive stage and yield stability under heat stress in wheat.

The QTLs were identified using 180 RIL's that were previously improved by AgriLife as a heat tolerant panel. Correlations were made between the QTLs for wax content and composition, heat stress tolerance, spectral reflectance, spike canopy temperature depression, and yield stability.

This project is novel because it focuses on the glume epicuticular wax as a new way in moderating heat stress in wheat. The *significance* of these results is in providing wheat breeders with reproducible high heat tolerant cultivars using manageable breeding techniques with maintained high yields and quality. The presence of high glumes wax content will keep the developing grains at desired temperatures under heat stress conditions which will improve heat and drought tolerance. The higher content of glume wax will also moderate the

dependency on evaporative cooling which reduces irrigation needs from underground and surface water resources. This is important because according to an estimate published by Richards et al (1986), high wax could save as much as 31,000 liters/acre (8,000 U.S. gal/acre) or 1/3 inch of rain/irrigation per day of water loss.

1.2. Literature review

1.2.1. Wheat

Wheat (Triticum spp.) is one of the predominant staple food crops grown worldwide. It covers more cultivated land in the world than any other crop. Average world production is nearly 600 million tons per annum. In terms of total production for use in cereal products, wheat ranks second in importance directly after maize while rice ranks third, according to the Food and Agriculture Organization of the United Nations (FAO 2006). Wheat is cultivated on more than 240 million hectares, larger than any other crop, and its global trade is greater than all other crops combined. Furthermore, wheat is the single most important source of plant protein in human diet due to its high protein content. This makes wheat an important cereal food and provides that provides more nourishment for humans than any other food source. Additionally, wheat is the source of almost 20% of total necessary calories for the world's population (Naseem et al. 2001). Wheat is the top source of carbohydrate in most countries; it provides energy and protein for more people than any other single food crop (Anon. 1985).

1.2.2. Wheat genetics

The wheat genome is a complicated field crop, since it possesses three ancestral genomes or six sets of seven related chromosomes. Hexaploid wheat was developed from domesticated

emmer or durum wheat hybridized with another wild diploid grass (*Aegilops tauschii*) (Hancock et al. 2004). Common bread wheat *Triticum aestivum* is a hexaploid, with three complete related genomes termed A, B and D each consisting of seven pairs of chromosomes (Chromosome number 2n =6x= 42 Genome: AABBDD) (Sears 1952). Wheat is a relatively recent product of hybridizations between three diploid ancestors which are the cultivated tetraploid wheat T. turgidum subsp. dicoccoides (AABB) and the wild diploid goat grass Aegilops tauschii (Lubbers et al. 1991, Dvorak et al. 1998). Each of these genomes size is estimated at 17, 44 Mb, more or less almost twice of the human genome and consists of around 5,500 million base pairs (Kaitao et al. 2012). Despite bread wheats hexaploid chromosome number, it is an amphidiploid behaves meiotically like a diploid with 21 bivalents, because of the presence of Ph1 gene which is a single dominant locus located on the long arm of chromosome 5B. For hexaploid wheat to be highly fertile, only true homologues may pair within each set of the seven related chromosomes during meiosis (Al-Kaff et al 2008). Ph1 is a major regulator of chromosome pairing and recombination during meiosis (Roberts et al, 1999).

1.2.3. High temperature stress

Abiotic stress is a common occurrence for wheat in many places worldwide. High temperature and drought are the most significant stresses that limit world crop production (Blum 1988). In addition, high temperature and drought stresses will increase as a result of global warming arising from elevated CO_2 concentration in the atmosphere, and could reduce its agricultural productivity and threatens its future (Iba 2002). High temperature and drought stress frequently occur simultaneously and result in reduced growth, productivity, and quality of crops; however severe stresses can lead to catastrophic losses. Dudal (1976)

estimated that 90% of world's arable lands may be categorized as a stress effected by abiotic stress including high temperature and drought stress. Wheat production is negatively affected by exposure to high temperature stress (Wardlaw and Wrigley 1994). Furthermore, around 7 million hectares of wheat are affected by persistent heat stress, whereas in 40% of temperate environments high temperature stress occurring waves affects over 36 million hectares. High temperature stress is currently recognized as a major limitation to wheat productivity in the drier and warmer climatic regions of the world (Fischer 1986). At the same time, there is a need to expand production in these hot climate regions (Mohammadi et al. 2008). Accordingly, the release of a new high temperature tolerance cultivars is a vital objectives in wheat breeding programs (Wardlaw et al. 2002). Many of the good agronomic HRWW varieties show susceptibility to high temperature stress in terms of their inability to maintain yield, primary components of grain yield and the duration of grain filling under heat stress (Hays et al. 2007b).

1.2.4. High temperature stress effect

High temperature stress has a large negative impact on wheat grain yield. Every degree rise in temperature above 15°C exhibits a 3% reduction in yield (Wardlaw et al. 1989). In addition, a persistent high temperature stress during the wheat life cycle leads to a failure in the cultivation of wheat in many countries. Consequently, many of these countries import more than 20 million tons of wheat per year (CIMMYT 1995). This impact becomes obvious through reduction in the potential number of grains, which is considered as a major abiotic stress factor that reduces wheat production mainly during grain filling (Fokar et al. 1998 b).

Wheat growth and development stages may be divided to three phases: vegetative, reproductive, and grain filling or ripening. The vegetative stage consists of tillering and stem

elongation/jointing. The reproductive stage is booting heading or flowering. High temperature stress affects plant growth at all developmental stages, however it is more problematic at the flowering and grain filling stages (Wahid et al 2007). Among the phases that are influenced by high temperature stress are the reproductive and grain filling stages. During the vegetative stage, high temperature stress has no major effect because of sowing wheat during the winter or spring months (Satorre and Slafer 1999). Wheat plants suffer serious injuries when high temperature stress occurs during reproductive stages. Abortion of floral buds, pollen and anther sterility, and restricted embryo development are examples of injuries caused by heat stress. These injuries lead to yield loss because of a reduction in grain number and yield. In wheat, both weight and grain number are sensitive to high temperature stress (Ferris et al. 1998). Even though high temperature stress accelerates growth (Fischer, 1980; Kase and Catsky 1984) temperature stress during reproductive stage can also cause pollen sterility, tissue dehydration, lower CO2 assimilation and increased photorespiration (Wardlaw and Wrigley 1994). The most favorable temperature for wheat flowers and grain filling ranges started from 12℃ to 22℃. If wheat plants are exposed to temperatures above this, it can significantly reduce grain yield (McDonald et al., 1983; Mullarkey and Jones, 2000; Tewolde et al., 2006). Also grain filling rate and duration are affected by high temperature stress. This stress can accelerate the rate of grain filling, shortening the grain filling duration, and resulting in decreased grain yield (Dias and Lidon, 2009). This reduction of the duration of grain fill by high temperature leads to shortening in the time to endosperm apoptosis and harvest maturity (Altenbach et al. 2003). For example, in wheat, the grain filling rate reduced by 12 days, temperatures increase 5℃ above 20℃ (Yin et al. 2009). High temperature stress also severely reduces grain yield throughout grains

per spike and individual grain weight. When high temperature stress remains for 10 days at 35℃ day and 20℃ night, the kernel weight is reduced by 29% and the kernel number by36% (Assad and Paulsen 2002). The degree of high temperature stress affects depend on the intensity and duration of stress. High temperature stress also induces an early abortion of tapetal cells, which causes the pollen mother cells to rapidly progress toward meiotic prophase and finally undergo programed cell death, a consequence leading to pollen sterility (Oshino et al. 2007).

1.2.5. Canopy Temperature Depression (CTD) and its role for diagnosing high temperature stress

Wheat has many adaptive mechanisms to overcome high temperature stress. One widely observed mechanism is leaf rolling. This enables wheat plant to decrease leaf area that is exposed to high light high temperature stress by rolling the leaves, thus decreasing transpiration. Also rolled leaves are cooler than un-rolled leaves. Therefore, plant genotypes with rolled leaves will be more tolerant to high temperature stress. For that reason, canopy temperature depression (CTD) can be very suitable tool to select between tolerant and susceptible genotypes for high temperature depression. Also, CTD acts as a useful tool for selecting high temperature stress tolerant genotypes, as it is considered a reliable and constant; this because when CTD is used at different stages, it shows a robust correlation to temperature tolerance (Reynolds *et. al.* 1997; Fischer et al. 1998).

Plant leaf temperature is accurate measurements of the plant response to its environment since it is influenced by radiational, convectional, and transpirational processes. Certainly, transpiration of water from leaves can reduce its temperature. Continual plant leaf transpiration reduces soil moisture, which will eventually reduces transpiration rate and

decrease degree of leaf temperature depression. Hence, canopy temperature depression should be proportional to the evapotranspiration rate (Reynolds et al 1997). Scientists have shown that there is a significant association between grain yield and CTD in hot environments (Reynolds et al. 1994; Fischer et al. 1998). Canopy temperature depression can be measured either with a hand-held infrared thermometer, or with remote sensing thermal camera. The readings are calculated by subtracting the temperature of the canopy from the surrounding ambient air temperature and can be used to evaluate hundreds of lines in a short period of time (Ayeneh et al. 2002; Balota et al. 2007; Bilge et al. 2008).

1.2.6. Epicuticular wax

Despite variability, all cuticles consist of the same two types of highly lipophilic materials. One of them, cutin, is a polymer consisting mainly of a very long -chain hydroxyl and epoxy fatty acids (Heredia *et al* 2003; Nawrath 2006). In contrast, the second component, cuticular wax, is monomeric and can be extracted by many organic solvents. Cuticular wax is a complex mixture of straight chain aliphatics and may include secondary metabolites such as triterpenoids, phenylpropanoids, and flavonoids (Jetter R et al. 2006). Jetter R et al. 2001 demonstrated that the intracuticular wax, interspersed within the cutin polymer, has a distinct chemical composition from the epicuticular wax lying on the outer surface of the cutin polymer. According to Stark RE *et al* 2006 the cutin is a fatty acid–based polyester that forms the structural skeleton of the cuticle, while wax is a mixture of highly lipophilic aliphatics surrounding and covering the cutin and sealing the plant surface. Ishag HM (2003) suggested that the leaf waxes may reduce high temperature stress input, thus lowering leaf temperatures.

Leaf waxyness has been associated with cooler canopies under both high temperature and drought conditions (Richards et al. 1986; Bennett et al. 2012); however, the effect of epicuticular wax in reducing canopy temperature under high temperature stress is still poorly studied. Jordan et al. (1984) reported that epicuticular wax plays significant role in reducing leaf transpiration and decreasing dehydration. Leaf epicuticular wax can lower evapotranspiration rate and reduce the risk of irreversible photo-inhibition through decreasing radiation load to leaf surface (Richards, 1996).

Periodic drying increased the total wax and light reflectance (genotype x environment) in tobacco (Kimberly et.al 2005) and in barley (Gonzalez & Ayerbe 2010). The elevated wax presence enhances heat stress avoidance by altering transpiration cooling needs. It acts as a reflective surface to excess photosynthetic and infrared energy, and thus, dissipates heat during high light, high temperatures, and drought stresses.

Epicuticular wax is an important adaptive trait that covers both sink and source organs such as leaves and glumes. This layer covers in a way that forms a protective barrier between the environment and the plant, offering protection against both abiotic, and biotic stress (Jenks et al. 1992; Kunst and Samuels, 2003; Shepherd and Griffiths 2006).

1.2.7. Quantitative trait loci (QTL) analyses of traits related to heat

One of the major challenges in wheat breeding is to find a linkage between genotype and phenotype in the context of the biotic or abiotic stresses. The polyploid nature of the wheat genome makes molecular analysis more difficult (Barnabas´ et al. 2008). The basic theories of identifying QTL were developed almost a century ago (Sax 1923). There are two methods for complex trait analysis. The first one is through using QTL analysis in biparental (RILs) mapping populations. The second one is a genome wide association study over a set of

unrelated individuals. Therefore, QTL studies are very important to recognize genomic regions of interest that can be used for marker assisted breeding (Cardon and Bell 2001). However, the majority of the traits related with yield, yield component, and high temperature stress tolerance are controlled by a number of genes each with a very small individual effects but with significant effect when acting simultaneously. In wheat, there are many factors that can negatively affect molecular analysis. The QTLs analysis has, partly, been hindered by the large genome size (Bennett et al. 1982). Generally, mapping studies of plant QTL is implemented using a population of RILs resulting from a biparental cross of two inbred lines that possess different traits (Jansen 2001). Many studies have shown that high temperature stress tolerance, physiological traits that responded to high temperature stress and yield components are inherited quantitatively (Maestri et al. 2002). This is consistent with what has been reported in previous studies that grain yield in wheat is controlled by multiple QTL and is highly affected by the environmental stresses, making it difficult to make satisfactory gains in yield improvement (Kato et al. 2000). Determining the physiological traits associated with high temperature stress tolerance and finding QTL associated with these traits might be a crucial result for high temperature tolerance in wheat breeding. Especially when we know that heat stress tolerance is a quantitatively inherited and environmentally influenced (Blum 1988; Yang et al. 2002). Because of repetitive DNA sequences, natural genetic variation may be used in the course of direct selection under high temperature stress through the reproductive phase or throughout QTL mapping. Molecular analysis for QTL mapping can be a useful tool to provide a realistic estimation of numbers, locations, scale of phenotypic effects, and models of gene action (Vinh and Paterson 2005). For instance, there is a 17% variation under high temperature stress for yield QTL's and canopy temperature

QTL's at the same location (Pinto et al. 2010). A number of publications recommend the use of main spike for the identification of QTLs genomic regions associated with high temperature tolerance (Mason et al. 2010).

Recently, a number of QTLs have been identified in wheat for high temperature stress tolerance during the reproductive stage. Such QTL were used by Ottaviano et al. (1991) to understand and delineate heat stress tolerance in cereals. Also QTL on chromosome 4A for canopy temperature under heat has been identified (Pinto et al., 2010). Yield stability heat tolerance QTL's were found to overlap with QTL for epicuticular wax (Suchismita et al. 2011). Detection of two major grain yield QTL's were identified in bread wheat under heat, drought and high yield potential environments (Bennett et al. 2012). Detection of QTL on bread wheat chromosomes 1B, 3D and 5A associated with constitutive production of leaf cuticular wax may contribute to lower leaf temperatures under heat stress (Suchismita et al. 2015).

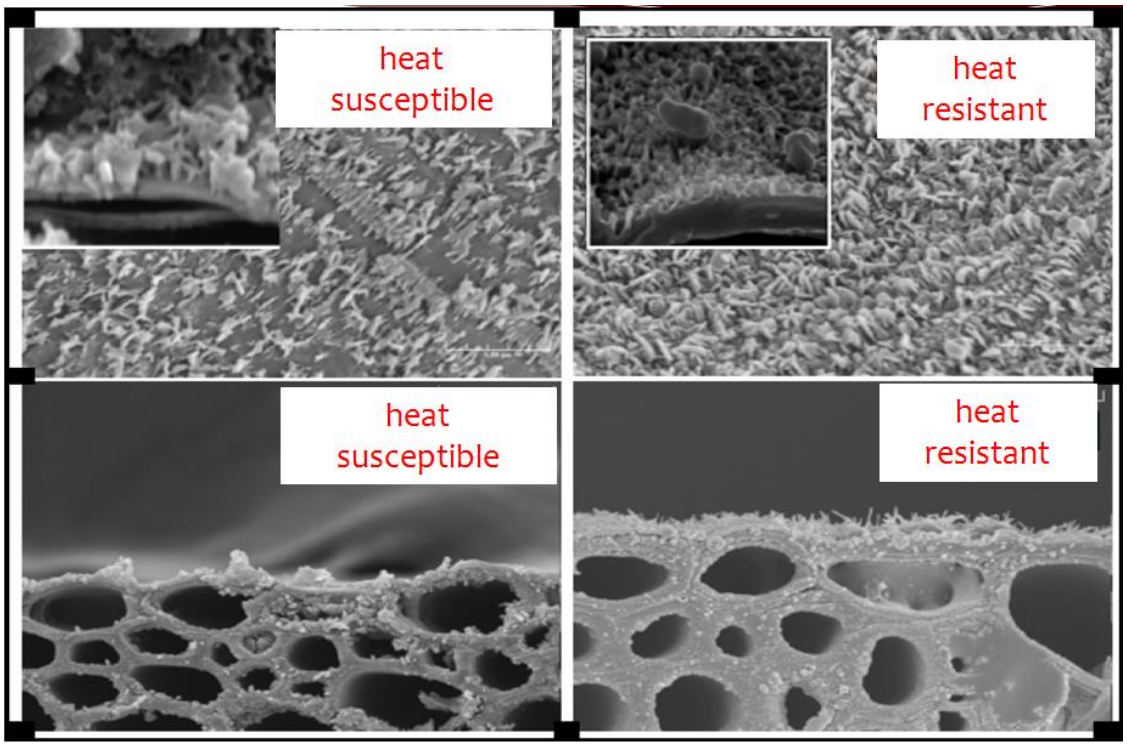


Figure 1.1. Scanning electron microscopy (SEM) imaging of epicuticular wax of heat tolerance and heat susceptible wheat varieties (S. Mondal- Thesis 2011).

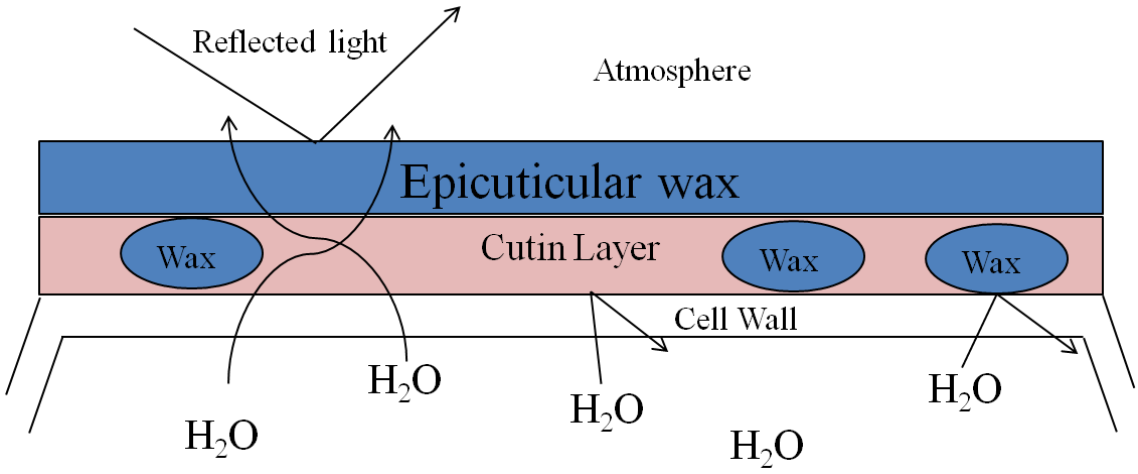


Figure 1.2. Epicuticular wax on leaf (Koch et al. 2006)

CHAPTER II

SPECTRAL REFLECTANCE INDEXES ANALYSIS ON GLUME AND LEAF EPICUTICULAR WAX AND THEIR RELATION TO HEAT TOLERANCE UNDER HIGH TEMPERATURE STRESS IN WHEAT

2.1. Introduction

High temperature and drought are major factors limiting wheat yields in many countries worldwide. Boyer 1982 reported that heat and drought stresses are primary abiotic factors in limiting plant growth and crop productivity worldwide. Moreover, they are complex morphological and physiological phenomena in plants. At the plant cell level, water shortage results in osmotic stress. A flux of water from the cells results from an alteration in extracellular solute concentrations. The loss of water causes a decrease in turgor and increase in concentrations of intracellular solutes, putting a stress on membranes and macromolecules. If chloroplasts are exposed to excessive excitation energy at the same time, water deficiency leads to the production of cell-toxic substances such as superoxide and peroxides that damage cell membranes (Holmberg and Bülow 1998). Consequently, high temperature and drought tolerant wheat varieties a valuable resource, especially when they are farmer friendly and based on seed technology that is easy to disseminate. In harsh climatic zones where plants are exposed to heat and drought stress adjusting stress adaptive strategies should include traits that reduce radiation load such as wax, pigments composition, leaf angle and rolling to increased transpiration efficiency (Richards 2006).

Epicuticular wax covers plant aerial organs to protect them from various biotic and abiotic stresses. EW is a very thin film upon the cutin matrix. This thin layer appears as microscopic mixtures called EW crystals (Barthlott et al. 1998; Jetter & Schäffer 2001). The

structure of epicuticular waxes is determined by the shape and density of single wax crystals. However, the morphology of such crystals depends on the plant's species, specific chemistry (Baker, 1982), and predominant wax compounds (Jetter & Riederer 1994; 1995). However, many factors can affect the amount and chemical composition of the EW surface. EW is not only species and organ specific, but it also varies due to the plant growth environment and development stage. The amount and orientation of the leaf wax crystals changes between leaf regions of variable age (Rhee et al. 1998).

Temperate cereals like wheat are relatively well adapted to high temperature and dry environments, being grown widely throughout the world. A high temperature and drought resistant genotype yield significantly higher than average under conditions where crop water availability is limited. While current breeding work has made steady progress, performance of cereals still show substantial loss to high temperatures (Reynolds et al. 1994). Moreover, significant breeding effort will be required to maintain their productivity under warmer conditions. Although breeders regard improved high temperature and drought resistance as specific target in their breeding programs, progress towards this objective is often hard to improve and achieve.

Plant phenotyping is a useful approach that can be applied to physiological breeding for the improvement of yield gains and traits that are related to the adaptation of different environments, such high temperature and dry environments. Plus it is a non-destructive, fast and often easy to implement (Reynolds et al. 1998).

Applying several physiological traits such as measuring plant water status is a vital evaluation for monitoring the physiological status of plants, and assisting wheat breeder in

selecting for high temperature and drought wheat species, as verities (Peñuelas et al. 1993, 1996).

Understanding water relations traits has been used to identify complementary parents in breeding for improved adaptation of wheat to water limited environments. Optimization of phenotyping methodologies has offered easy and rapid screens that permit precision phenotyping of large numbers of genotypes within a time frame that does not confuse measurement that are environmentally unstable (Pinto et al. 2010). Relative water content (RWC) for wheat plants after a period of drought-stress represents the ability of a genotype to retain water in their tissues. It appears to be a better indicator of turgor pressure and consequently cell volume as a result of drought-stress. Nevertheless, this trait and most of water relations traits such as leaf water potential, leaf relative water content (RWC), root characteristics, and osmotic adjustment generally require destructive preparation of sampling, are labor-intensive, and time consuming for an applied routine in breeding programs. However, these physiological traits are very useful tools and accurate indicators of stress levels in field trials (Peñuelas et al. 1993; Reynolds et al. 1994). By applying high-throughput phenotyping tools for measuring such physiological traits e.g. spectral reflectance indices (SRI), and canopy temperature depression CTD could rapidly overcome these drawbacks. Understanding these relationships has permitted the identification of efficient tools that are used in plant selection for adaptation to high temperature and drought.

Blum et al (1982) reported that using of infrared imaging to quantify differences in the CTD of wheat genotypes under drought is a useful tool for identifying cultivars. Canopy temperature depression can be measured either with a hand-held infrared thermometer, or with a remote sensing thermal camera. The readings are calculated by subtracting the

temperature of the canopy from the surrounding ambient air temperature and can be used to evaluate hundreds of lines in a short period of time (Ayeneh et al. 2002; Balota et al. 2007; Bilge et al. 2008).

It has been found that phenotypic correlations of CTD with grain yield were occasionally positive (Reynolds 1997). The suitability of CTD as an indicator of yield and stress tolerance, however, must be determined for individual environments. For example, it can be a poor indicator where yield is highly dependent on hygroscopic water vapor pressure deficit CTD, while net radiation, air temperature, and wind speed have slight effects (Smith et al., 1986). CTD is effected by biological and environmental factors such as soil water status, wind, evapotranspiration, cloudiness, conduction systems, plant metabolism, air temperature, relative humidity, and continuous radiation (Reynolds et al. 2001). CTD measurements are actual integrative and scoring many leaves at once, thus reducing error associated with leaf to leaf variation. The main disadvantage is CTD readings are somewhat sensitive to the environment, needing relatively stable weather to obtain reliable data (Reynolds 2012).

However, spectral radiometry can detect with high resolution an even greater range of light reflected from the canopy in the range of 350–2500 nm. Many indices have been calculated using different wavelengths that relate to different traits including photochemical reflective index (PRI) (Penuelas et al. 1995), normalized difference vegetation index (NDVI) (Gao, B.C. 1995), and the water index (Peñuelas et al. 1993). Accordingly spectral reflectance techniques can be easy to apply in the field and provides several rapid, reliable, and non-intrusive measurements by quantifying the patterns in both the visible (400-750 nm) and the near-infrared (750-1100 nm) wavelengths. Plots can be smaller, and measurements

can be repeated many times (Mullan et al 2010). Previous study have already shown effects of nutrient and water deficiencies on the spectral reflectance and transmittance of single leaves (Peñuelas 1994). High-resolution reflectance between 750-1100 nm has already been proven to be a reliable method for estimating plant water concentration (Carter 1991; Danson et al. 1992). Water absorption bands throughout the mid-infrared region 1300-2500 nm showed the highest sensitivity to leaf water concentration (Carter 1991).

While field sampling of single leaves provides the most accurate assessment of plant water status, it is time consuming especially for large areas. Whereas the spectral radiometry techniques offer alternative of a non-destructive and instantaneous method to assess water status of vegetation over large spatial scales (Gao 1995).

2.2. Material and methods

2.2.1. Plant material

In this study a set of 180 recombined Inbred lines (RILs) of wheat were used. These RILs were derived from crossing of two spring wheat lines, Halberd and Len. Halberd is an Australian spring wheat (Triticum aestivum L) as a donor cultivar with the pedigree Scimitar/Kenya/C6042/ Bobin/2/Insignia49 (Paull et al. 1998). Halberd is a heat and drought tolerant cultivar, and maintains carbohydrate accumulation during moisture stress (Ji et al. 2010). Len is hard red spring wheat as a recurrent cultivar developed in North Dakota with the pedigree ND499/3/Justin/RL4205/W1261. Len is a semi-dwarf that is a drought and heat susceptible; however, it is known for its good agronomic characteristics (Hossain et al. 2012). The two parents were chosen due to similarities in flowering period and maturity. The 180 RILs were developed by preceding the F1 progeny through single seed descent in

head rows to the F5 generation. Seeds from the F5 generation were bulked to develop 180 F5:6 RILs. The F6 lines were advanced in the field and were evaluated during 2010 as an F5:7 generation. During 2011 and 2012, F8 and F9 generations were used respectively, to conduct experiments (S. Mohammed *et al* 2014).

2.2.2. Growing environment

The RILs and the two parents were grown in the field during the winter season. The crop growing seasons for all experiments are referred to as year 2014 seasons at CIMMYT's experimental station Ciudad Obregon, Northwest Mexico (27.3° N, 109.9o W, 38 m above sea level). Weather conditions were mostly sunny and dry during the winter cropping cycle. The soil type is coarse sandy clay, low in organic matter, and slightly alkaline (pH 7.7) in nature (Sayre et al. 1997). Nitrogen and phosphorus were applied to the plots at a rate of 150 kg ha−1 and 22 kg ha−1, respectively. Field plots consisted of two raised beds (28 cm apart) each 5 m long and 80 cm wide. An alpha lattice design with two repetitions was used for experiment. The planting dates were in February and plants reached booting and heading during April–May and were harvested in May.

2.2.3. Agronomic and physiological measurements

Physiological traits measured at 10 DAP are CT, SRI measurements, glume EW and leaf EW. Measurements were always taken at a specific time between 1 pm and 3 pm.

2.2.3.1. Airborne data collection thermal infrared imagery (thermal index)

Aerial imagery was collected via the AscTec Falcon 8 Unmanned Aerial Vehicle (UAV) Ascending Technologies GmbH., Krailling, Germany). Thermal camera measures the emitted thermal radiation. The thermal index (TI) was calculated using the sum of the green

and blue bands of the plot averaged values of the processed images acquired from the recorded video.

$$\boldsymbol{TI} = \boldsymbol{TG} + \boldsymbol{TB}, 2$$

Where $\boldsymbol{TG}$ *and* $\boldsymbol{TB}$ are the averaged 'plot' values at the green and blue bands respectively. The thermal index is positively related to temperature, so the higher the temperature, the higher the thermal index.

2.2.3.2. The ground based measurements of canopy temperature

CT were recorded at 10 DAP. Measurements were taken using a portable infrared thermometer a hand-held infrared thermometer (Fluke 561 HVACPro Combination IR Non-Contact and K -type thermocouple thermometer, Fluke Infrared Instrument Co. Inc., Everett, WA). The measurements were obtained from the same side of each plot at an angle of approximately 45° for 30 s. With respect to the horizontal angle to integrate as many spikes as possible without capturing the soil in the measurement. The measurements were taken in the afternoon at a specific time between (13.00–14.00 h) when the crop experienced maximum transpiration rates. Hot weather, sunny, non-cloudy, and low wind conditions were taken into consideration during CT measurements.

2.2.3.3. Spectral reflectance measurements

Spectral of canopy reflectance was measured in the 350–1100 nm range and collected at 1.5 nm intervals using a high-resolution spectro-radiometer ASD handheld 2 Field Spec spectroradiometer (Analytical Spectral Devices, Boulder, CO). Data was calculated from four readings per plot at10 days after pollination (10DAP) at midday (between 10.30 h and 14.00 h) to avoid differences due to the solar inclination. After the machine was calibrated using a white plate of barium sulphate (BaSO4) which provides maximum reflectance

(Labsphere Inc., North Sutton, USA). Measurements were taken at a height of 0.5 m above the canopy and with a field of view of 25°. The water index proposed by Peñuelas et al. (1993) was calculated (WI=R970/R900) and four normalized water indices (NWIs) were also calculated according to Babar et al. (2006) and Prasad et al. (2007) (NWI-1=[R970–R900]/[R970+*R*900], NWI-2=[*R*970–*R*850]/[*R*970+*R*850], NWI–3=[*R*970–*R*880]/[*R*970+*R*880], and NWI-4=[*R*970–*R*920]/[*R*970+*R*920]). Simple ratio (SRI) calculated according to Peñuelas et al. (1998) was calculated R980 / R680 for estimation of canopy photosynthetic area. Photochemical reflective index (PRI) was calculated according to Peñuelas et al. (1995) was calculated by (R531 – R570/ (R531+R570) for estimation of radiation-use efficiency. Normalized difference vegetation index (NDVI) was calculated (R780 – R670)/ (R780+ R670) for estimation of canopy photosynthetic area Peñuelas et al. (1993).

2.2.4. Wax sample collection

Samples were collected at 10 DAP. Four glumes were collected for wax analysis using tweezers to carefully remove glume without touching the glume surface avoiding any slightest scratches on the EW layer. Leaf discs for wax analysis were collected from flag leaves using a disc punch with a 1 cm diameter drum (Rabbit Tool USA, Rock Island IL USA). Four 1cm (diameter) leaf punches was collected from the primary inflorescence, and placed in Borosilicate Glass Vails with Screw Caps. The sample vials were placed in the laminar flow to air dry and stored at normal room temperature.

2.2.5. Wax extraction

EW concentrations were determined using the colorimetric method (Ebercon et al., 1977). Glume and leaf EW was extracted by submerging glumes and leaf discs in 1 ml HPLC grade

chloroform for 30 s, the submersion time previously determined to completely remove the epicuticular wax from the leaf (Mayeux and Jordan, 1984). The resulting mixture was transferred to a clean 1.8 ml glass GC vial (VWR Auto sampler Vial, Radnor, PA). The chloroform solvent was removed under a continuous flow of nitrogen gas by leaving the vial without cap overnight in the laminar flow hood.

2.2.6. Wax quantification

The resulting extract was oxidized by adding 300µl acidified potassium dichromate and heated for 30 min water bath at 100 ^{0}C. After boiling, vials were allowed to cool for a 1hr period and 700µl of deionized water was added to each vial, allowing color development for another hour. A spectrophotometer (PHERAstar plus, BMG LABTECH, Offenburg, Germany) was used to determine the optical density for each sample at 590 nm. Samples were loaded in 96 well, sanitized, clear flat bottom microplates (Greiner Bio-One, Monroe, NC, USA). A standard curve was developed from randomly selected wheat flag leaves from Halberd. Samples were placed in large glass vials and 20 ml HPLC grade chloroform was added to remove EW. The resulting chloroform-wax solution was proportioned based on the serial dilution technique. The standard curve was used to calculate wax levels based on leaf area (Mondal et al. 2014).

2.2.7. Statistical analyses

Statistical analyses of all phenotypic traits were performed using JMP Pro 11.2.1 (JMP Version 11, SAS Institute Inc., Cary, NC). All phenotypic traits were tested for normality. The generalized linear model (GLM) was used for analysis of variance and the means were compared using Fischer's least significant difference (LSD). A combined analysis of variance was also done using GLM procedure considering genotype and year as fixed effects.

Pearson's correlations were estimated for determining the association between leaf and glume epicuticular waxes and the physiological and phenological responses under high temperature stress.

2.3. Results

2.3.1. Leaf and glume epicuticular wax load

Analysis of wax load in both leaf and glume showed normally distributed data with significant differences observed between lines (Fig 2.1.). Contrary to what is expected, comparison between wax content of glume and that of the leaf showed no significant variation between the two data sets. ANOVA test put the means of both set at F correlation value of 0.7126 with glume wax mean of 5.37 mg/dm^2 and leaf wax mean of 5.97 mg/dm^2 (Fig 2.2.). Glume and leaf wax had minimums of 1.04 mg/dm^2 and 1.56 mg/dm^2, and maximums of 12.92 mg/dm^2 and 11.00 mg/dm^2 respectively. A significant but weak correlation exists between glume wax and leaf wax with r of 0.0304 at $p \leq 0.05$ significance level (Fig 2.3.). However, the same sample did not produce matching leaf and glume wax content throughout the lines (Fig2.4).

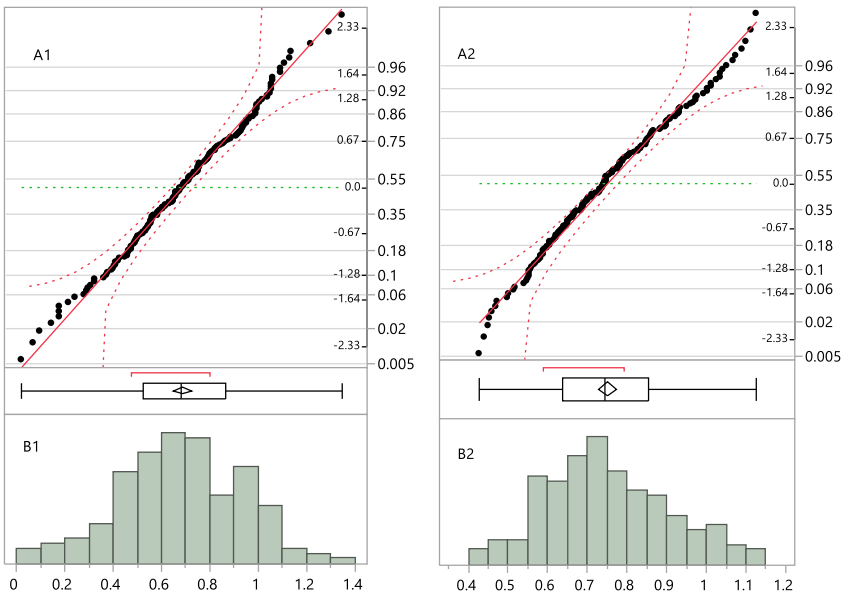


Figure 2.1. Quantile plot of log 10 transformation of glume and leaf wax load mg/dm2 (A1, A2); Normal distribution of glume and leaf wax load mg/dm2 (B1, B2).

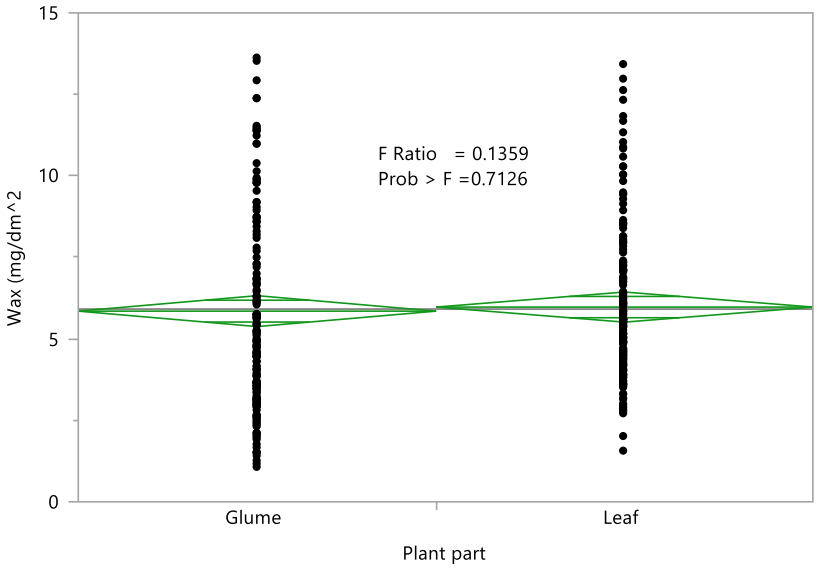


Figure 2.2. One-way analysis of wax (mg/dm2) by plant part.

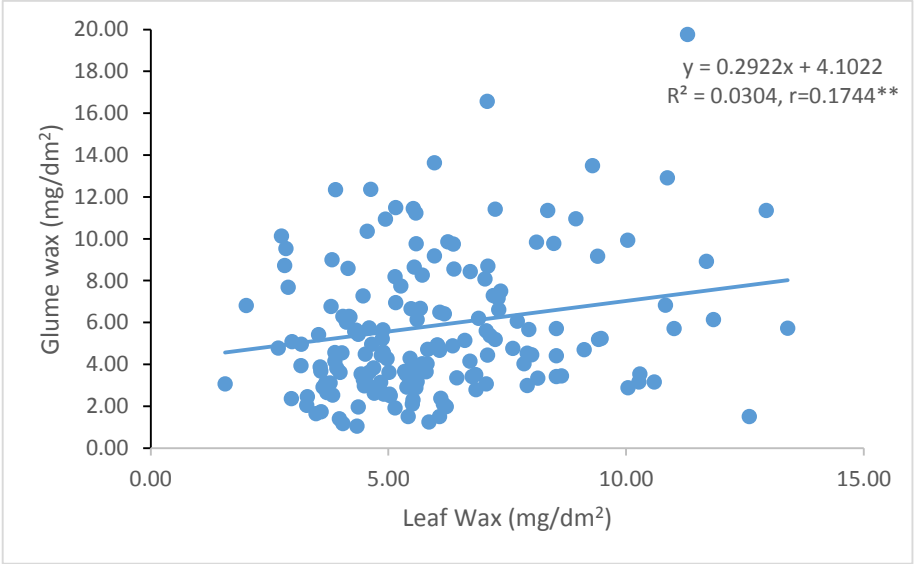


Figure 2.3. Linear correlation between glume wax and leaf wax, **significant at p≤ 0.05 with Pearson's correlation.

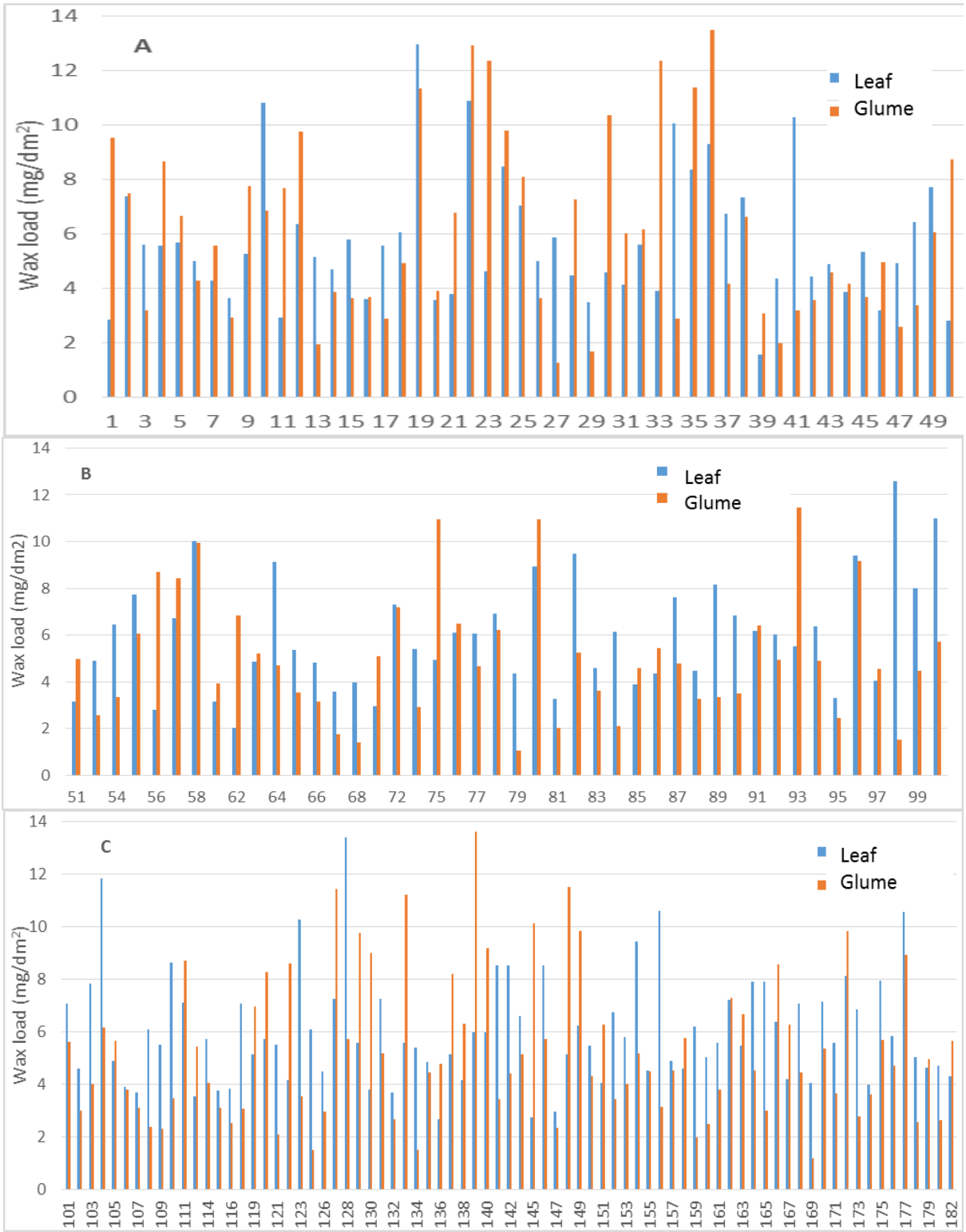


Figure 2.4. Leaf and glume wax load for wheat lines 1-50 (A), 51- 100 (B), and 101 -182 (C).

2.3.2. Canopy spectral reflectance and wax correlation

Differences were observed in reflectance between the lines across the photosynthetic active region and the near infrared region NIR with reflectance beginning at 2% and reaching 35% (Fig 2.5). Reflectance bands in the NIR overlapped for some lines but completely varied between the lowest readings and the highest readings. The percent reflectance in the NIR ranged from a minimum of 25% and a maximum of 35% for lines 122 and 168 respectively with average percent change of 33% (Fig 2.6.). Parent line (182) Hallberd achieved reflectance at 30% in the NIR which is approximately midway between the lowest observed reading and highest observed reading. On the other hand, parent line (181) Len reflectance was in the lower at 27%.

All spectral indices data showed variation between the lines to a certain extent (Fig 2.8.). WI showed the largest range among the water status indices of 0.63 as compared to the smallest range by NWI-4 with of 0.035 (Table 2.1.). Both NWI-2 and NWI-3 did not vary significantly for across for the different line with similar ranges of 0.0462 and 0.0432 respectively. (Figure 2.8.) shows that other spectral indices NDVI, PRI, and SRI also varied across the lines yet only SRI had significant variation and a larger range of 1.58 (Table 2.1.).

Water status indices related negatively with the wax load of both the leaf and the glume (Fig 2.10., 2.11.). Moreover, the correlation was significant at $p \leq 0.05$ for all water status indices associated with glume wax except NWI-2 which was not significant. R^2 values for the correlation ranged from 0.274 to 0.2198 for WI and NWI-1 respectively (Table 2.2.). Whereas all the correlation of the water status indices with the leaf wax were not significant with R^2 values ranging from 0.0076 to 0.0708 for NWI-3 and WI respectively (Table 2.3.). The normalized difference vegetation index followed the same pattern as the

water status indices by being positively correlated to glume wax and negatively correlated to leaf wax (Fig 2.12.). Again, the correlation was significant at $p \leq 0.05$ with Pearson's correlation value of 0.5304 for NDVI with glume wax as compared to none significant Pearson's correlation value of -0.0750 for NDVI with leaf wax (Table 2.3.). While the correlation between simple ratio index and glume wax was also positive, it was not significant like the others, neither was that of the leaf wax with $r = 0.2664$ and $r = -0.1066$ respectively (Fig 2.13.). Photo reflective index repeated that pattern of having a positive significant correlation with glume wax with ($r = 0.5334$) and negative none significant correlation with leaf wax (Table 2.3. and Fig 2.14.).

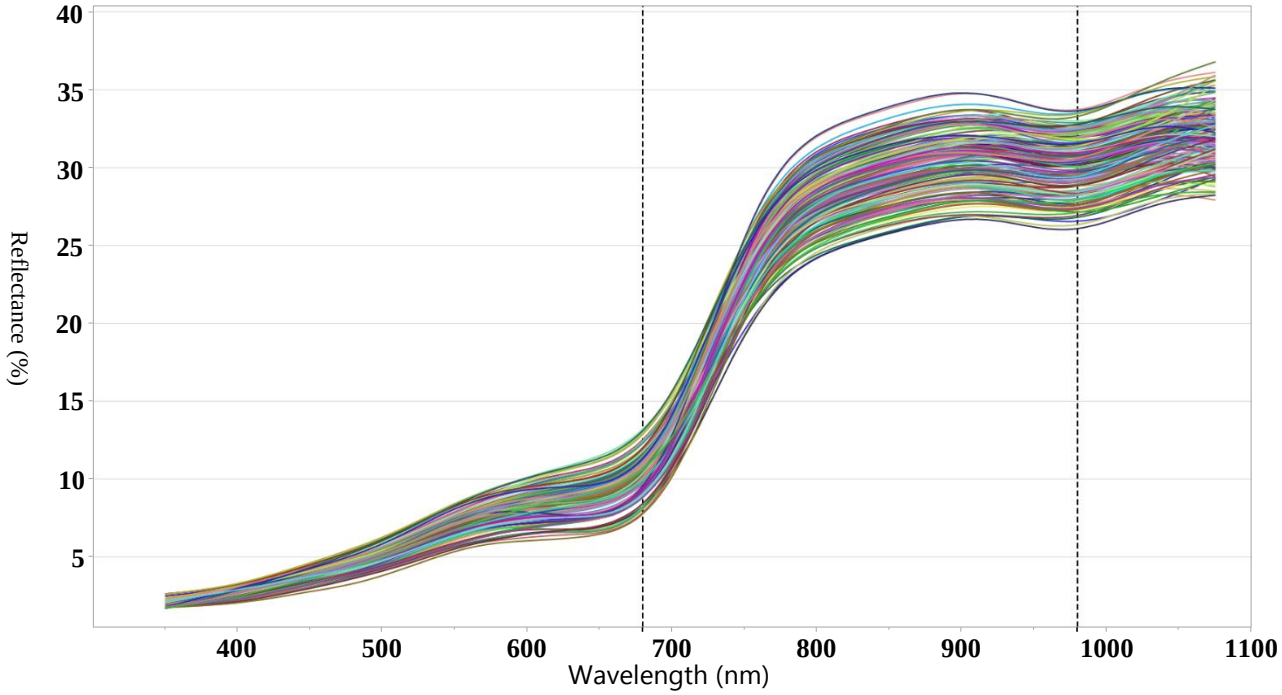


Figure 2.5. Percent reflectance of all lines and two parent lines in photosynthetic active region and near infrared region.

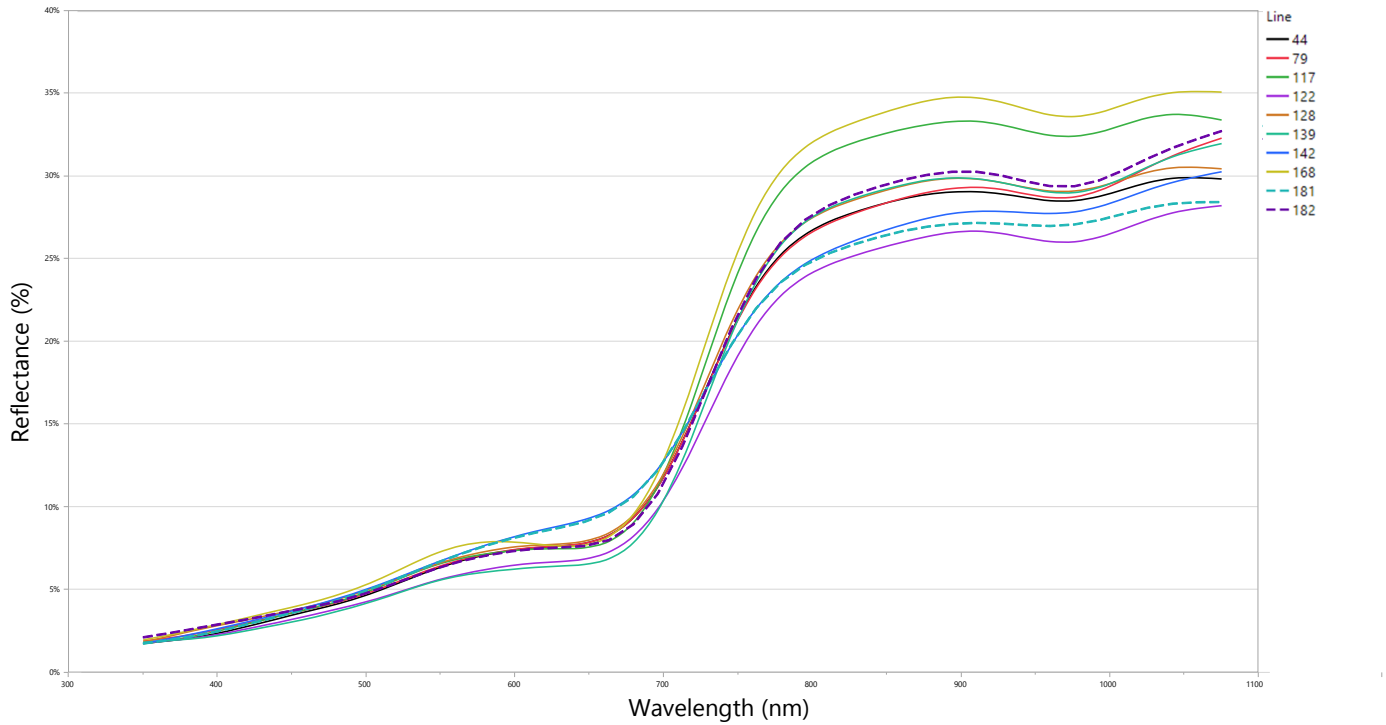


Figure 2.6. Percent reflectance of selected lines of high leaf wax load (128), low leaf wax load (44), high glume wax load (139), low glume wax load (79), two parent lines (181and 182), higher reflectance (168 and 117), lower reflectance (122 and 142).

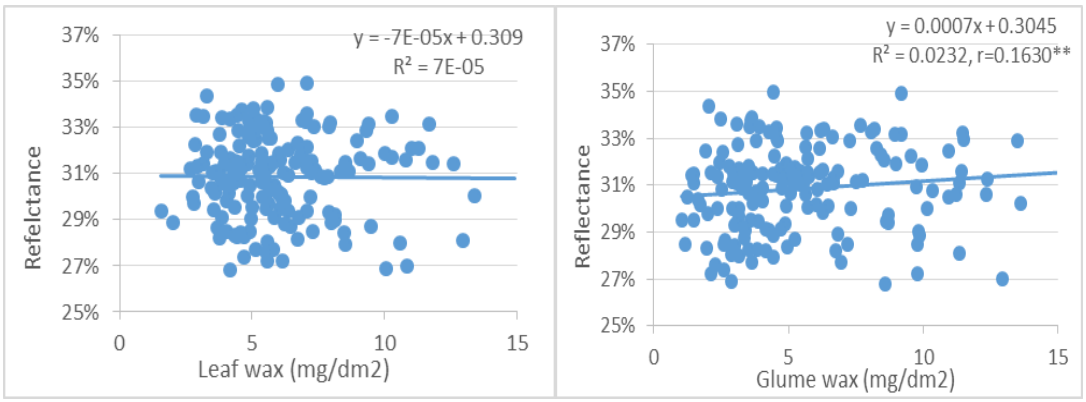


Figure 2.7. Linear relationship between reflectance at 900 nm and leaf wax (left), reflectance at 900 nm and glume wax (right), ** significant at p≤ 0.05.

Table 2.1. Spectral reflectance indices quantiles and summery of statistics

Index	Mean	STD	Maximum	Minimum	Range
WI	0.963	0.0153	0.998	0.925	0.063
NWI-1	-0.0188	0.00794	-0.001	-0.0388	0.0378
NWI-2	0.00248	0.00919	0.0248	-0.0214	0.0462
NWI-3	-0.0118	0.00937	0.00921	-0.034	0.0432
NWI-4	-0.0193	0.00742	-0.0028	-0.0378	0.035
NDVI	0.527	0.0421	0.621	0.415	0.206
PRI	-0.0849	0.0066	-0.07	-0.100	0.03
SRI	3.15	0.338	4.05	2.47	1.58

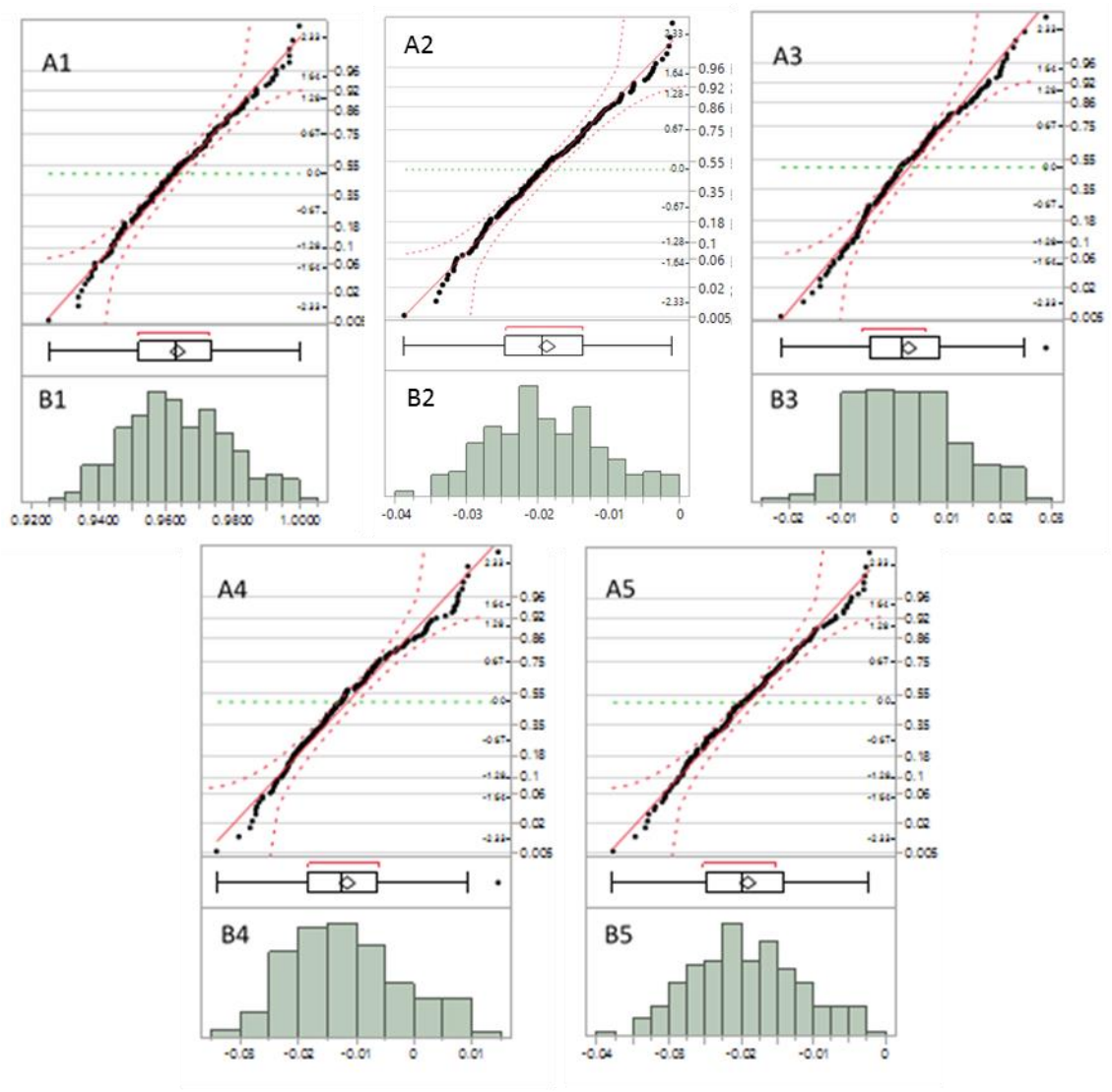


Figure 2.8. Distribution and quantile plots of spectral reflectance plant water status data. Normal distribution and quantile plot of WI (A1, B1), Normal distribution and quantile plot of NWI-1 (A2, B2) ; Normal distribution and quantile plot of NWI-2 (A3,B3), Normal distribution and quantile plot of NWI-3 (A4,B4), Normal distribution and quantile plot of NWI-4 (A5,B5),

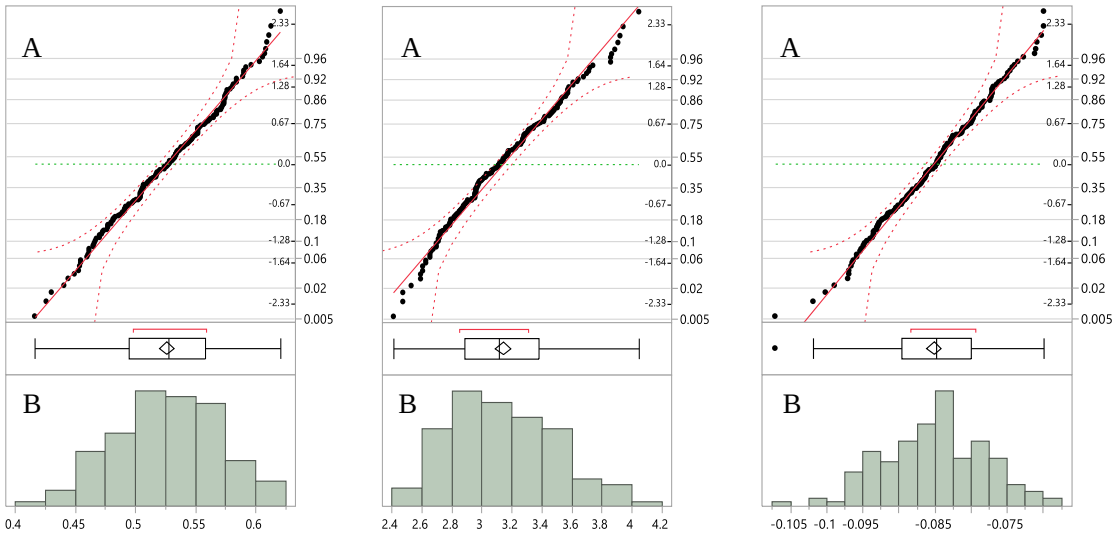


Figure 2.9. Spectral reflectance distribution and quantile plots of NDVI (A1, B1), SR (A2, B2), PRI (A3, B3).

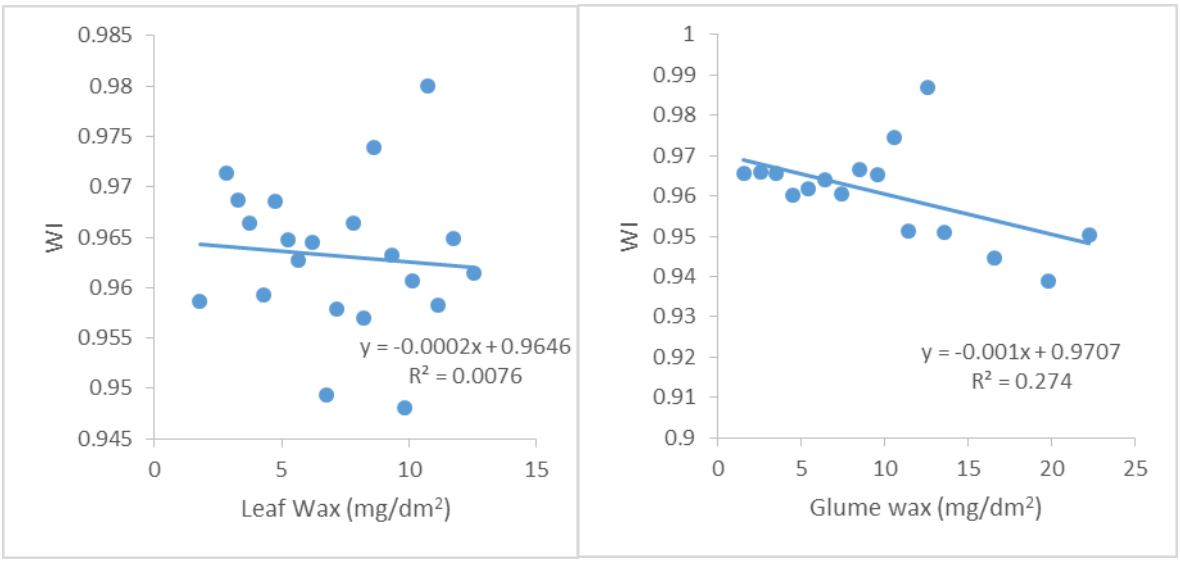


Figure 2.10. Linear relationship between water index and leaf wax (left) and glume wax load (right).

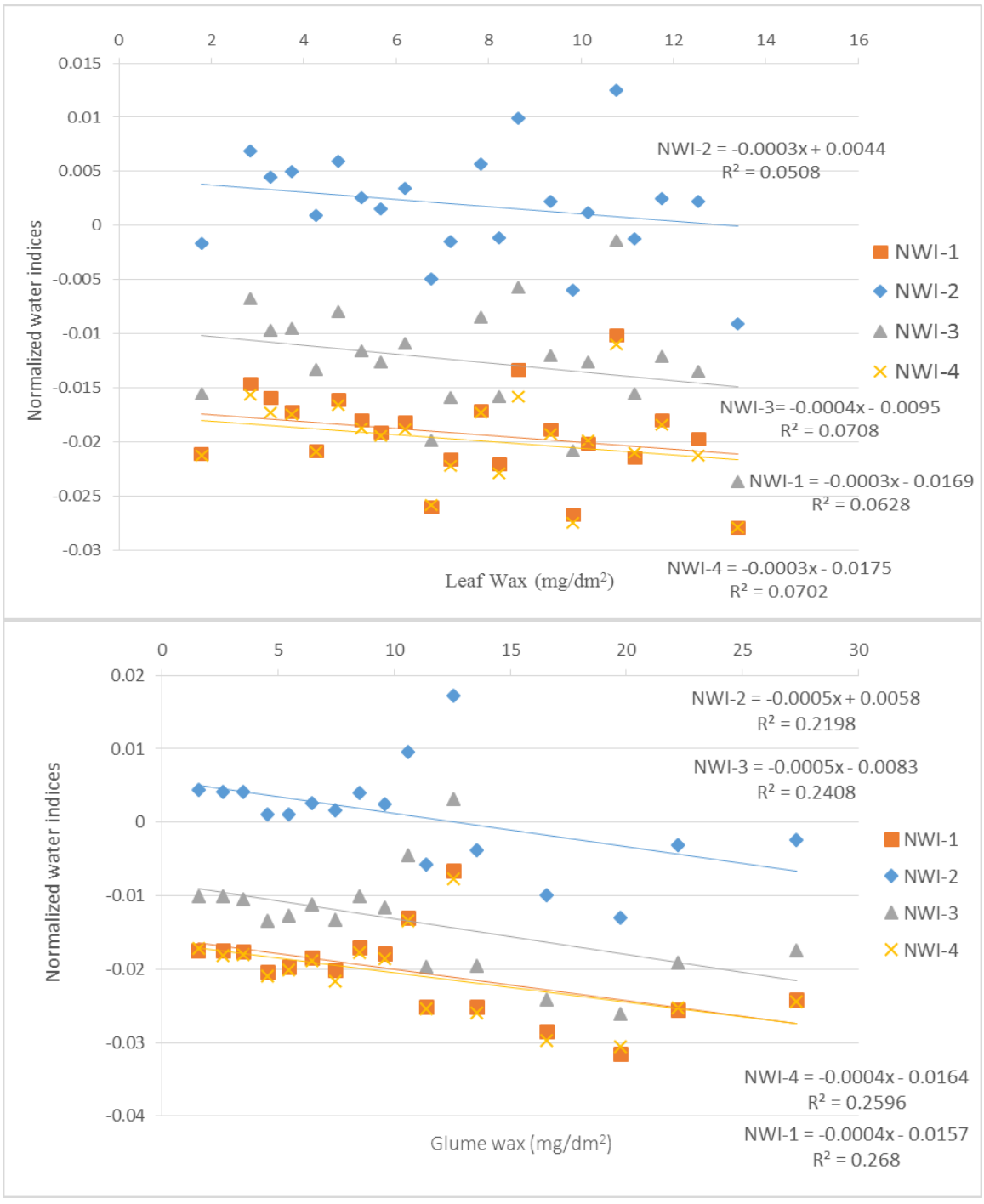


Figure 2.11. Linear relationship between normalized water indices and leaf wax (top) and glume wax load (bottom).

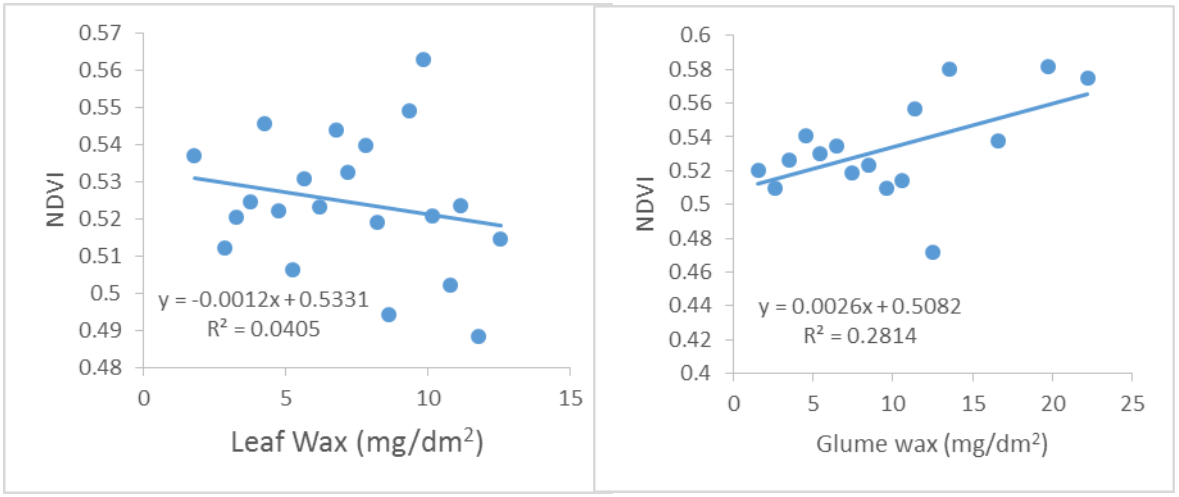


Figure 2.12. Linear relationship between normalized difference vegetative index (NDVI) vs. leaf wax load (left) and glume wax load (right).

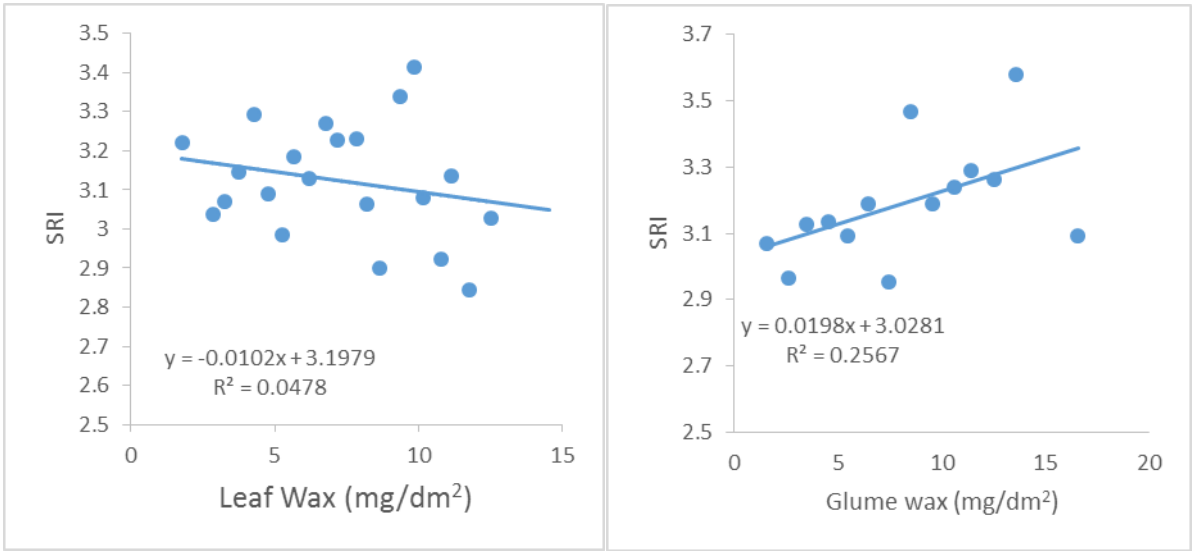


Figure 2.13. Linear relationship between simple ratio index (SR) vs. leaf wax load (left) and glume wax load (right).

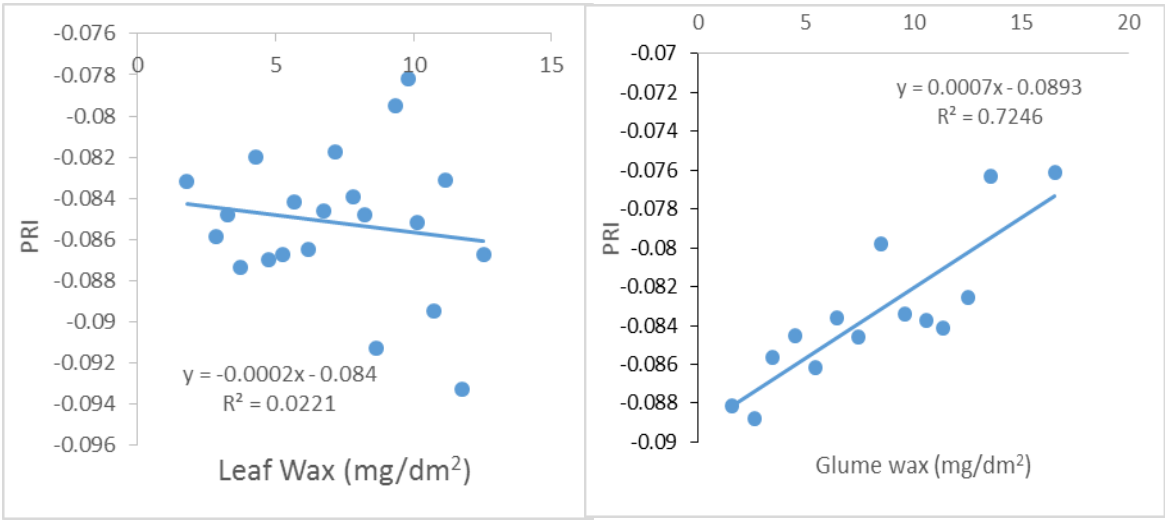


Figure 2.14. Linear relationship between photochemical reflection index (PRI) vs. leaf wax load (left) and glume wax load (right).

Table 2.2. Pearson's correlation coefficients of glume epicuticular wax load, water status indices (WI, NWI-1, NWI-2, NWI-3, and NWI-4), NDVI, SRI, and PRI. *, **, *** significant at p≤ 0.01, 0.05 and 0.001 respectively. NS not significant.

Indices	WI	NDVI	NWI-1	NWI-2	NWI-3	NWI-4	Glume wax	SRI
NDVI	-0.8826***							
NWI-1	1.0000***							
NWI-2	0.9924***	-0.8409***	0.9926***					
NWI-3	0.9977***	-0.8665***	0.9976***	0.9953***				
NWI-4	0.9950***	-0.8574***	0.9948***	0.9898***	0.9968***			
Glume wax	-0.5235**	0.5304**	-0.5264**	-0.4959NS	-0.5098**	-0.5186**		
SRI	-0.0926 NS	0.2891 NS	-0.0964 NS	-0.1171 NS	-0.1043 NS	-0.0876 NS	0.2664	
PRI	-0.3260 NS	0.2903 NS	-0.3287 NS	-0.3447	-0.3540NS	-0.3763 NS	0.5334**	0.6422***

Table 2.3. Pearson's correlation coefficients of leaf epicuticular wax load, water status indices (WI, NWI-1, NWI-2, NWI-3, and NWI-4), NDVI, SRI, and PRI. *, **, *** significant at p≤ 0.05, 0.01 and 0.001 respectively. NS not significant.

Indices	WI	NDVI	NWI1	NWI2	NWI3	NWI4	Leaf wax	SRI
NDVI	-0.6975***							
NWI-1	1.0000***	-0.6983***						
NWI-2	0.9870***	-0.6599***	0.9867***					
NWI-3	0.9899***	-0.6365***	0.9896***	0.9950***				
NWI-4	0.9893***	-0.6665***	0.9893***	0.9725***	0.9863***			
Leaf wax	-0.2502^{NS}	-0.0750^{NS}	-0.2507^{NS}	-0.2254^{NS}	-0.2660^{NS}	-0.2650^{NS}		
SRI	-0.6860***	0.9926***	-0.6870***	-0.6514***	-0.6279***	-0.6538***	-0.1066^{NS}	
PRI	-0.6619***	0.9191***	-0.6625***	-0.6587***	-0.6272***	-0.6403***	-0.0350^{NS}	0.921***

2.3.3. Canopy temperature and wax correlation

Both canopy thermal index (TI) and spike temperature (CT) had a normally distributed data with significant variation across the lines (Fig 2.15.). TI ranged from a minimum of 92.55 to a maximum of 128.0 while CT ranged from a minimum of 30.25 to a maximum of 36.2℃ including the ambient air temperature (Table 2.4.). The variation of CT appeared mostly at a 0.5℃ level of measurement.

Thermal index had negative correlation with both leaf and glume wax content with an R^2 values of 0.0185and 0.3532 respectively. The correlation was only significant with glume wax content with Pearson's coefficient r = -0.5943 and significance level of P≤0.001 (Fig2.16, A). On the other hand, spike temperature had a positive correlation with both leaf and glume wax content with an R^2 values of 0.078 and 0.1952 respectively. The correlation

was not significant for both leaf and glume wax content at any significance level (Fig 2.16, B).

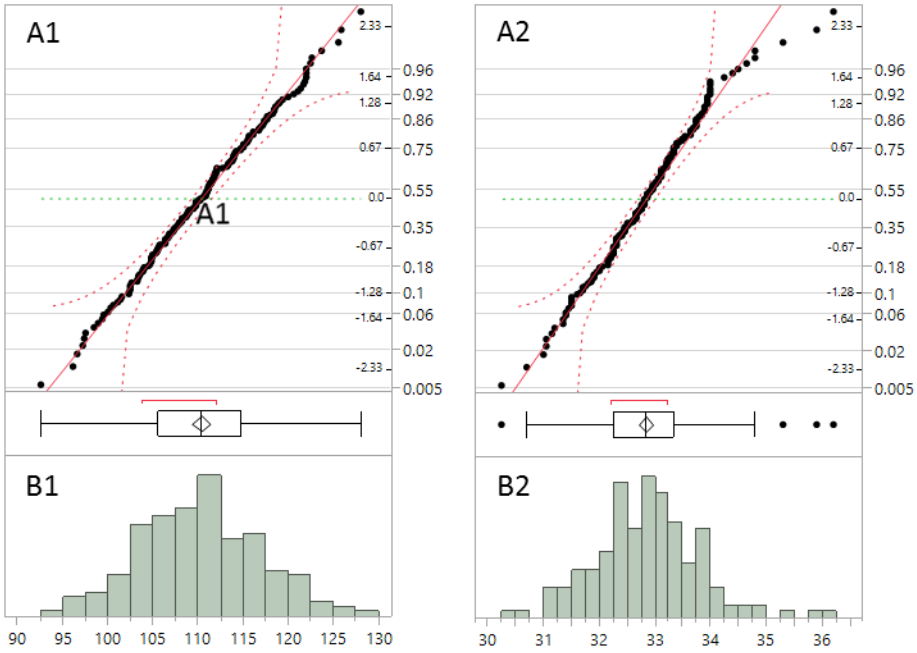


Figure 2.15. Canopy thermal index distribution and quantile plot (A1, B1) and spike and ambient air temperature distribution and quantile plot PRI (A2, B2).

Table 2.4. Simple statistics of TI and CT including ambient air temperature.

Trait	Mean	STD	Maximum	Minimum	Range
TI	110.3	6.597	128.01	92.55	35.46
CT (˚C)	32.83	0.92	36.2	30.25	5.95

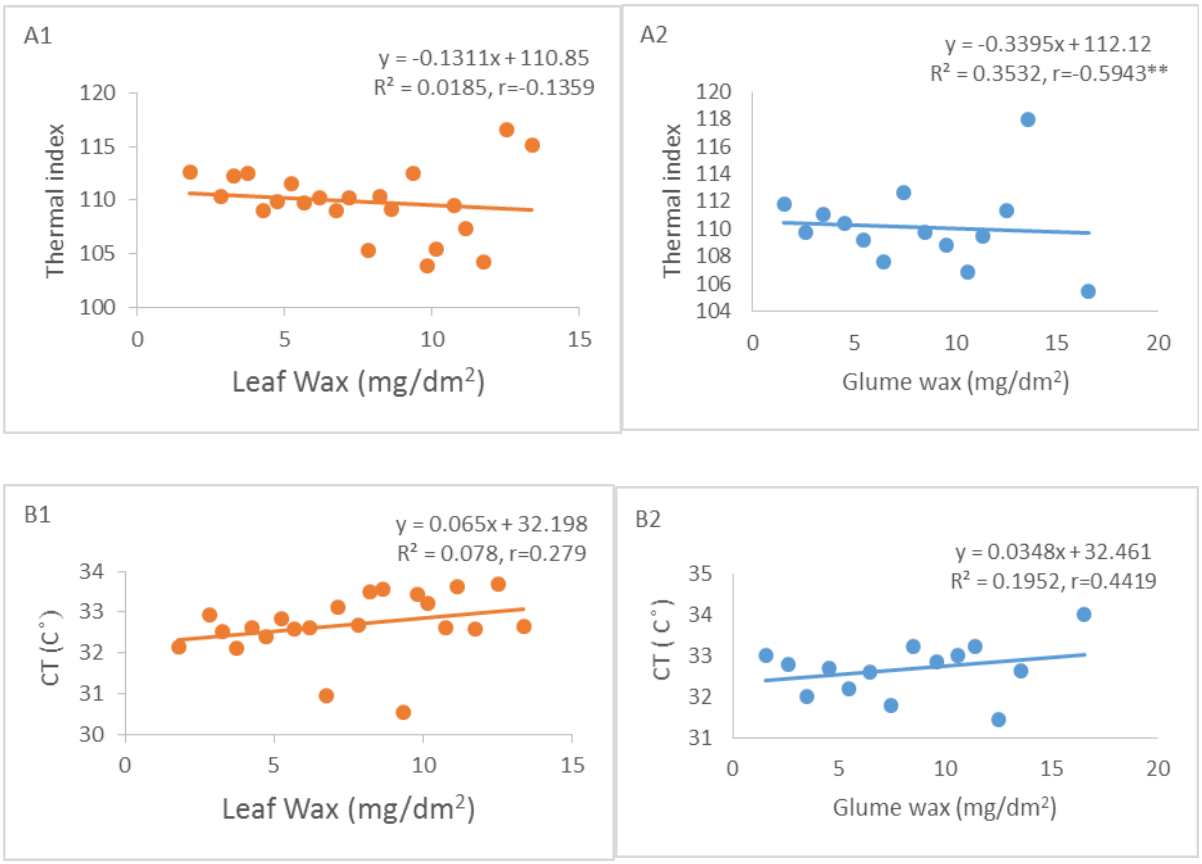


Figure 2.16. Linear relationship between Thermal index vs. leaf wax load (A1) and glume wax load (A2), and spike temperature vs. leaf wax load (B1) and glume wax load (B2).

2.3.4. Grain yield and wax correlation

Grain yield had wide range of 544 and varied significantly among the lines with minimum yield of 213 and maximum yield of 757. The mean for the yield was 496 with a standard deviation of 106 (Fig 2.17.). The linear regression of grain yield with leaf wax was not significant with R2 of 0.009. The regression for grain yield with glume wax was significant and negatively correlated with R2 of 0.3156 and Pearson's correlation of -0.5935 significant at $p \leq 0.05$ (Fig 2.18.).

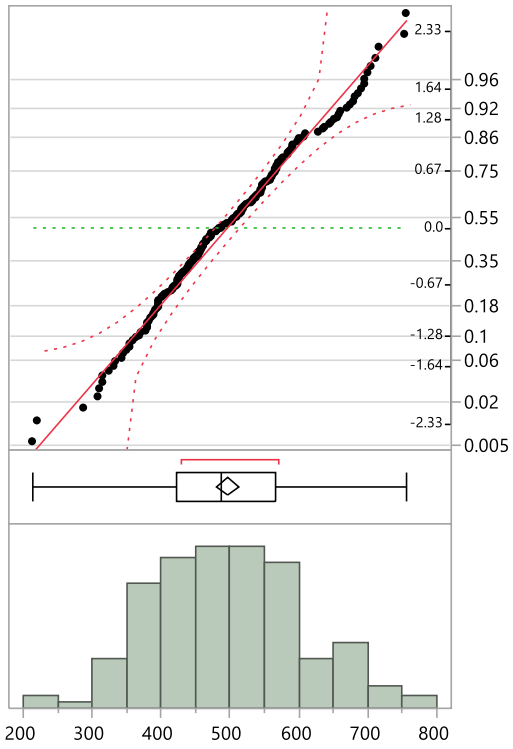


Figure 2.17. Grain yield distribution and quantile plot (maximum = 757.5, minimum = 213.5, Mean = 496.2, standard deviation = 106.5).

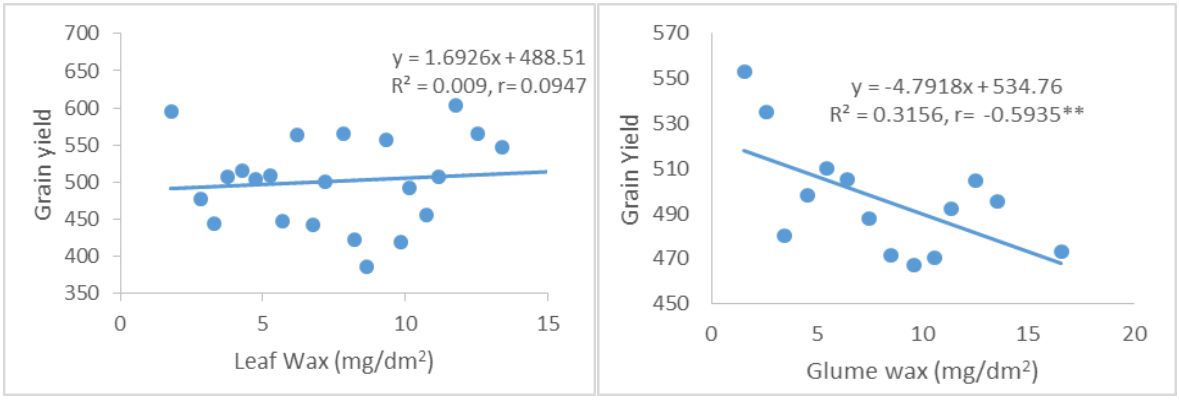


Figure 2.18. Linear relationship between grain yield vs. leaf wax load (left) and glume wax load (right), ** significant at p≤ 0.05.

2.4. Discussion

2.4.1. Leaf and glume epicuticular wax load

The physical and chemical makeup of epicuticular wax in plants is effected by both genetic and environmental factors (Whitecross et al 1972). Some environmental conditions that effect wax content and composition are relatively high-humidity conditions, such as in tissue culture, suppress wax production (Sutter and Langhans, 1979, 1982), and the photoperiod affects the chain length of wax components (von Wettstein-Knowles et al. 1980).Waxes differ widely among plant species and among the organs and tissues of a single plant, attesting to the genetic diversity and developmental influences (von Wettstein-Knowles 1995; Lemieux 1996; Post-Beittenmiller 1996). Different organs on the plant, or different parts of the plant, may have different proportions of wax component (Tulloch 1973). Analysis of data on waxes from different parts of the plant can contribute to assessment of physiological traits (Baum et al 1975). In this study, a comparison was made between the epicuticular wax load on wheat glume and flag leaf of 180 RILs and their parents under high temperature environment. Analysis of wax load in both leaf and glume showed normal distributed data with significant differences observed across the 180 RILs. Wax load for both glume and leaf reached readings as high as ~13 mg/dm^2 and as low as ~ 1 mg/dm^2. There was no apparent consistency in the glume wax to leaf wax ratio within the line. For example some lines had 1:1 ratio in the wax load of glume to leaf like lines 58, 72 and 91. Others had more varied ratios like 1:3, 1:4, and 1:12 for lines 84, 79, and 98 where leaf wax was noticeably higher than that of the glume. Another pattern where the glume wax was higher than the leaf wax like in lines 33 and 139 with ratios of 2:1 and 3:1 respectively. This observed variation is most likely due to genetic makeup of the different RILs.

The anticipated results of crossing between Halberd X Len is in agreement with previously reported studies on flag leaf wax content in wheat due to genetic variability (Uddin and Marshall 1988; Clarke et al. 1993). ANOVA test; on the other hand, suggests that there is no significant deference between the means of glume wax and leaf wax load (Fig 2.2.). In addition, when fitting the data to a linear model, a significant positive relationship for glume wax vs. leaf wax was observed with Pearson's correlation r of 0.0304 at $p \leq 0.05$ significance level and R2 of 0.03 (Fig 2.3.). This small correlation may be due to a previous reported correspondence between the presence of wax filaments on glumes and the occurrence of relatively high amounts of β-diketones among Triticeae (Simpson et al 1980).

2.4.2. Canopy spectral reflectance and wax correlations

The thin layer of epicuticular wax that is secreted on the tissue surface of the plant organ plays an important role as a protective layer against excess radiation and water loss through the reflection of visible and infrared wavelengths (Shepherd and Griffiths 2006). Thus, it makes sense to relate between epicuticular wax load and reflected radiation using high-resolution spectroradiometer device. In this experiment, correlations where made between the variable wax load in flag leaf and glume produced by different RILs and canopy reflectance. Differences were observed in percent reflectance between the lines where RILs exhibited a minimum ranges in the 2% to 3% reflectance in the photosynthetic active region and maximum ranges of 25% to 35% in the near infrared region. Reflectance of RILs were compared with leaf and glume wax loads at wavelength 900 nm, where there was maximum reflectance and there is no absorption by water concentration but reflectance is affected in the same way with respect to plant structure only (J. Peñuelas et al. 1997). Interestingly, a significant positive relationship was found between wax content of glume and the reflectance

but no relationship was detected between wax content of leaf and reflectance at 900 nm. The finding that higher epicuticular wax content gave rise to higher reflectance is in agreement with previous studies (Koch and Ensitak 2008). However, what is catching here is that the reflectance correlated to glume wax instead of leaf wax. Almost all the spectral indices gave similar results to this correlation when related with glume wax and leaf wax. This suggests that this correlation was not coincidental but as a matter of fact it is specific to this study. Water index and its normalized derivatives were significantly and negatively associated with glume wax but not significant with leaf wax. The similarities between the water indices indicated that the choice of wavelength for a water index was less important for thin canopies, and the best wavelengths were those where water absorbance was weak to moderate (Sims et al. 2003). Moreover, the values indicates that while the canopy was able to maintain good water status under heat stress conditions during grain filling stage (Prasad et al. 2009) it is the glume that can be used as an indicator of that status. This may be due to early senesces of leaf as compared with glume's viability and higher stay-green which makes it more photosynthetically active during this stage than the leaf (Kong et al. 2015). Since photosynthetic activity is in direct relationship to chlorophyll content, then vegetation indices will also show stronger relation with glume wax content than leaf wax content. This is because strong absorption by chlorophyll in the red region of the spectrum results in the well-known saturation of the normalized difference vegetation index (NDVI) and simple ratio index at high leaf area indices (Gamon et al. 1995; Sellers 1985). The results of this study did agree with this prediction where both NDVI and SR related positively and significantly with glume wax content but did not relate to leaf wax content again during grain filling and under high heat stress. The vegetative indices, SR and NDVI, increased through the increase

in glume wax content under HT conditions, which suggest an increase in plant health for RILs with higher glume wax content. On the other hand, the index that measures radiation use efficiency, PRI in this case, may be used to measure changes in the status of xanthophyll pigments without canopy scale PAR manipulations (Araus, 1996). Heat stress causes plants to respond with the conversion of the xanthophyll cycle (Demmig-Adams & Adams 1992). Here, PRI had higher values for glumes with higher wax content. PRI is expected to increase compared to non-stressed conditions, indicating a reduction in radiation use efficiency (Penuelas et al. 1995; Babar et al. 2006). The variation between PRI was very small across the RILs because they were all under the same heat stress condition but the small differences detected by the spectrophotometer correlated significantly with wax content of glume, reinforcing the outcome of the study where glume wax content was observed to have correlations with all spectral indices analyzed.

2.4.3. Canopy temperature and wax correlation

CT integrates many physiological functions that facilitates adaptations to different environment making it highly versatile measurement (Amani et al. 1996; Blum et al. 1982). Under heat stress, cooler canopies were associated with yield among random lines as well as providing a powerful tool for selecting advanced lines for performance at a number of heat-stressed target environments (Reynolds et al. 2001). In this study, CT by ground base showed no significant correlation with both leaf and glume wax content. The reason behind the lack of correlation using ground based imaging is likely due to dramatic change in weather conditions during data collection. The proses of collecting data via ground was time consuming and thus both wind and temperature varied considerably from the start to end of collection time.

Thermal index via airborne method is a well-known measure of stomatal conductance in plants. In this study, thermal index related negatively and significantly with glume wax content as compared to leaf wax content. Not only does this mean that wax content in general decreased the thermal index at the grain filling stage and under heat stress, but also it was the glume that was mostly "seen" by the sensor camera. This is similar to a previous study on cereals where it was found that developing and maturing seed heads tend to have stomata, and transpire less than the leaves resulting in an increased canopy temperatures (Milthorpe and Moorby 1979; H. G. Jones et al.2009).

2.4.4. Grain yield and wax correlation

Grain yield is one of the most important, yet complex traits in crops. It is a combination of interaction between environment and developmental processes during growth stages that occur throughout the life cycle of crop (Quarrie et al. 2006). Grain yield is directly determined by yield its component (such as SKW and seed number). Yield-related traits (such as biomass, harvest index, plant architecture, adaptation, resistance to biotic and abiotic constraints) may also indirectly affect yield by affecting the yield component traits or by other, unknown mechanisms. (Shi. et al 2009)

Grain yield related negatively and significantly with glume wax content as compared with none significant correlation with the leaf wax content in the present study. It was projected that the yield will increase for those RILS with higher glume wax content after all the above successes however, a closer look at the parent lines suggest otherwise. This is because parent Halberd and Len both had an average leaf wax content as compared to the rest of the RILs leaf wax content, but parent Len had a low glume wax content with very high yield (~700) while parent Halberd had an average glume wax content and grain yield (~400).

Thus, it is possible that high yield trait was associated with low glume wax trait. QTL analysis was performed on these RIL and their parent lines to further explore this relation.

CHAPTER III

A COMPARATIVE ANALYSIS ON THE ROLE OF LEAF EPICUTICULAR WAX TO GLUME EPICUTICULAR WAX ON IMPROVED ADAPTATION FOR HIGH TEMPERATURE STRESS IN WHEAT

3.1. Introduction

High temperature and drought are major factors limiting wheat yields in many countries worldwide. Yield components such as kernel weight, number of kern per spike, and number of spike per m^2 are assumed to be inherited quantitatively (Benmoussa et al. 2005). High temperature stress negatively effects these yield components during kernel development, and ripening. A study by Gibson and Paulsen (1999) showed that yield and yield component were reduced by 78% through a reduction in kernel weight and kernel number by 29% and 63%, respectively when exposed to a 35/20 °C (day/night) at 10 days after pollination (DAP) until ripeness. However, exposure of wheat grain to the same temperature in the later stages of seed development at 15 and 20 DAP, showed less yield loss. High temperature stress during grain filling stage lead to decrease in starch in endosperm cells, leading to a reduction in the amount of starch per granule which reduced grain weight (Jenner 1991). At the plant cell level, water shortage results in osmotic stress. A flux of water from the cells results from an alteration in extracellular solute concentrations. The loss of water causes decrease in turgor and increase in concentrations of intracellular solutes, putting a stress on membranes and macromolecules. If chloroplasts are exposed to excessive excitation energy at the same time, water deficiency leads to the production of cell-toxic substances such as superoxide and peroxides damaging cell membranes and enzymes (Holmberg and Bülow 1998).

Heat tolerance is also inherited quantitatively based on phenotypic traits like higher seed set, grain weight and an extended grain filling duration at higher temperatures (Yang et al. 2002). Controlling high temperature and drought tolerance in wheat is one way to improve breeding efficiency. Consequently, high temperature and drought tolerant wheat varieties are the most appropriate solution especially when they are farmer friendly and based on seed technology that is easy to disseminate. In harsh climatic zones where plants are exposed to heat and drought stress adaptive trait strategies should include breeding traits that reduce radiation load, such as increased wax pigment composition, leaf angle and rolling that increased transpiration efficiency (Richards 2006). Plants utilize stress adaptive physiological mechanisms to survive under high temperatures according to the degree of stress. Reduced photosynthetic rate, and waxy leaf and glume are some of the physiological responses that have been associated with yield under high temperature stress in wheat (Reynolds et al. 1994). In pea cultivars, EW influences grain yield indirectly by improving harvest index, decreasing residual transpiration rates, and leaf CT under water-deficit conditions (Sánchez et al. 2001). Increased leaf EW may compensate for increased stomatal conductance, thereby increasing leaf temperature depression and yield stability under heat stress conditions (Mondal et al. 2014).

Higher plants comprise a cuticular layer covered by a waxy deposit. Epicuticular wax (EW) plays a major role in the water balance of plants (Eglinton 1967). EW covers plant aerial organs to protect them from various biotic and abiotic stresses. EW could be described as a very thin film upon the cutin matrix, this thin layer appear as microscopic mixtures called EW crystals expanded from this thin film (Barthlott et al. 1998; Jetter & Schäffer 2001). The structure of epicuticular wax is determined by the shape and density of single

wax crystals. However, the morphology of such crystals depends on the plant's species and specific chemistry (Baker 1982), and by a predominating wax compounds (Jetter & Riederer 1994; 1995). However, many factors likely affect the amount and chemical composition of EW surface. EW is not only species and organ specific, but also vary due to plant growth environment and development stage. The amount and orientation of the leaf wax crystals changes between leaf regions of variable age in expanding leaves (Rhee et al. 1998). Leaf EW was consider to have low heritability in wheat compared to different crops (Mondal et al. 2014). Temperate cereals like wheat are relatively well adapted to high temperature and dry environments, being grown widely throughout the world. A high temperature and drought resistant genotype gives significantly higher yield than average under conditions where crop water availability is limited by environmental aspects. Ongoing breeding work has made steady progress, yet performance of cereals still shows substantial loss to high temperatures (Reynolds et al. 1994). Moreover, significant breeding effort will be required to maintain their productivity under warmer conditions. Although breeders regard improved high temperature and drought resistance as specific target in their breeding programs, progress towards this objective is often hard to improve and achieve. This study aims to compare and analysis the role of leaf epicuticular wax to glume epicuticular wax on improved adaptation for high temperature stress in wheat.

3.2. Material and methods

3.2.1 Plant material

In this study, a set of 180 recombined inbred lines (RILs) of wheat were used. These RILs were derived from cross of the heat tolerant spring wheat lines 'Halberd' with Len. Halberd

is an Australian spring wheat (*Triticum aestivum* L) as a donor cultivar with the pedigree Scimitar/Kenya/C6042/ Bobin/2/Insignia49 (Paull et al. 1998). Halberd is a heat and drought tolerant cultivar, also has ability to maintain carbohydrate accumulation during moisture stress (Ji et al. 2010). Len is hard red spring wheat as a recurrent cultivar developed in North Dakota with the pedigree ND499/3/Justin/RL4205/W1261. Len is a semi-dwarf that is a drought and heat susceptible however, it is known for its good agronomic characteristics (Hossain et al. 2012). The two parents were chosen due to similarities in flowering period and maturity. The 180 RILs were developed by preceding the F1 progeny through single seed descent in head rows to the F5 generation. Seeds from the F5 generation were bulked to develop 180 F5:6 RILs. The F6 lines were advanced in the field and were evaluated during 2010 as an F5:7 generation. During 2011 and 2012, F8 and F9 generations were used respectively, to conduct experiments (S. Mohammed et al. 2014).

3.2.2. Growing environment

The RILs and the two parents were grown in the field during the winter seasons. Yield trials were conducted at Uvalde Agrilife research station during 2013 and 2014 seasons, College Station Agrilife research station, and International Maize and Wheat Improvement Center (CIMMYT) Ciudad de Obregon, Mexico during 2014 season. All growing parameters were the same with variability being only in growing location.

Weather conditions are mostly sunny and dry during the winter cropping cycle. Table 1 list daily temperature for the three locations during grain filling stage. Nitrogen and phosphorus were applied to the plots at a rate of 150 kg ha−1 and 22 kg ha−1, respectively. Field plots consisted of two raised (28 cm apart) each 3m long and 1m wide and seeded at 50g per plot. An alpha lattice design with two repetitions was used for all experiments. The

planting dates were in February for Obregon location and plants reached booting and heading during April–May and were harvested in May. For College station and Uvalde, planting dates were in January and plants reached booting and heading during April–May and were harvested in May. Record was made for daily high temperature for the 10 DAP (table 3.1).

Table 3.1. Daily high temperature during grain filling stage for all growing environments**.**

DAP	Daily high temperature (F˚)			
	OBR14	UVL13	UVL14	CS13
1	95	93	77	86
2	98	81	79	72
3	89	79	93	69
4	91	88	93	82
5	94	88	95	73
6	91	93	96	78
7	91	100	95	86
8	92	103	93	87
9	101	95	88	80
10	106	97	93	84
Mean	94.8	91.7	90.2	79.7

3.2.3. Yield and yield component measurements

Measurements were taken after complete plant maturity, when leaves are completely dry and heads are chlorotic, ready to harvest. The plants were harvested and threshed separately for primary spike and all other spikes were bulked. Plot yield (gm^{-2}), thousand kernel weight (g) (TKW), and kernel number per spike (KNS) were estimated by harvesting 50 heads at each plot. Mean single head weight (MSHW) was the average weight of seed from 50 heads harvested from each plot. Main heads were harvested from the central region of the plot area uniformly, excluding secondary tiller heads. Grain weight for 100 kernels was measured using a seed-counting machine (SeedBuro TM 801 Count-a-Pak) and weighed to calculate the TKWs (g).

3.2.4 Wax sample collection

Samples were collected during early spring 10 DAP. Four glumes per RIL replicate were collected for wax analysis using tweezers to carefully remove the glume without touching the surface EW layer. Leaf discs for wax analysis were collected from flag leaves using a disc punch with a 1 cm diameter drum (Rabbit Tool USA, Rock Island IL USA). Four 1cm (diameter) leaf punches was collected from the primary inflorescence leaf per plant of four pates per plot and placed in Borosilicate Glass Scintillation Vials with Screw Caps. The sample vials were placed in the laminar flow to air dry and stored at room temperature.

3.2.5 Wax extraction

EW concentrations was determined using the colorimetric method of Ebercon et al., (1977). Glume and Leaf EW was extracted by submerging glumes and leaf discs in 1 ml HPLC grade chloroform for 30 s, the submersion time previously determined to completely remove the epicuticular wax from the leaf (Mayeux and Jordan, 1984). The resulting mixture was

transferred to a clean 1.8 ml glass GC vial (VWR Auto sampler Vial, Radnor, PA). The chloroform solvent was removed under a continuous flow of nitrogen gas by leaving the vial uncapped overnight in the laminar flow hood.

3.2.6 Wax quantification

The resulting extract was oxidized by adding 300µl acidified potassium dichromate and heated for 30 minutes in a water bath at 100 ^{0}C. After boiling, vials were allowed to cool for a 1 hour period and 700µl of deionized water was added to each vial, allowing color development for another hour. A spectrophotometer (PHERAstar plus, BMG LABTECH, Offenburg, Germany) was used to determine the optical density for each sample at 590 nm. Samples were loaded in 96 well, sanitized, clear flat bottom microplates (Greiner Bio-One, Monroe, NC, USA). A standard curve was developed from randomly selected wheat flag leaves from Halberd. Samples were placed in large glass vials and 20 ml HPLC grade chloroform was added to remove EW. The resulting chloroform-wax solution was proportioned based on the serial dilution technique. The standard curve was used to calculate wax levels based on leaf area (Mondal et al. 2014).

3.2.7. Statistical analysis

Statistical analyses of all phenotypic traits were performed using JMP Pro 11.2.1 (JMP Version 11, SAS Institute Inc.,Cary, NC). Also; all this phenotypic traits were determined and tested for normality. The generalized linear model (GLM) was used for analysis of variance and the means were compared using Fischer's least significant difference. A combined analysis of variance was also done using GLM procedure considering genotype and year as fixed effects. Pearson's correlations were estimated for determining the

association between leaf and glume epicuticular waxes and the physiological and phenological responses under high temperature stress for the three locations.

3.3. Results

3.3.1. Leaf and glume epicuticular wax load and temperature correlations

Analysis of wax load in both leaf and glume showed normally distributed data with differences observed between lines being significant at $p \leq 0.001$. A maximum of 4.89 and 5.036 mg/dm^2 for leaf and glume epicuticular wax load respectively were observed in CS13 which are the lowest readings of wax load maximums as compared to the other locations (Table 3.2.). Contrary to what is expected, wax load mean was higher in glume as compared to that of leaf for all locations except for CS13 (Fig 1). ANOVA test for leaf epicuticular wax ranks the mean of the locations from highest to lowest as OBR14, UVL13, UVL14, and CS13, with the mean of OBR14 being 5.37 and that of CS13 being 2.508 mg/dm^2 respectively (Fig 3.2.). This order was repeated for glume epicuticular wax load were OBR14 had a mean of 5.97 mg/dm^2 and CS13 mean of 2.38 mg/dm^2 (Fig 3.3.). However, the same line did not produce matching leaf and glume wax content throughout the lines and the locations as shown in Fig 4. A plot of glume wax load vs. leaf wax load showed no significant relationship when tested for CS13 and UVL14. However, there was a very strong correlation between the two wax loads for UVL13 with $R^2 = 0.8285$ and $r=0.9195$ significant at $p \leq 0.001$. A significant correlation also was observed for the two wax loads for OBR14 with $R^2 = 0.0304$ and $r=0.1744$ significant at $p \leq 0.05$ (Fig 3.5.). A strong correlation was also found between the mean wax load for leaf and that for glume as function of the mean high temperature recorded for grain filling stage days. While both leaf and glume wax

showed a strong correlation with temperature, the one for leaf wax load vs. temperature had an $R^2 = 0.6719$ while that of the glume wax was stronger at $R^2 = 0.8483$ (Fig 6).

Table 3.2. Summary of statistics of leaf and glume epicuticular wax for all growing locations.

Location	Leaf wax load (mg/dm^2)				Glume wax load (mg/dm^2)			
	Max	Min	Mean	STD	Max	Min	Mean	STD
CS13	4.890	1.353	2.508	0.696	5.036	0.918	2.386	0.873
UVL13	6.649	1.527	3.620	1.096	12.096	1.476	5.341	2.412
UVL14	10.7	0.303	3.107	2.12	14.29	0.325	3.644	2.55
OBR14	12.944	2.64	5.37	2.22	14.71	0.139	5.97	3.35

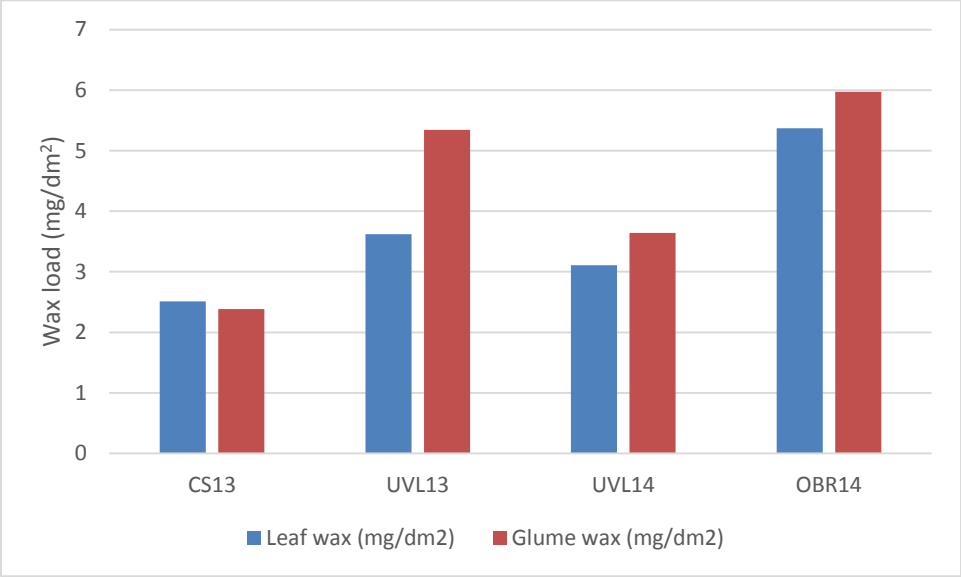


Figure 3.1. Leaf and glume mean wax load for all growing locations.

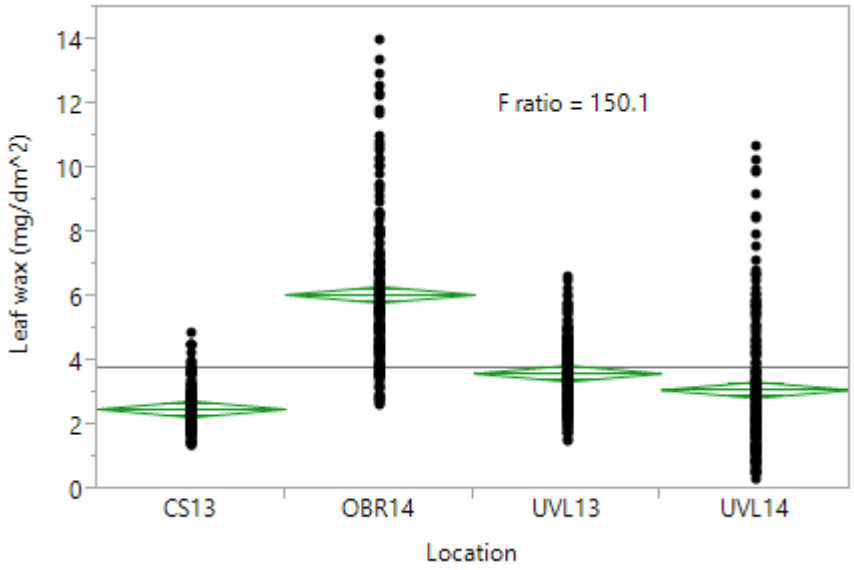


Figure 3.2. One-way analysis of leaf epicuticular wax (mg/dm2) by growing location, significant at p≤ 0.001.

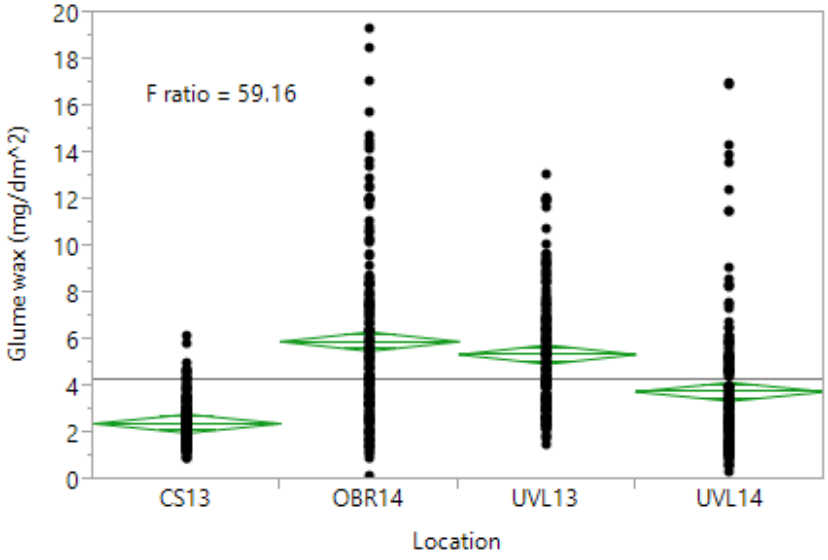


Figure 3.3. One-way analysis of glume epicuticular wax (mg/dm2) by growing location, significant at p≤ 0.001.

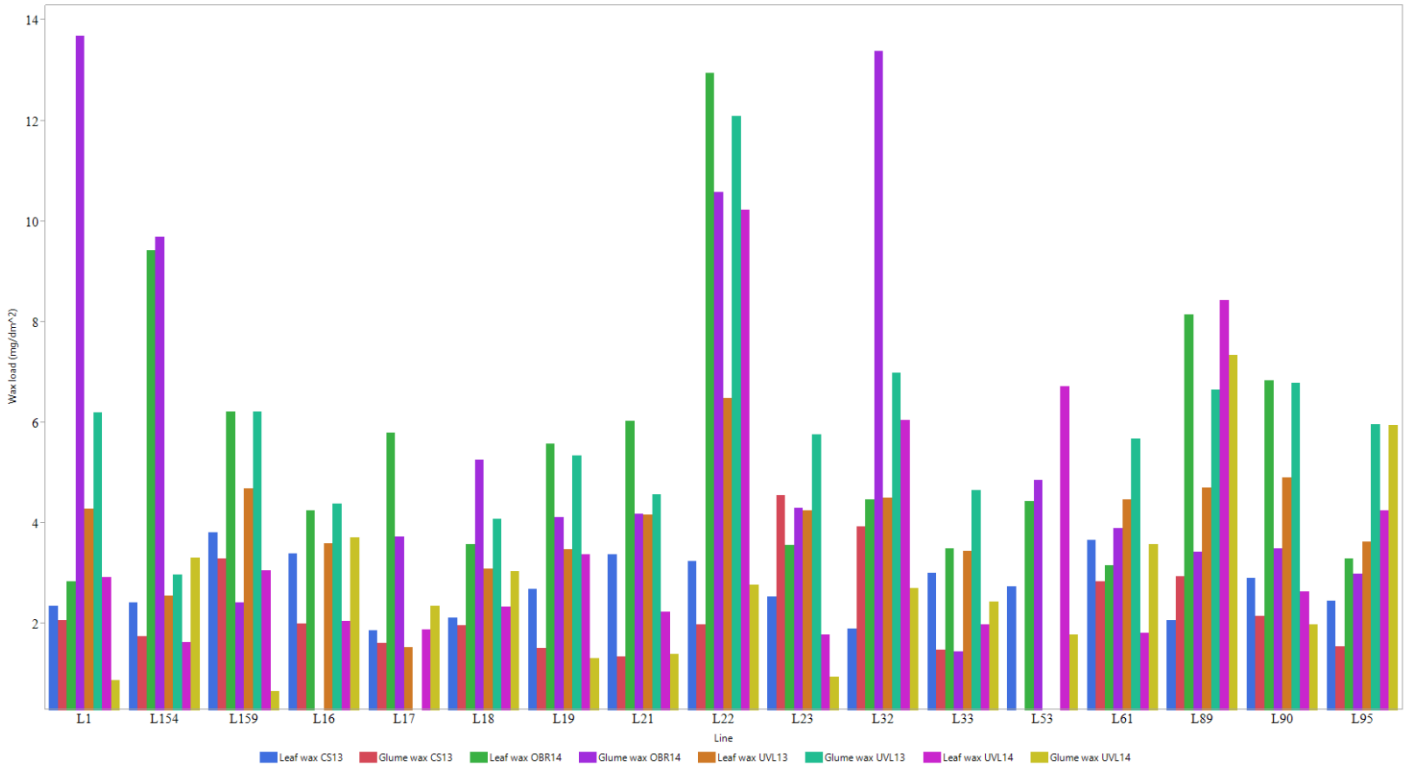


Figure 3.4. Leaf and glume wax load for sample wheat lines for all growing locations.

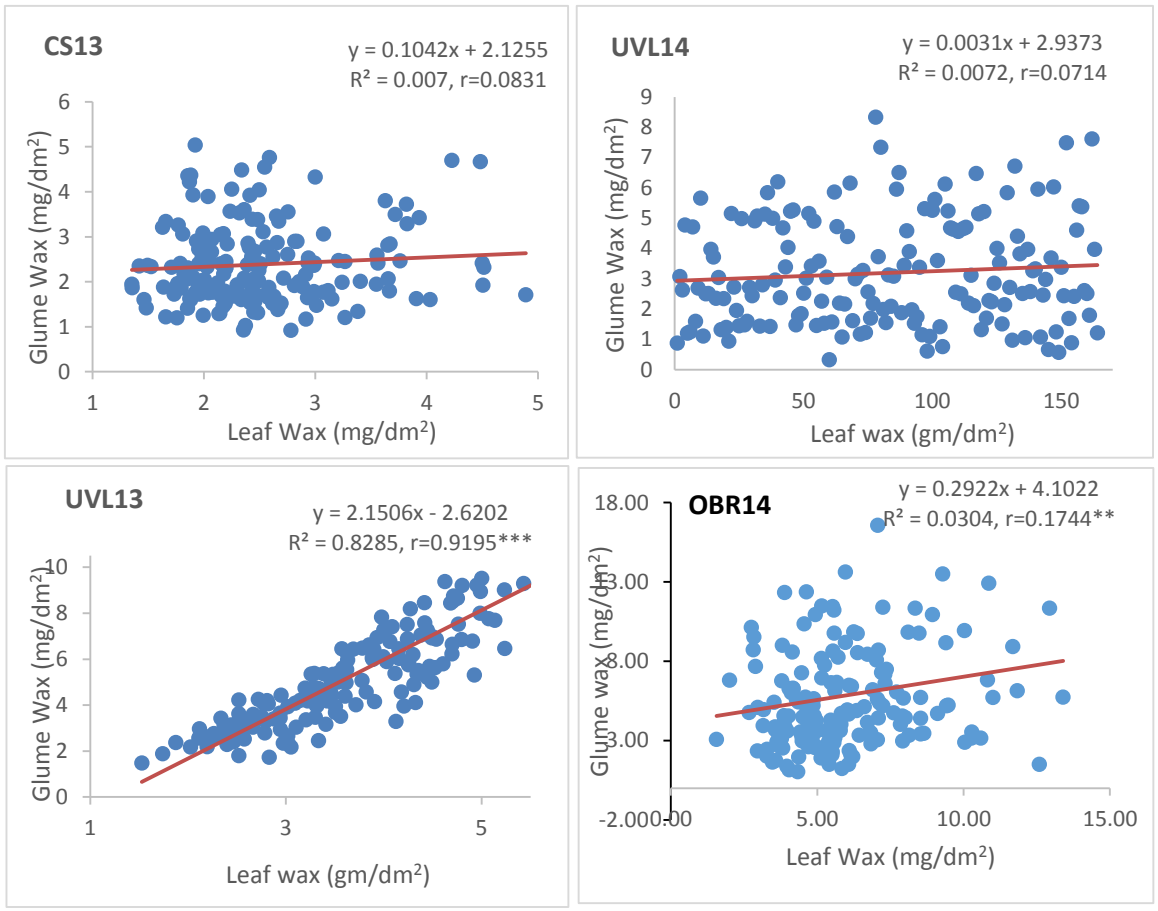


Figure 3.5. Linear correlation between glume wax and leaf wax for all growing locations, **significant at p≤ 0.05, ***significant at p≤ 0.001 with Pearson's correlation

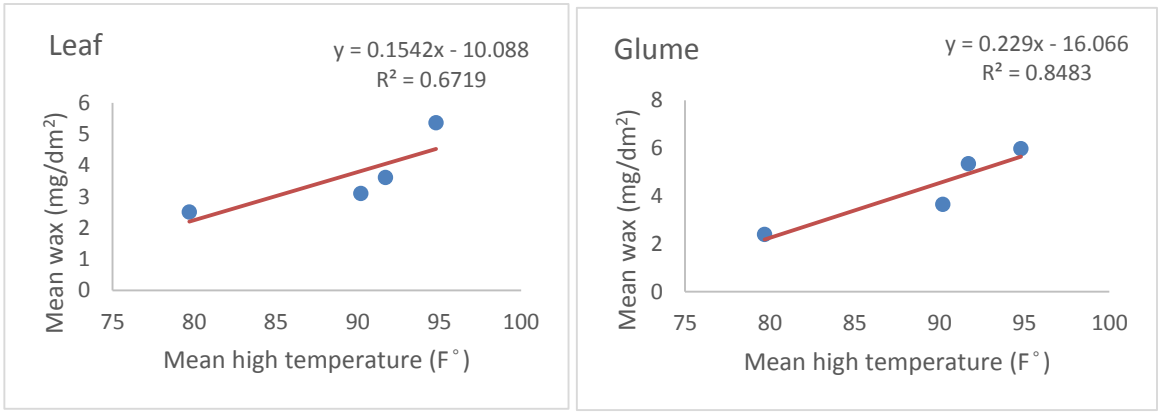


Figure 3.6. Mean wax load as a function of mean high temperature during grain filling stage for both leaf and glume.

3.3.2. Yield and yield component and wax correlation

All yield and yield components gave normally distributed data and showed variation between the different growing locations (Figures 3.7. and 3.12.). Means of ANOVA analysis on SKW for CS13 and UVL13 were very close with CS13 higher by only 0.002g. The minimum weight measured for both location was 0.028g (Table 3.3., Fig 3.8.). A similar result was observed when comparing MSHW of both CS13 and UVL13. In this comparison, CS13 had a mean of 40.14g as compared to 38.36g for UVL13 with F ratio of 7.28 significant at $p \leq 0.001$ (Fig 3.9.).

A plot of SKW as a function of wax load for both leaf and glume showed no significant relation at CS13 and UVL14. However, the relation was more apparent for UVL13 where R^2 for SKW vs. Leaf wax was 0.0202 and that for glume was 0.0286 with r = 0.1692 significant at $p \leq 0.05$ (Fig 3.10.). This pattern was repeated again when plotting MSHW vs. wax load for both leaf and glume, yet, the leaf-wax relationship was stronger at UVL13 with $R^2 = 0.023$ and r=0.1516 significant at $p \leq 0.001$(Fig 3.11).

Grain yield had a wide range and varied significantly among the lines for both OBR14 and UVL14. An ANOVA analysis of grain yield between UVL14 and OBR14 showed noticeable variation with F ratio = 252.3 significant at $p \leq 0.001$ (Fig 3.13.). In this analysis OBR14 had a lower mean of 496g as compared to the mean of UVL14 which was 729g. Also, OBR14 reached a maximum of 757g while UVLD14 maximum was 1047g (Table 3.3.). A comparison of the grain yield behavior for each line between the two locations showed a higher reported yield on average UVL14 as compared for the yield of OBR14 for the same line (Fig 3.14.). This was reinforced when plotting yield of UVL14 vs

that of OBR14 where there was a significant correlation at $p \leq 0.001$ with $R^2 = 0.0295$ and $r=0.1638$ (Fig 3.15.).

Linear regression of grain yield with leaf wax was not significant for both OBR14 and UVL14 with R^2 of 0.0013 and 0.0069 respectively. The regression for grain yield with glume wax was significant and negatively correlated with R^2 of 0.3156 and Pearson's correlation of 0.5935 significant at $p \leq 0.05$ for OBR14 but was not significant for UVL14 (Fig 3.16.).

Table 3.3. Summary of statistics of yield and yield components for all growing locations.

Location	Yield Component	Max	Min	Mean	STD
CS13	SKW (g)	0.048	0.028	0.038	0.0037
UVL13		0.044	0.028	0.036	0.0031
CS13	MSHW (g)	58.45	23.20	40.14	6.12
UVL13		52.30	20.85	38.36	6.29
UVL14	Grain Yield	1047	374.5	729.96	153.59
OBR14	(g)	757.5	213.5	496.60	106.68

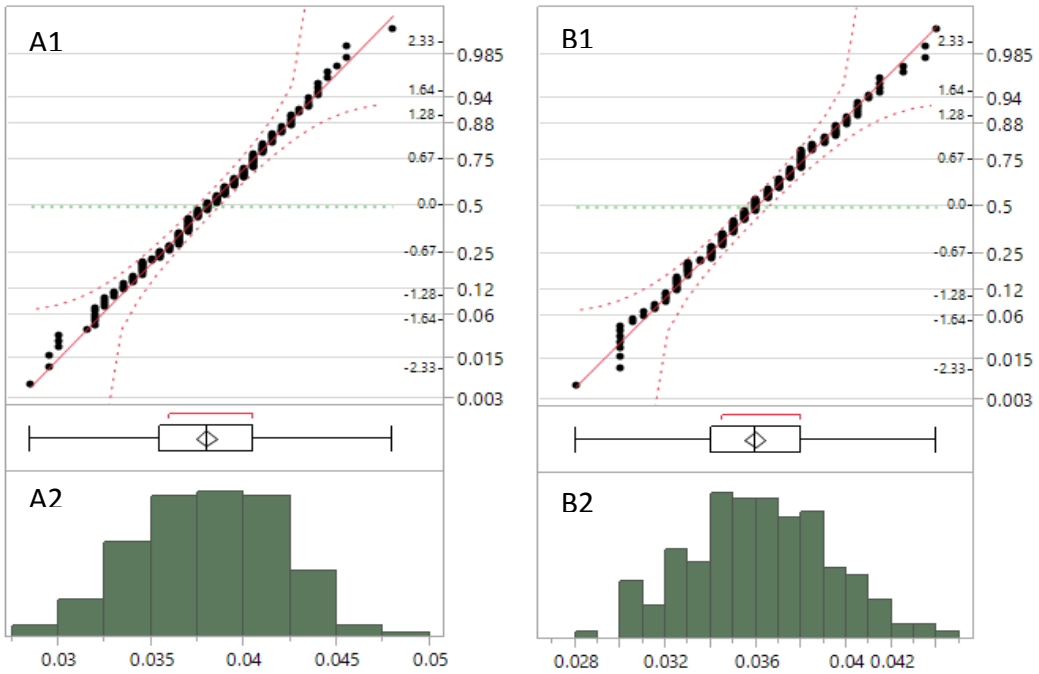


Figure 3.7. Distribution and quantile plots of SKW data; Normal distribution and quantile plot of SKW for CS13 (A1, A2), Normal distribution and quantile plot of SKW for UVL13 (B2, B2).

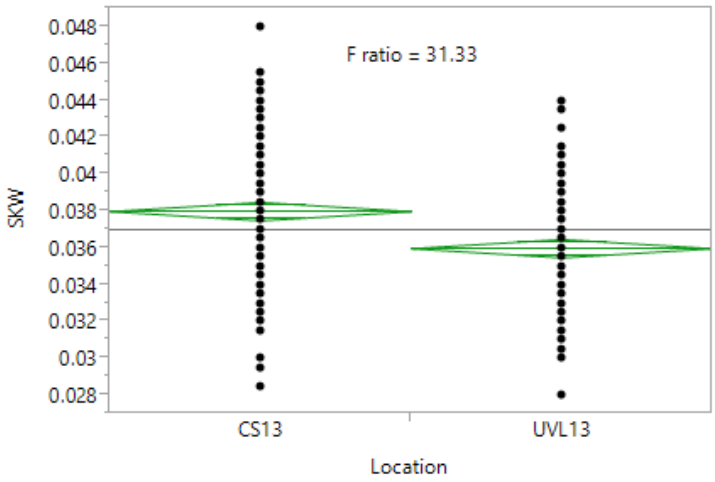


Figure 3.8. One-way analysis of SKW (g) by growing location for CS13 and UVL13, significant at $p \leq 0.001$.

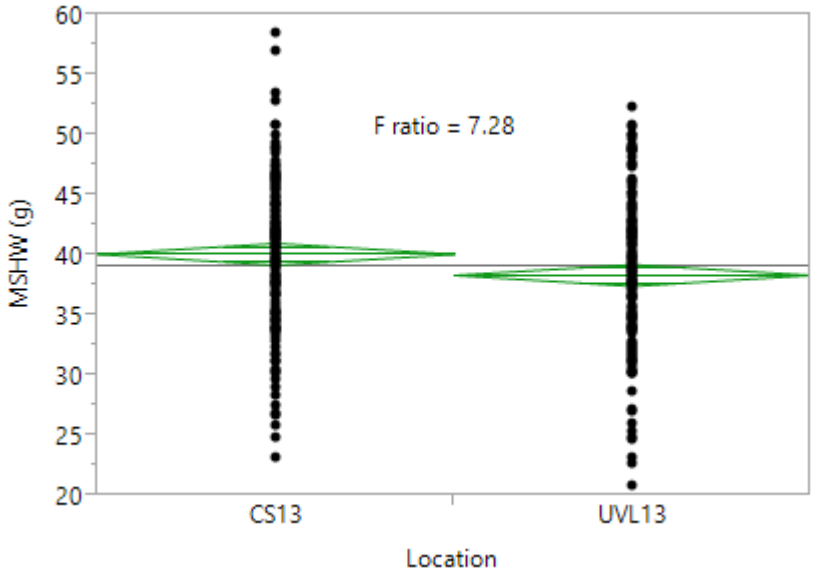


Figure 3.9. One-way analysis of MSHW (g) by growing location for CS13 and UVL13, significant at $p \leq 0.001$.

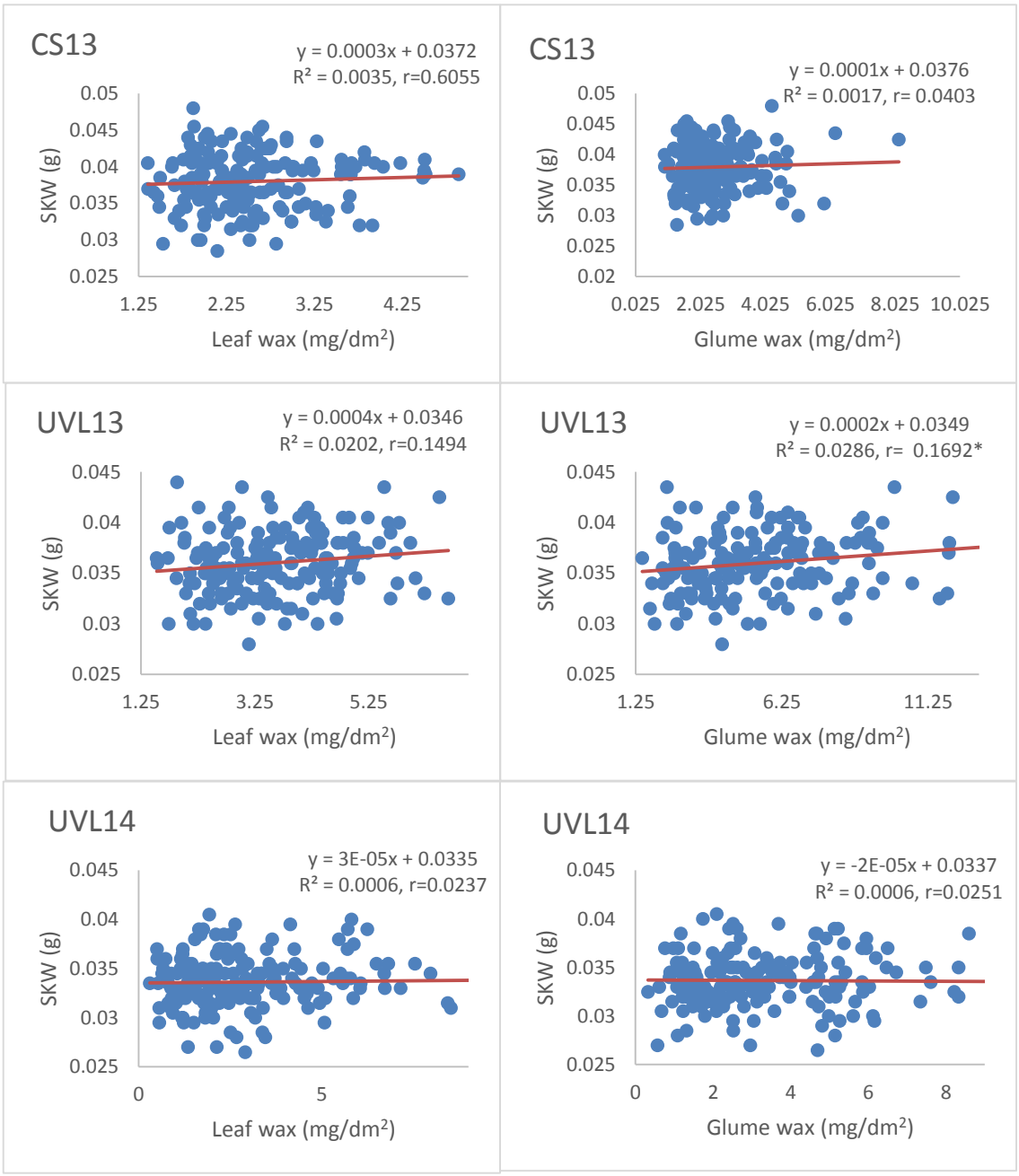


Figure 3.10. Linear relationship between SKW (g) and wax load (mg/dm2) for both leaf and glume at CS13, UVL13, and UVL14, * significant at p≤ 0.05.

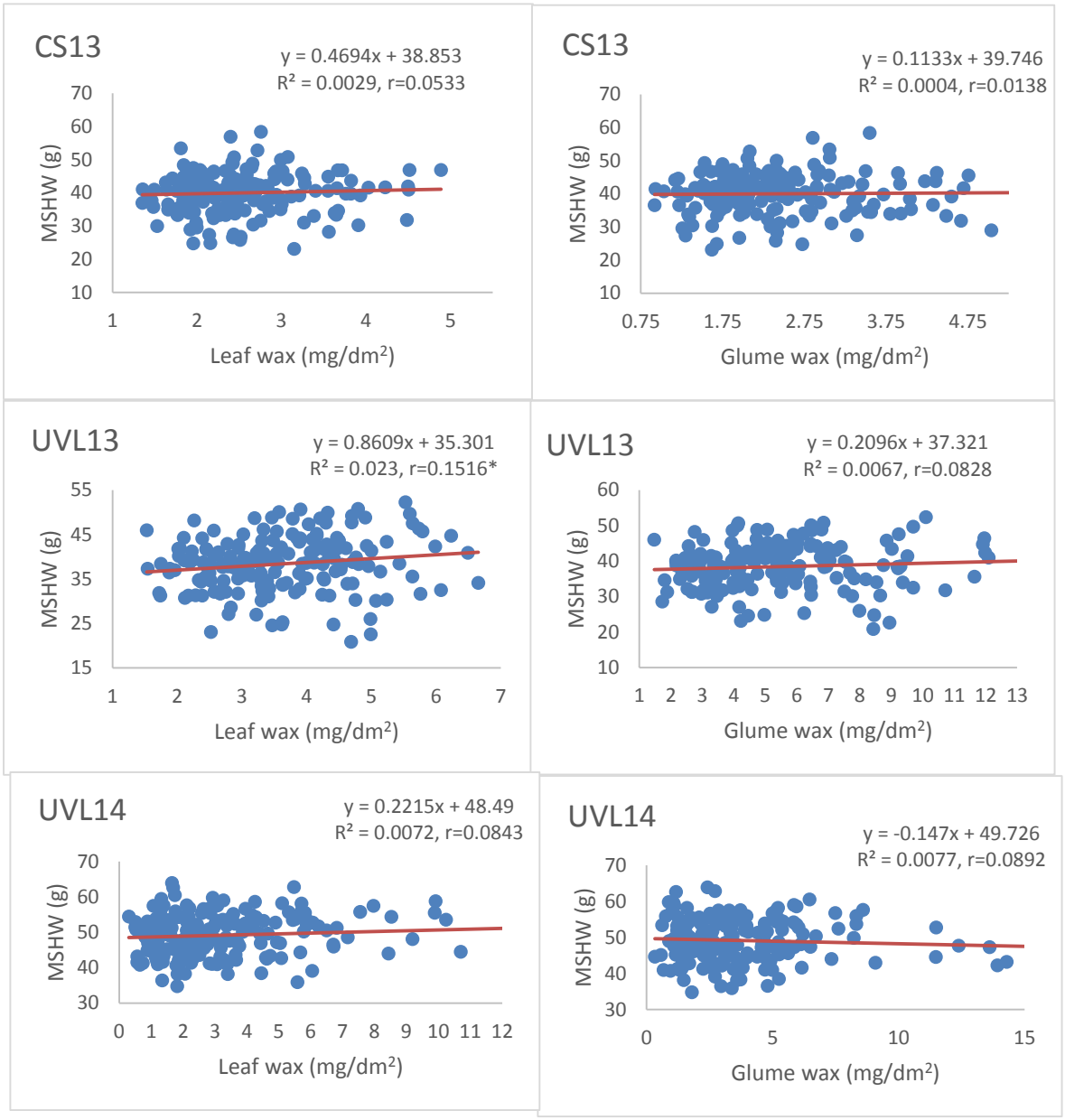


Figure 3.11. Linear relationship between MSHW (g) and wax load (mg/dm2) for both leaf and glume at CS13, UVL13, and UVL14, * significant at p≤ 0.001.

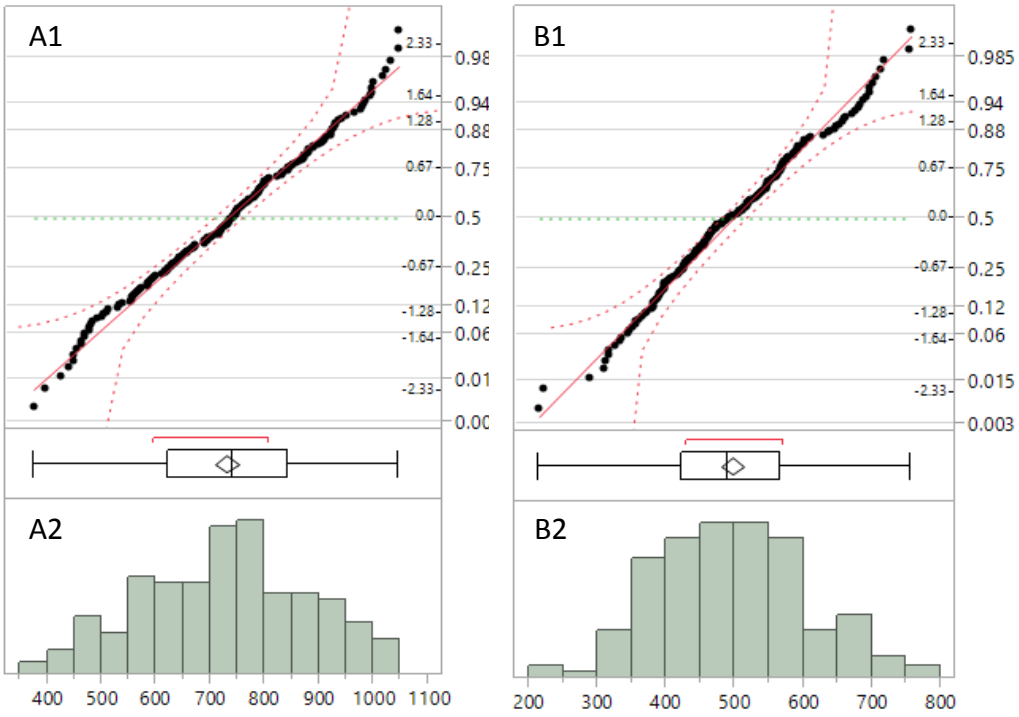


Figure 3.12. Distribution and quantile plots of grain yield data; Normal distribution and quantile plot of grain yield for UVL14 (A1,A2), Normal distribution and quantile plot of grain yield for OBR14 (B2,B2).

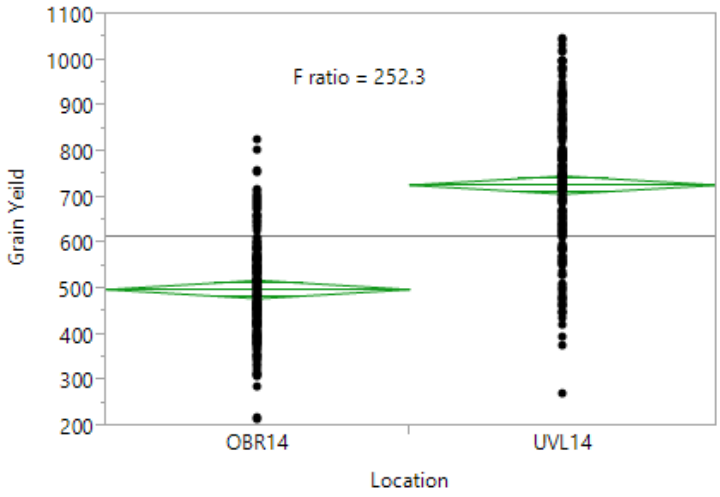


Figure 3.13. One-way analysis of grain yield (g) by growing location for OBR14 and UVL14, significant at $p \leq 0.001$.

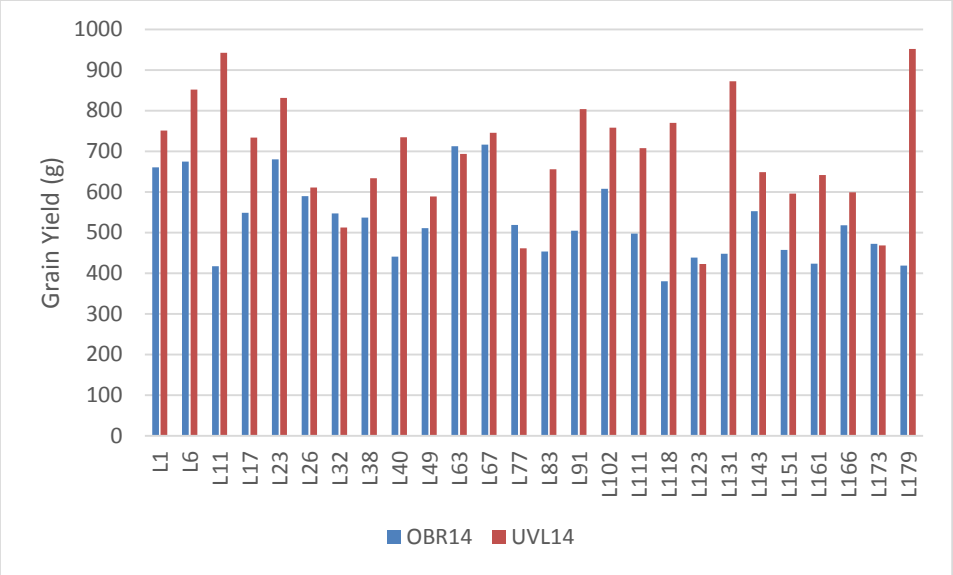


Figure 3.14. Random sample of lines showing grain yield (g) for each line for OBR14 and UVL14.

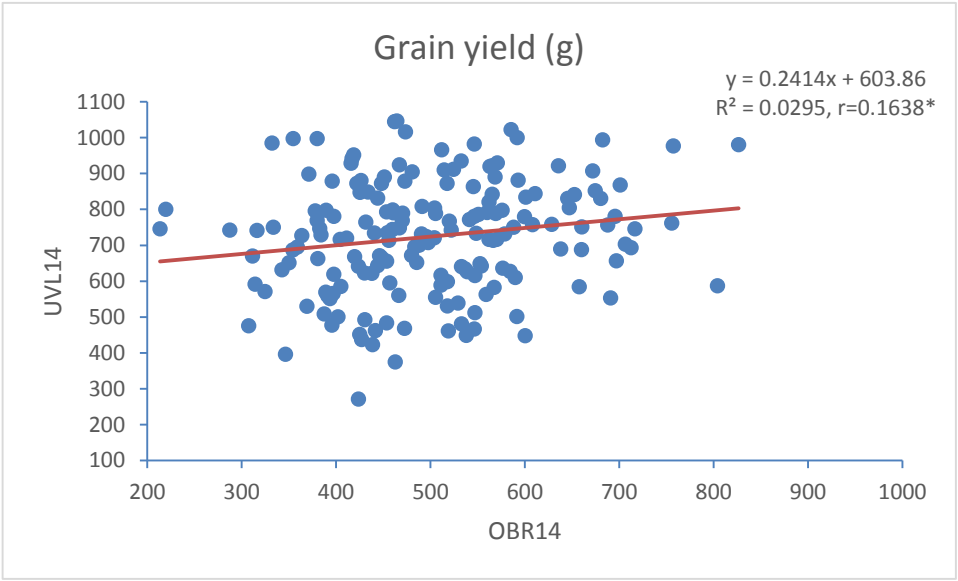


Figure 3.15. Linear relationship between grain yield for UVL14 (g) and grain yield for OBR14 (g), * significant at $p \leq 0.001$.

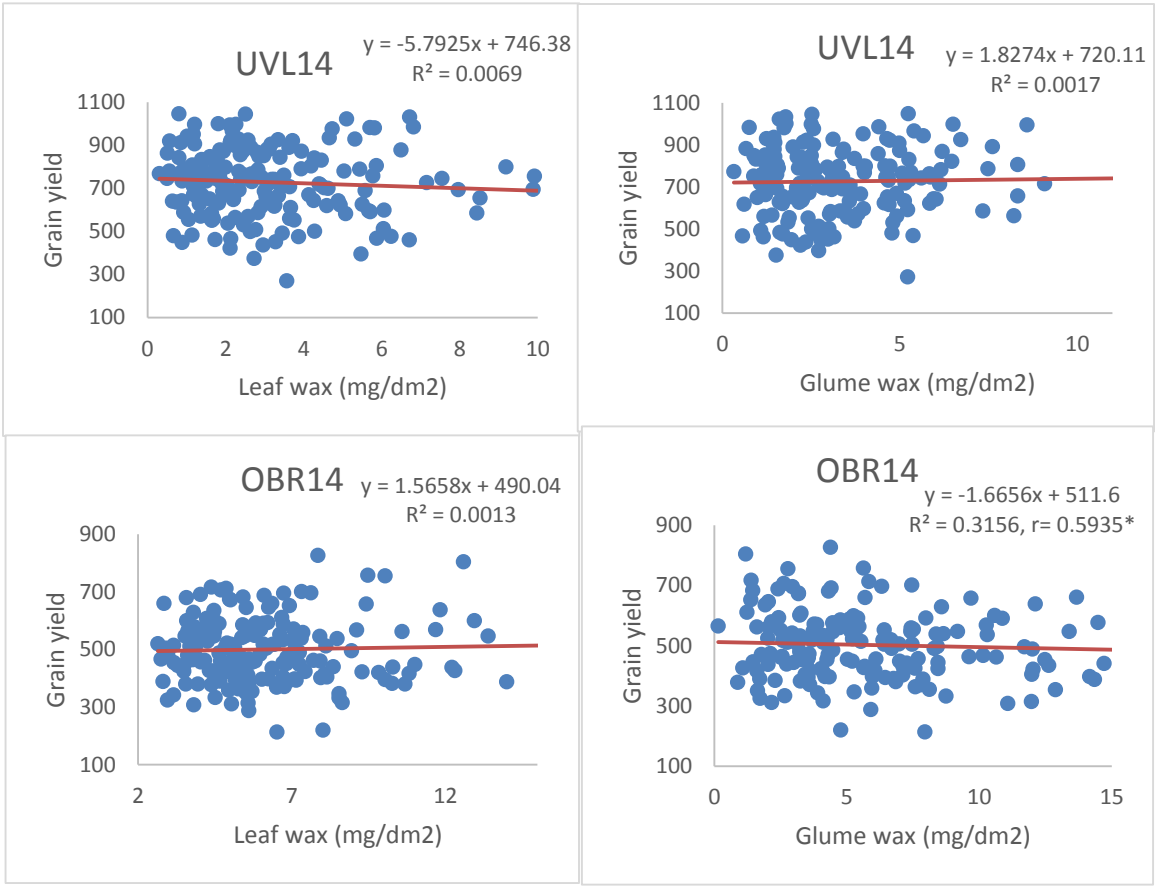


Figure 3.16. Linear relationship between grain yield vs. leaf wax load (left) and glume wax load (right) for UVL14 and OBR14, *significant at $p \leq 0.05$.

3.4. Discussion

3.4.1. Leaf and glume epicuticular wax load and temperature correlations

The physical and chemical makeup of epicuticular wax in plants is effected by both genetic and environmental factors (Whitecross et al 1972). Some environmental conditions that effect wax content and composition are relatively high-humidity conditions, such as in tissue culture, suppress wax production (Sutter and Langhans 1979, 1982), and the photoperiod affects the chain length of wax components (von Wettstein-Knowles et al. 1980).Waxes differ widely among plant species and among the organs and tissues of a single plant,

attesting to the genetic diversity and developmental influences (von Wettstein-Knowles 1995; Lemieux 1996; Post-Beittenmiller 1996). Different organs on the plant, or different parts of the plant, may have different proportions of wax component (Tulloch 1973). Analysis of data on waxes from different parts of the plant can contribute to assessment of physiological traits (Baum et al 1975).

In this study, a comparison was made between the epicuticular wax load on wheat glume and flag leaf of 180 RILs and their parents under high temperature environment. The effect of temperature on wax load for both plant parts was studied by planting the RILs and their parents in four different growing environments, College Station, and Uvalde, Texas in 2013 and Uvalde, Texas and Obregon, Mexico in 2014. Analysis of wax load in both leaf and glume showed a normally distributed data with significant differences observed across the 180 RILs and across the environments. The mean wax load for leaves and glumes was higher as the temperature of grain filling stage increased for the environment. CS13 reported the lowest mean temperatures for this study and thus those RILs for CS13 were considered under normal temperature. The stress increased for UVL14, UVL13, and OBR14 consecutively and those RILs of OBR14 were considered under very high temperature stress. The mean for both leaf and glume wax followed the order of temperature where the lowest mean was for CS13 and the highest for OBR14. However, as the stress increased, the mean of glume wax became higher than that of leaf wax for all stressed environments. This suggests that while epicuticular wax load production is more active during high temperature stress (Shepherd et al 2006), the glume response to temperature stress is even more prominent than the leaf. Yet, no apparent consistency in the glume wax to leaf wax ratio between the lines was observed for the different stressed environments (Fig 3.4.). This

observed variation is most likely due to genetic makeup of the different RILs and the fact that wax load is effected by multiple environmental and genetic factors (Whitecross et al 1972). The anticipated results of crossing between Halberd X Len is in agreement with previously reported studies on flag leaf wax content in wheat due to genetic variability (Uddin and Marshall 1988; Clarke et al. 1993). In addition, different behavior was observed when fitting data of glume wax and leaf wax to a linear model. The model suggests that under no high temperature stress, like in CS13, or moderate high temperature stress, like UVL14, no significant relation exists in the amount of glume epicuticular wax load to leaf epicuticular wax load. However, a significant strong positive relationship for glume wax vs. leaf wax was observed at high temperature stress, like UVL13, with Pearson's correlation $r = 0.9195$ at $p \leq 0.001$ significance level and $R^2 = 0.8285$ (Fig 3.5.). Then, as the stress increased more, this relationship became weaker while still significant with $R^2 = 0.0304$ and $r=0.1744$ significant at $p \leq 0.05$. This correlation may be due to a previous reported correspondence between the presence of wax filaments on glumes and the occurrence of relatively high amounts of β-diketones among Triticeae genera (Simpson et al 1980). Also, Jenks *et al. (*1994) reported an increase in the local density of vesicles adjacent to the site of wax excretion when wax production in sorghum was induced by light. This could explain the coherent in production of wax load on glume and on leaf under high temperature stress. Yet, when stress is even higher, the mechanisms of deposition and secretion probably differ between the glume and the leaf. Another important factor that may play a role in these relations is the chemical composition of glume wax as compared to leaf wax. The higher correlation between glume wax with leaf wax is likely due to difference in structure and composition of epicuticular waxes for the two parts (Kong et al. 2015). Studies by Riederer

& Schneider (1990) showed that in addition to affecting wax quantity, temperature influence composition of wax.

The study showed that changes in daytime and nighttime temperatures may differentially effect wax composition. Higher daytime temperature during leaf development reduced the quantities per unit area of alkanes, primary alcohols, fatty acids and alkyl esters, whereas, except for the esters, the amounts of these components increased with higher nighttime temperatures. Therefore, the induced heat stress will most likely induce a change in the chemical makeup of wax on the different plant parts making a study of its make up as important as a study of its quantity.

3.4.2. Grain yield and wax correlation

Grain yield is one of the most important, yet complex, trait in crops. It is a combination of interaction between environment and developmental processes during growth stages that occur throughout the life cycle of crop (Quarrie et al. 2006). Grain yield is directly and multiply determined by yield component traits (such as SKW and MSHW). In this study, yield and yield components of RILs and their parents planted in CS13 were compared to those planted in UVL13, UVL14 and, OBR14 to examine the effect of temperature on yield and yield components with relation to epicuticular wax on both leaves and glumes. The data for all growing environment for all measured yield and yield components had significant variation between the lines with normally distributed data. However, analyzed yield components did not vary significantly across the locations. When comparing SKW of RILs in CS13, being the least temperature stressed location, to those of UVL13, being the high temperature stressed location, CS13 mean SKW was higher than that of UVL13 by only 0.002g. A similar observation was made when comparing MSHW of CS13 with that of

UVL13 where the two means differed by only 1.78g. This was expected because in many temperate cereal crops, both grain weight and grain number appear to be impacted by heat stress, with a decline in grain number directly proportional with increasing temperatures during flowering and grain filling stages (Porter and Semenov 2005; Mahmood et al. 2010).

A linear regression of the SKW data vs. epicuticular wax load for both leaf and glume for CS13, UVL13, and UVL14 gave significant positive correlation for glume wax at UVL13 with $R^2 = 0.0286$ and $r = 0.1692$ at $p \leq 0.05$. The correlation was also stronger at UVL13 vs. leaf wax load yet not statistically significant. The positive, yet nonsignificant correlation between SKW and wax load across the locations along with the significant correlation at UVL13, suggests that wax load and SKW are somewhat related. It seems that at high temperature stress, i.e. UVL13, the amount of wax and SKW increased proportionally to each other. Again, this was repeated when plotting a linear regress of MSHW vs. epicuticular wax load of leaf and of glume for the three locations. This time, it was the leaf wax at UVL13 that showed a significant positive relationship. This implies that epicuticular wax, on glume or leaf, may have participated in the increased tolerance for high temperature stress of wheat. The participation of epicuticular wax in the rise of SKW and MSHW is most likely via mechanical mechanisms. For example, it was previously reported that wheat plants with higher leaf epicuticular wax content had lower canopy temperatures under heat stress conditions (Mondal 2011). Spiertz et al. (2006) reported that high growth temperatures reduced the grain dry mass because of limited supply of assimilates. High temperature stress 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). Thus, a lower canopy temperature will prevent

or minimize the reduction high molecular starch and as a result will prevent decrease in SKW or MSHW accordingly.

Grain yield was reduced significantly when comparing grain yield of UVL14, with moderate high temperature stress, to that of OBR14, with very high temperature stress. The same RIL gave lower yield on average for OBR14 as compared to that of UVL14 as shown in Figure 15. This observation was expected since reduction of grain yield has been associated with high temperature stress in cereals (Viswanathan and Khanna-Chopra 2001). Grain yield related negatively and significantly with glume wax content only for OBR14 as compared with non-significant positive correlation with the leaf wax content of OBR14 and UVL14 and glume wax content of UVL14. The negative significant correlation between glume wax content and yield suggests that reduction in yield may be caused by reduction in single head number rather than reduction in single kernel weight. This is reinforced by the above observation of the positive significant correlation between SKW in UVL13 and wax load. It is suggested here that epicuticular wax, on glume specifically, is an important factor in the tolerance mechanisms for high temperature stress at the grain level. An increase in wax load was observed when RILs were exposed to high and very high temperature stress in the field during grain filling stage yet no significant variation in SKW was observed when compared to lower stress environments, namely CS13. Thus, the reduction in yield was most likely caused by grain count rather than grain weight. Barnabás et al. (2008) had reported that high temperature stress induces changes in respiration and photosynthesis and thus leads to a shortened life cycle and diminished plant productivity which in return reduces yield. However, because reduction in yield was still apparent and significant at very high

temperature stress, the reduction of yield cause by seed abortion overcame the increased yield through maintenance of grain weight.

CHAPTER IV

MAPPING THE GENETIC LOCI REGULATING HIGH TEMPERATURE TOLERANCE FOR LEAF AND GLUME EPICUTICULAR WAX IN WHEAT (TRITICUM AESTIVUM L.)

4.1. Introduction

Model simulation experiments on global climates projects an average increase of ambient temperatures of 0.2°C per decade for the 2000 to 2100 period. This translates into an increase of about 1.7 and 5.8°C of overall global temperature by the end of this century (IPPC, 2007). Such prediction emphasizes the importance of crop varieties that have high heat tolerance. Wheat is one of the very important crops because it is a staple food for the world population. Heat and drought are major factors limiting wheat yields worldwide, especially in regions where 60% of global land area is classified as arid or semiarid. Controlling heat and drought tolerance in wheat is one way to improve breeding efficiency.

Generally speaking, most rain-fed farmers are limited in resources, own small land holdings, and have minimal capacity to adopt high input technologies. Thus, heat and drought tolerant wheat varieties are appropriate solution because they are farmer friendly and are based on seed technology that is easy to disseminate. Although steady progress has been made with up to date breeding work (e.g. Trethowan et al., 2002; Ammar et al., 2008), overall performance of cereals still shows considerable grain yield loss to high temperatures (Wardlaw et al., 1989; Reynolds et al., 1994).

Increase in temperature induces heat stress in wheat particularly during the reproductive and grain-filling stages (Wollenweber et al. 2003). The ideal temperature for wheat anthesis and grain filling is between 12 to 22◦C. Temperatures above this reduce grain

yield significantly (McDonald et al., 1983; Macas et al. 1999, 2000; Mullarkey and Jones 2000; Tewolde et al. 2006). Heat stress during anthesis can cause increased embryo abortion, pollen sterility, tissue dehydration, lower CO2 assimilation and increased photorespiration (Wardlaw and Wrigley, 1994). It was found that many current Hard Red Winter Wheat (HRWW) cultivars grown in the Southern Great Plains are heat susceptible in terms of sterility, abortion and an early transition to the dry seed stage (Hays et al, 2007a, and b).

In order for wheat to maintain growth and productivity, it must adapt to heat stress conditions. Some of these mechanisms involve the alteration of various photosynthetic attributes and physiological traits under heat stress exposure. Also, changes after perception of high heat signals occur at the molecular level altering the expression of genes and accumulation of transcripts, thereby leading to the synthesis of stress-related protein as a stress tolerance strategy (Iba 2002). As an example of tolerance adaptation, germplasm from Australia, CIMMYT, and ICARDA were found to exhibit heat and drought tolerance while in the reproductive stage. These lines maintained photosynthesis and yield through production of high seed set, grain weight, and an extended grain filling under heat stress conditions.

The first line of defense against high temperature environment in wheat is the epicuticular wax that covers the plants cuticle in various areas of the plant. The presence of this waxy layer on the leaf and glume can reduce heat stress by epidermal transpiration and excess light energy. Studies show that surface reflectance was reduced when the waxy layer from the leaf was removed with chloroform was for the abaxial and adaxial surface (Uddin, M. Nizam et.al 1988). This indicates that epicuticular wax is a major factor in leaf rolling

mechanism and abaxial reflectance. As an adaxial and abaxial reflective surface to excess energy, epicuticular wax reduces transpirational cooling needs and stomatal conductance. The scattering of heat during high temperatures by the epicuticular layer help maintain a temperate cellular environment, minimizing water loss and optimizing metabolic function. Consequently, epicuticular wax can hinder launching of drought indicators that reduce photosynthesis and promote seed abortion. Therefore, it is important to preform studies that focus on epicuticular wax to optimize structural content and chemical composition in crops such as wheat.

Many important traits for abiotic stress tolerance like yield, leaf wax, and anthesis time, are controlled by many genes known as quantitative traits. High density genetic maps (linkage maps) constructed with molecular markers are useful in facilitating the detection and estimation of the effect of QTL controlling those traits, and as tools for. One of the major challenges in wheat breeding is to find a linkage between genotype and phenotype in the context of the biotic or abiotec stresses. The polyploid nature of the wheat genome makes molecular analysis more difficult (Barnabas´ et al. 2008). Thus, finding linkage while under high temperature stress by using the analysis of complex trait variation aims toward identifying alleles that control variation for a high temperature stress tolerance phenotypes. Many studies have shown that high temperature stress tolerance, physiological traits that responded to high temperature stress yield and yield components are inherited quantitatively (Maestri et al. 2002). Determining the physiological traits associated with high temperature stress tolerance and finding QTL associated with these traits might be a crucial result for high temperature tolerance in wheat breeding.

Recently, a number of QTLs have been identified in wheat for high temperature stress tolerance during the reproductive stage. Such QTL were used by Ottaviano et al. (1991) to understand and explain heat stress tolerance in cereals. Also QTL on chromosome 4A for canopy temperature under heat has been identified (Pinto et al. 2010). Yield stability heat tolerance QTL's were found to overlap with QTL for epicuticular wax (Mondal et al. 2011). Two major grain yield QTL's in bread wheat where detected in heat, drought and high yield potential environments (Bennett et al. 2012). Detection of some QTL are associated with constitutive production of leaf cuticular wax and may contribute to lower leaf temperatures under heat stress, on three bread wheat chromosomes which is 1B, 3D and 5A (Mondal et al. 2015).

The goal of the present study was to define QTL regulating high temperature tolerance for leaf and glume epicuticular wax in wheat (Triticum aestivum L.), and identify stable loci associated with yield and/or physiological traits such as CTD that will contribute to improve heat tolerance under field conditions.

4.2. Material and methods

4.2.1 Plant material

In this study, a set of 180 recombined inbred lines (RILs) of wheat were used. These RILs were derived from cross of the heat tolerant spring wheat lines 'Halberd' with Len. Halberd is an Australian spring wheat (*Triticum aestivum* L) as a donor cultivar with the pedigree Scimitar/Kenya/C6042/ Bobin/2/Insignia49 (Paull et al. 1998). Halberd is a heat and drought tolerant cultivar, also has ability to maintain carbohydrate accumulation during moisture stress (Ji et al. 2010). Len is hard red spring wheat as a recurrent cultivar developed in North

Dakota with the pedigree ND499/3/Justin/RL4205/W1261. Len is a semi-dwarf that is a drought and heat susceptible however, it is known for its good agronomic characteristics (Hossain et al. 2012). The two parents were chosen due to similarities in flowering period and maturity. The 180 RILs were developed by preceding the F1 progeny through single seed descent in head rows to the F5 generation. Seeds from the F5 generation were bulked to develop 180 F5:6 RILs. The F6 lines were advanced in the field and were evaluated during 2010 as an F5:7 generation. During 2011 and 2012, F8 and F9 generations were used respectively, to conduct experiments (S. Mohammed *et al* 2014).

4.2.2. Growing environment

The RILs and the two parents were grown in the field during the winter seasons. Yield trials were conducted at Uvalde Agrilife research station during 2013 and 2014 seasons, College Station Agrilife research station, and International Maize and Wheat Improvement Center (CIMMYT) Ciudad de Obregon, Mexico during 2014 season. All growing parameters were the same with variability being only in growing location.

Weather conditions are mostly sunny and dry during the winter cropping cycle. Table 1. list daily temperature for the three locations during grain filling stage. Nitrogen and phosphorus were applied to the plots at a rate of 150 kg ha−1 and 22 kg ha−1, respectively. Field plots consisted of two raised (28 cm apart) each 3m long and 1m wide and seeded at 50g per plot. An alpha lattice design with two repetitions was used for all experiments. The planting dates were in February for Obregon location and plants reached booting and heading during April–May and were harvested in May. For College station and Uvalde, planting dates were in January and plants reached booting and heading during April–May and were harvested in May.

Table 4.1. Daily high temperature during grain filling stage for all growing environments**.**

DAP	Daily high temperature (F˚)			
	OBR14	UVL13	UVL14	CS13
1	95	93	77	86
2	98	81	79	72
3	89	79	93	69
4	91	88	93	82
5	94	88	95	73
6	91	93	96	78
7	91	100	95	86
8	92	103	93	87
9	101	95	88	80
10	106	97	93	84
Mean	94.8	91.7	90.2	79.7

4.2.3 Wax sample collection

Samples were collected during early spring 10 DAP. Four glumes per RIL replicate were collected for wax analysis using tweezers to carefully remove the glume without touching the surface EW layer. Leaf discs for wax analysis were collected from flag leaves using a disc punch with a 1 cm diameter drum (Rabbit Tool USA, Rock Island IL USA). Four 1cm (diameter) leaf punches was collected from the primary inflorescence leaf per plant of four pates per plot and placed in Borosilicate Glass Scintillation Vials with Screw Caps. The sample vials were placed in the laminar flow to air dry and stored at room temperature.

4.2.4 Wax extraction

EW concentrations was determined using the colorimetric method of Ebercon et al. (1977). Glume and Leaf EW was extracted by submerging glumes and leaf discs in 1 ml HPLC grade chloroform for 30 s, the submersion time previously determined to completely remove the epicuticular wax from the leaf (Mayeux and Jordan 1984). The resulting mixture was transferred to a clean 1.8 ml glass GC vial (VWR Auto sampler Vial, Radnor, PA). The chloroform solvent was removed under a continuous flow of nitrogen gas by leaving the vial uncapped overnight in the laminar flow hood.

4.2.5. Wax quantification

The resulting extract was oxidized by adding 300µl acidified potassium dichromate and heated for 30 minutes in a water bath at 100 ^{0}C. After boiling, vials were allowed to cool for a 1 hour period and 700µl of deionized water was added to each vial, allowing color development for another hour. A spectrophotometer (PHERAstar plus, BMG LABTECH, Offenburg, Germany) was used to determine the optical density for each sample at 590 nm. Samples were loaded in 96 well, sanitized, clear flat bottom microplates (Greiner Bio-One, Monroe, NC, USA). A standard curve was developed from randomly selected wheat flag leaves from Halberd. Samples were placed in large glass vials and 20 ml HPLC grade chloroform was added to remove EW. The resulting chloroform-wax solution was proportioned based on the serial dilution technique. The standard curve was used to calculate wax levels based on leaf area (Mondal et al. 2014).

4.2.6. Statistical analysis

A log 10 base was performed on all data collected for a data normalization and fit purposes. Statistical analysis was carried out using the PROC MIXED model procedure. Data from all

traits were subjected to analysis of variance (ANOVA) for augmented design using the Mixed procedure of the (SAS v9.4) (Institute 2014). The genetic variance of the yield components and physiological traits was calculated by considering the treatments as fixed and genotypes, years, and replications as random effects. Simple contrast analysis was performed on QTL associated with parental alleles to determine phenotypic means of different traits.

4.2.7. Molecular analysis

DNA extraction was performed on the 180 RIL population including the parents using the DArT method (Doyle 1990; Jaccoud et al. 2001). Extraction buffer stock (0.35M sorbitol, 0.1 M Tris HCl, 5mM EDTA), lysis buffer stock (0.2M Tris HCl, 0.05 M EDTA, 2 M NaCl, and 2% CTAB) and sarcosyl stock 5% (w/v) solutions were prepared accordingly. In addition, a fresh solution of 0.5% w/v sodium disulfite, 2% w/v PVP-40 (Polyvinylpyrrolidone) (sigma chemicals) was added to the extraction, lysis, and sarcosyl buffers. Fresh leaf tissue of 2 week old RIL seedlings were harvested and placed in 2 ml eppendorf tubes. Then 1ml of the freshly prepared extraction buffer solution at 65ºC was added and the tissue was disrupted using a Fastprep -24 homogenizer at 4.0 Movement/s for a 2 min period. The resulting mixtures were incubated in a water bath at 65ºC for 1 hr. After cooling, 1 ml of chloroform:isoamyl alcohol (24:1) mixture was added to the samples and then centrifuged at 10,000 rpm for 20 min. The supernatant of each tube was transferred into new 2 ml eppendorf tubes, and then an equal volume of ice cold isopropanol was added. Tubes were then centrifuged at 10000 rpm for 30 min to precipitate the DNA. The supernatant was discarded, and the precipitate pellet was washed with 1.5 ml 70% ethyl

alcohol. The resulted nucleic acid pellet was air dried and then dissolved in 200 µl of 1 X TE (10mM TrisHCl pH 8.0, 1 mM EDTA pH 8.0). (Mohamed.S. 2013)

DNA samples have been taken from the 180 RIL population and send to the USDA-ARS, Fargo for genotyping using silica bead chips containing 90K SNPs (Single nucleotide polymorphism) array through Illumina Infinium Golden Gate assay. The sequencing proses done by using next generation sequencing (Akhunov et al. 2009; Cavanagh et al. 2013). The SNPs clustering and annotations was analyzed using GenomeStudio v2011.1 software. Each SNP was annotated based on the clustering of individual alleles across the population. After scoring and annotating of 90K SNPs, SNPs that showed monomorphic clustering, SNPs showing more than 20% missing points, SNPs with vague calling, and SNPs that had a minor allele frequency < 10% were discarded. The resultant data set of 2,700 polymorphic SNPs was exported from GenomeStudio. Linkage map was created by using JoinMap software version 4.0 (Van Ooijen 2006) using recombinant events and the different reference population maps, such as a map from 9K SNPs (Gregersen et al. 2005), Avalon X Cadenza (Nelson et al. 1995), Savannah X Rialto (Snape et al. 2007), and Synthetic X Opeta (Allen et al. 2011). Finally, 22 linkage groups were identified at a significance level of 0.05 and 10,000 permutations across the wheat genome. These linkage groups were mapped with phenotypic data across five environments to identify possible QTL using MapQTL v6 (Van Ooijen 2004). The traits with significant segregation/genetic variations or low genetic by environment interactions or normally distributed populations were utilized for QTL mapping. The Kosambi function was used to calculate the recombinant event distances with a critical LOD score value of 2.5. The mapping method MQM (multiple QTL mapping) was used, where markers of non-linkage groups were used as cofactors, which reduces noise on the

genetic background (Jansen and Stam 1994). The QTL identified in four different individual environment were considered to be 'stable'. Co-localized QTL with major effects identified across the wheat genome for yield, and different heat stress environment traits were represented graphically using the software map chart (Voorrips, R.E. 2002).

4.3. Results

Among the four growing environments, Obregon had the highest recorded temperature, 41.1°C, and the highest mean temperature, 34.4°C, during the first ten days of grain filling stage. Following in recorded temperature was Uvalde 2013 with high temperature of 39.4°C and mean temperature of 34.4°C. While Uvalde 2014 had a mean temperature close to that of Uvalde 2013, 34.4°C, the highest recorded was only 35.5°C as compared to that of Uvalde 2013 of 39.4°C. Collage Station had the coolest growing environment with temperatures ranging between 20.5 and 30°C only and mean of 34.4°F (Table 4.1.).

Phenotypic analysis of wax data collected for the 180 RILs and its parental lines for leaf and glume showed normally distributed data with differences observed between lines being significant at $p \leq 0.001$. A maximum of 4.89 and 5.036 mg/dm2 for leaf and glume epicuticular wax load respectively were observed in CS13 which are the lowest readings of wax load maximums as compared to the other locations (Table 4.2.). Contrary to what is expected, wax load mean was higher in glume as compared to that of leaf for all locations except for CS13 (Fig 4.1.). ANOVA test for leaf epicuticular wax ranks the mean of the locations from highest to lowest as OBR14, UVL13, UVL14, and CS13, with the mean of OBR14 being 5.37 and that of CS13 being 2.508 mg/dm2 respectively (Fig 4.2.). This order was repeated also for glume epicuticular wax load were OBR14 had a mean of 5.97 mg/dm2

and CS13 mean of 2.38 mg/dm2 (Fig 4.3.). However, the same line did not produce matching leaf and glume wax content throughout the lines and the locations as shown in (Fig 4.4.) A plot of glume wax load vs. leaf wax load showed no significant relationship when tested for CS13 and UVL14. However, there was a very strong correlation between the two wax loads for UVL13 with R2 = 0.8285 and r=0.9195 significant at $p \leq 0.001$. A significant correlation also was observed for the two wax loads for OBR14 with $R^2 = 0.0304$ and r=0.1744 significant at $p \leq 0.05$ (Fig 4.5.).

Table 4.2. Summary of statistics of leaf and glume epicuticular wax for all growing location

Location	Leaf wax load (mg/dm^2)				Glume wax load (mg/dm^2)			
	Max	Min	Mean	STD	Max	Min	Mean	STD
CS13	4.890	1.353	2.508	0.696	5.036	0.918	2.386	0.873
UVL13	6.649	1.527	3.620	1.096	12.096	1.476	5.341	2.412
UVL14	10.7	0.303	3.107	2.12	14.29	0.325	3.644	2.55
OBR14	12.944	2.64	5.37	2.22	14.71	0.139	5.97	3.35

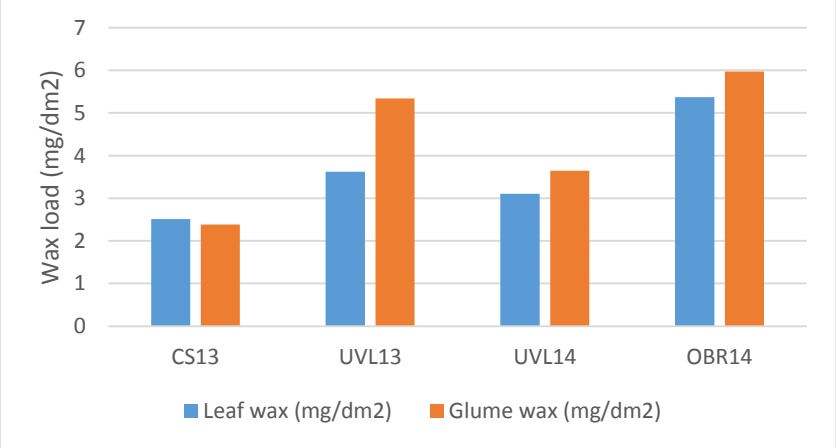


Figure 4.1. Leaf and glume mean wax load for all growing locations.

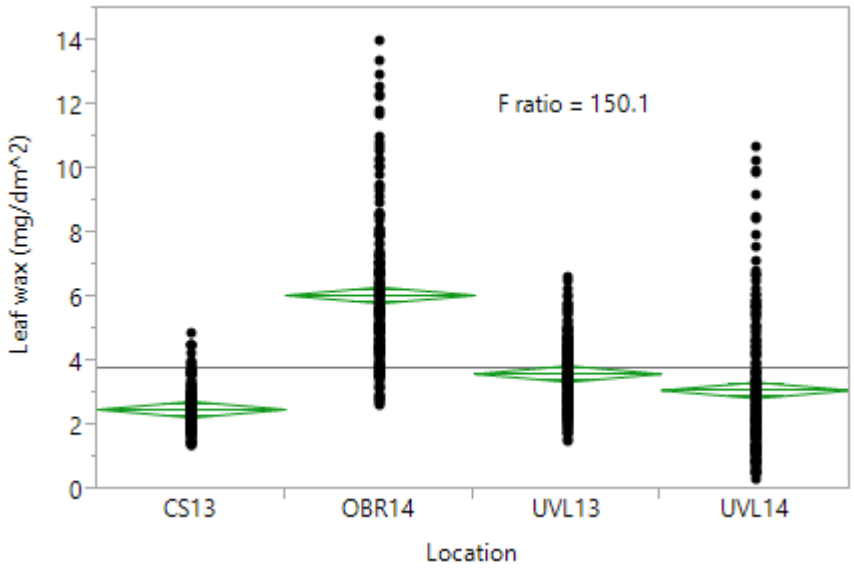


Figure 4.2. One-way analysis of leaf epicuticular wax (mg/dm2) by growing location, significant at p≤ 0.001.

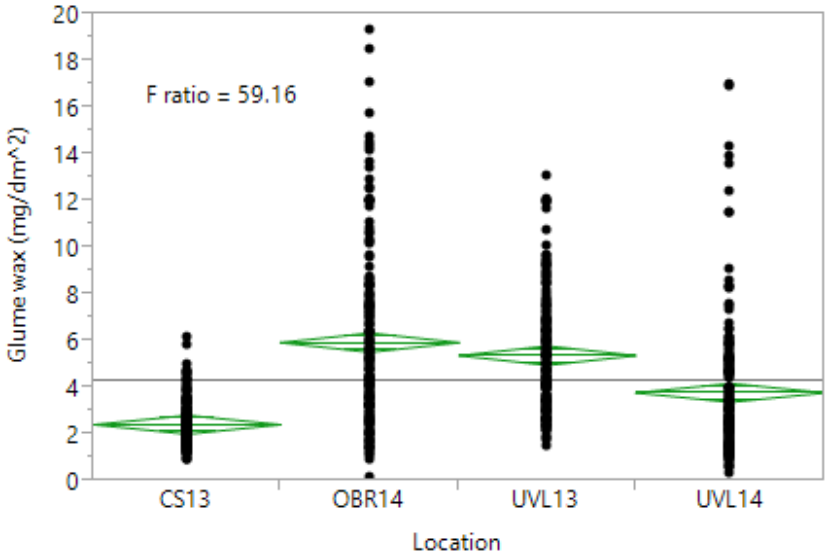


Figure 4.3. One-way analysis of glume epicuticular wax (mg/dm2) by growing location, significant at p≤ 0.001.

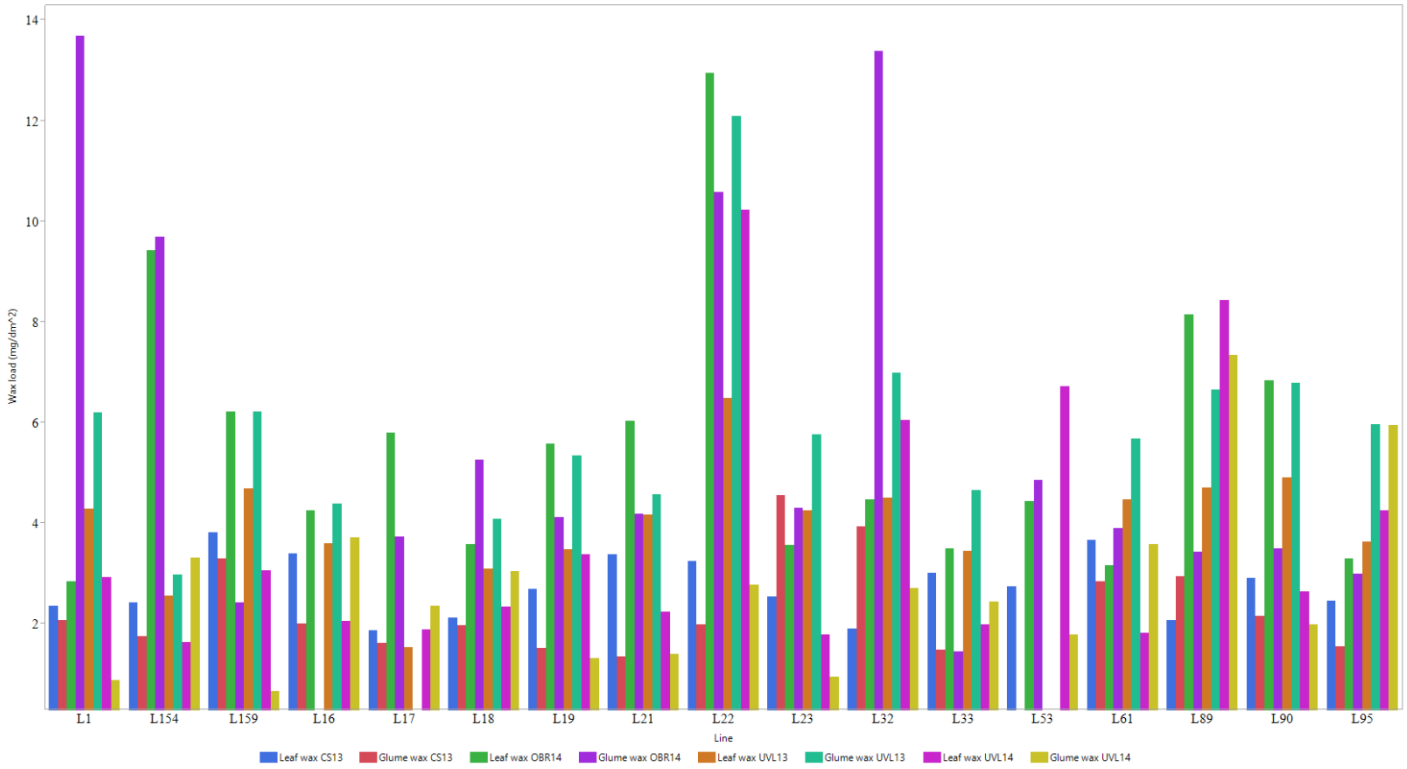


Figure 4.4. Leaf and glume wax load for sample wheat lines for all growing locations.

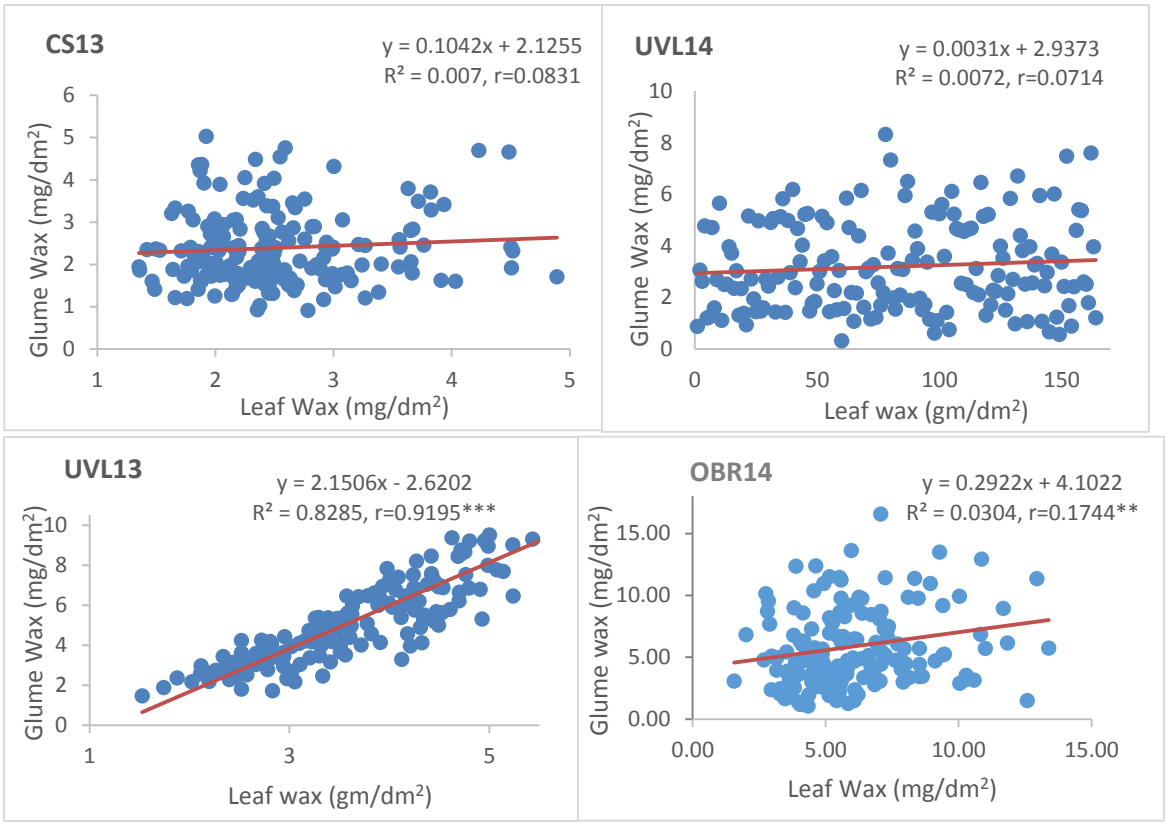


Figure 4.5. Linear correlation between glume wax and leaf wax for all growing locations, **significant at p≤ 0.05, ***significant at p≤ 0.001 with Pearson's correlation

Table 4.3. QTLs associated with Leaf and Glume wax load in a Halberd X Len Recombinant Inbred Line

QTL name	Position	Chr	Marker(s)	Trait	LOD	R^2	Additive
QLWax.tam-5B	104.584	5B	*BS00003243*	UVL13_LF	2.66	0.0170	-0.0356
QGWax.tam-5B	102.098	5B	*RAC875_c27743_249*	UVL13_GL	2.6	0.0171	-0.0348

Table 4.4. QTL detected with insignificant LOD scores in a Halberd X Len Recombinant Inbred Line

Treat	Group	Chr	Marker	Position	Additive
Yield_UVL_13*	13	7A	*wsnp_Ex_c106_217340*	54.879	-
SKW_CS_13**	1	2B	*BS00009460*	100.936	0.0194806
SKW_CS_13	3	3A	*BS00059618*	17.985	0.00716533
GY_OBR_14***	1	2B	*wsnp_Ex_rep_c101349_86725007*	351.674	0.00721278
GY_OBR_14	2	1A	*BS00065750_51*	74.945	-
GY_OBR_14	5	7A	*wsnp_Ra_c8394_14242358*	24.404	-
GY_OBR_14	14	6A	*Excalibur_c37240_609*	121.51	-0.0132277
TI_OBR_14****	3	3A	*wsnp_Ex_c35457_43602830*	115.52	-

*50 spike yield, **SKW single kernel weight, *** GY grain yield, ****TI thermal index

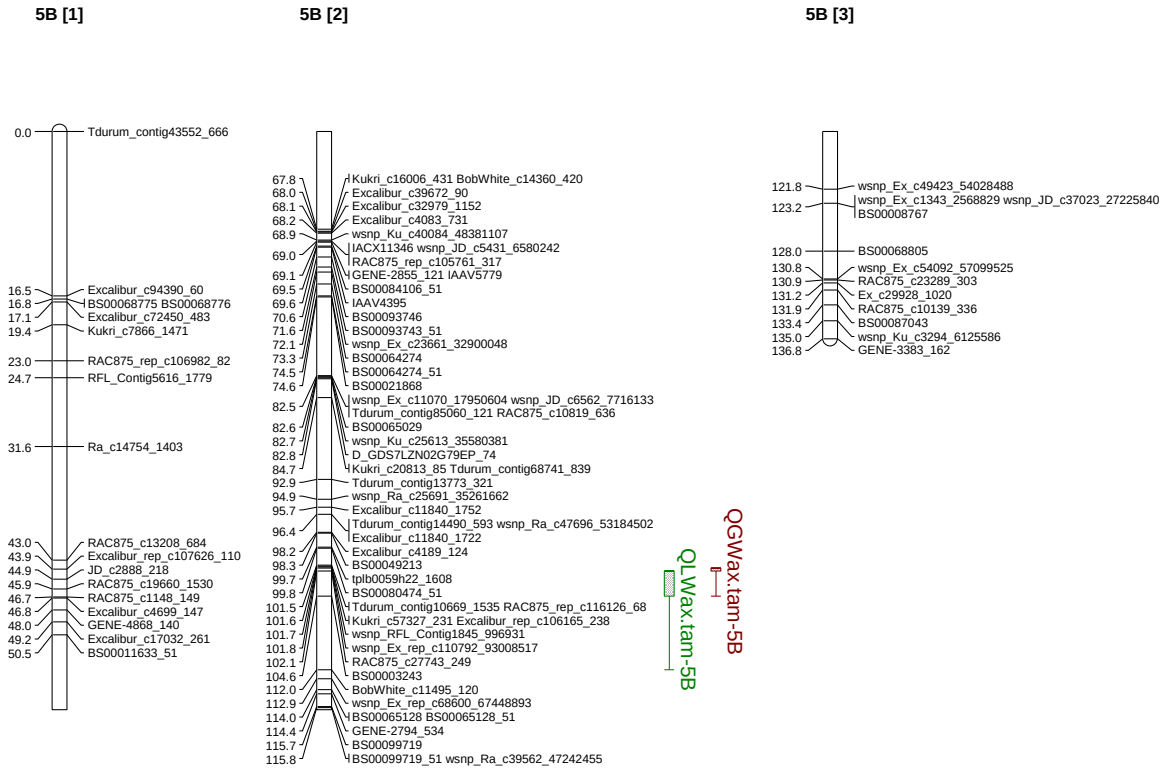


Figure 4.6. QTL for glume and leaf epicuticular wax in the 180 RIL population derived from Len and Halberd cultivars for UVL13. Identified co-localized QTLs were traced across different linkage groups of wheat genome with > 2.5 LOD scores.

Two significant QTL were detected on the same chromosome, 5B, at a distant of less than 20cM, one for leaf epicuticular wax and the other for glume epicuticular wax (Table 3.4.). Both QTLs favorable allele was contributed by 'Len' and were identified only in one individual environment of UVL13. Leaf epicuticular wax, QTL QLWax.tam-5B, was located on position 104.584 of chromosome 5B and explained 6.8% of the variation with 2.66 LOD score (Figure 4.6.). Glume epicuticular wax QTL, QGWax.tam-5B, was located on position 102.098 and explained 6.6% of the variation with 2.6 LOD score (Figure 4.6.). Eight more QTLs were detected with insignificant LOD scores, yet they showed ideal looking curve. Tow QTLs related to SKW of CS13 were detected on chromosomes 2B and

3A. Four QTLs related to grain yield in Obregon location were located on chromosomes 2B, 1A, 7A, and 6A. One QTL related to combined yield for UVL13 was detected on chromosome 7A, and another one related to thermal index of OBR14 was detected on chromosome 3A (Table 4.4.).

4.4. Discussion

Molecular mechanisms play a major rule in production of high temperature stress response elements during particular physiological stages of the plant cycle (Ai Li Qu et al 2013).wheat, like many other plants, have used epicuticular wax expression as a tolerant adaptive method to high temperature stress(Shepherd et al 2006). Waxes differ widely among plant species and among the organs and tissues of a single plant, attesting to the genetic diversity and developmental influences (von Wettstein-Knowles 1995; Lemieux 1996; Post-Beittenmiller 1996). Different organs on the plant, or different parts of the plant, may have different proportions of wax component (Tulloch 1973). Analysis of data on waxes from different parts of the plant can contribute to assessment of physiological traits (Baum et al 1975). This study focused on mapping QTLs localized for epicuticular wax on leaf and glume of 180 RILs and their parents under high temperature environment. The effect of temperature on wax load and QTL linkage to the wax for both plant parts was studied by planting the RILs and their parents in four different growing environments, College Station, Texas and Uvalde, Texas in 2013 and Uvalde, Texas and Obregon, Mexico in 2014. Phenotypic analysis of wax load in both leaf and glume showed that mean wax load for leaf and glume was higher as the temperature of grain filling stage increased for the environment. CS13 reported the lowest mean temperatures for this study and thus those

RILs for CS13 were considered under normal temperature. The stress increased for UVL14, UVL13, and OBR14 consecutively and those RILs of OBR14 were considered under very high temperature stress. The mean for both leaf and glume wax followed the order of temperature where the lowest mean was for CS13 and the highest for OBR14. However, as the stress increased, the mean of glume wax became higher than that of leaf wax for all stressed environments. This suggests that while epicuticular wax load production is more active during high temperature stress (Shepherd et al 2006), the glume response to temperature stress is even more prominent than the leaf. Yet, no apparent consistency in the glume wax to leaf wax ratio between the lines was observed for the different stressed environments except in UVL13 where strong relation between glume wax load and leaf wax load was detected. UVL13 was also the only location where QTL signal was observed. Interesting enough, QTLs for both leaf and glume wax where in close proximity to each other at the UVL13 location. They were both expressed on 5B chromosome and, explained about 7% of the variation, and had a LOD score of 2.6. The detection of the QTL on chromosome 5B is in agreement with a study where QTL for heat tolerance under hot and dry conditions were detected on chromosomes 2B and 5B in a spring wheat population (Butler JM et al 2002). The co-localization of leaf and glume QTLs suggests that they not controlled by the same gene. In this case, it was the Len cultivar that contributed the two QTLs of leaf wax on 5B, this QTL was previously detected by Mohamed (2013) in the same mapping population.

While analyzing QTLs of all co-located data, few apparent peaks with low LOD scores were detected. These peaks were relevant because they coincide with findings in previous study (Mohamed S. 2013). The observations of low LOD scores across the four different environments and phenotypic variations could be a result of high genotype by

environment interactions, suggesting that traits for environmental adaptation or minimum effect QTLs, will be difficult to select for (Romagosa and Fox 1993).

CHAPTER V

CONCLUSIONS

The anticipated rise in global temperature in the coming years along with increased demand of wheat production place heavy emphasis on the importance of wheat improvement programs. The ability to produce economically significant yield for high temperature environments relays on several plant physiological parameters and mechanisms that contribute to heat tolerance in the field. In many cases, a heat-tolerant variety is characterized by higher photosynthetic rates, increased membrane thermostability and heat avoidance (Nagarajan et al., 2010; Scafaro et al., 2010). In this study, a comparison was made between epicuticular wax on leaf as compared to that on glume under high temperature stress. Epicuticular wax content and position proved to be useful indicator for different phenotyping measurements. Although it is not being suggested that glume wax content should be mainly used for physiological studies in breeding program, it can be an indirect criteria and a powerful added tool for the program at hand. Epicuticular wax is presumed to come of a relatively simple genetic makeup and is easy to select for visually. The study employed this presumption by investigating high temperature-adaptive traits with significant genetic variation. The significant phenotypic correlations of physiological and agronomic traits give an indication to the existence of genetic linkage for high temperature -adaptive and potential yield attributes across different environments. Both leaf and glume epicuticular wax have significant association with cooler canopies, likely by reflecting high energy wavelengths and reducing excess heat energy on the plant productive part's surfaces.

Integrating genetic loci that regulate high levels of leaf and glume epicuticular wax and cooler canopies in the genetic background of abiotic susceptible elite lines can be achievable.

Further research is needed to quantify the cost benefit of different types of wax and its deposition strategies in variant environment. This is to say that even with their vital importance to plant survival and protection, and extensive studies of wax composition, very little is known about the initiation of epicuticular wax production and how production may be influenced by developmental and environmental factors.

REFERENCES

Ai L. Qu, Yan F. Ding, Qiong Jiang, Cheng Zhu. (2013) Molecular mechanisms of the plant heat stress response Biochemical and Biophysical Research Communications, 432: 203-207.

Akhunov E, Nicolet C, Dvorak J (2009) Single nucleotide polymorphism genotyping in polyploid wheat with the Illumina GoldenGate assay. Theoretical and Applied Genetics 119: 507-517.

Allen AM, Barker GL, Berry ST, Coghill JA, Gwilliam R, Kirby S, Robinson P, Brenchley RC, D'Amore R, McKenzie N (2011) Transcript-specific, single-nucleotide polymorphism discovery and linkage analysis in hexaploid bread wheat (Triticum aestivum L.). Plant Biotechnology Journal 9: 1086-1099.

Altenbach SB, DuPont F, Kothari K, Chand R, Johnson E, Lieu D (2003) Temperature, water and fertilizer influence the timing of key events during grain development in US spring wheat. J Cereal Sci. 37: 9-20.

Amani, I., Fischer, RA. and Reynolds, MP. (1996) Canopy temperature depression associati on with yield of irrigated spring wheat culti vars in a hot climate. Journal of Agronomy and Crop Science 176: 119–129.

Ammar, K., Lage, J., Villegas, D., Crossa, J., Hernandez, H. and Alvarado, G. (2008) Association among durum wheat international testing sites and trends in yield progress over the last twenty–two years. In Reynolds, M.P., Pietragalla, J. and Braun, H.–J. (Eds.) International Symposium on Wheat Yield Potential: Challenges to International Wheat Breeding. Mexico D.F., Mexico, CIMMYT: 108–119.

Anon. 1985. Ending hunger: an idea whose time has come. The hunger project. Special studies. Praeger, New York: 101.

Araus, J. L. 1996. Integrative physiological criteria associated with yield potential. Pages 15-167 in M. P. Reynolds, S. Rajaram, and A. McNab, eds. Increasing yield potential in wheat: Breaking the barriers. CIMMYT, Mexico, DF.

Assad, M.T., Paulsen, G.M. (2002) Genetic changes in resistance to environmental stresses by U.S. Great Plains wheat cultivars Euphytica 128(1): 87-96.

Babar MA, Reynolds MP, van Ginkel M, Klatt AR, Raun WR, Stone ML. (2006). Spectral reflectance indices as a potential indirect selection criteria for wheat yield under irrigation. Crop Science 2006;46: 578-588.

Baker EA. (1982). Chemistry and morphology of plant cuticular waxes. In: The plant cuticle (eds Cutler DF, Alvin KL, Price CE), Academic Press, New York : 139-166.

Barnabás, B. - Jäger, K. - Fehér, A. (2008) The effect of drought and heat stress on reproductive processes in cereals. Plant, Cell and Environment, 31: 11-38.

Barthlott W, Neinhuis C, Cutler D et al. (1998). Classification and terminology of plant epicuticular waxes. Botanical Journal of the Linnean Society 126: 237–260.

Baum, B. R., and V. E. Hadland (1975). The epicuticular waxes of glumes of Avena : a scanning electron microscopic study of the morphological patterns in all the species. Can. J . Bot. 53: 1712-1718.

Benmoussa, M., A. Achouch, and J. Zhu.(2005) QTL analysis for yield compoments in rice (Oryza sativa L.) under different environments. Journal Central European Agriculture. 6: 317 322.

Bennett MD, Smith JB, Heslop-Harrison JS (1982) Nuclear DNA amounts in angiosperms. Proc R Soc London Ser Biol 216:179– 199.

Bennett B, Johnson TE (1998) Development of congenics for hypnotic sensitivity to ethanol by QTL-marker-assisted counter selection. Mamm Genome 9: 969–974.

Bennett D, Reynolds M, Mullan D (2012) Detection of two major grain yield QTL in bread wheat (*Triticum aestivum* L.) under heat, drought and high yield potential environments. *Theoretical and Applied Genetics*. 125(7): 1473–1485.

Blum, A., Mayer, J. and Gozlan, G. (1982) Infrared thermal sensing of plant canopies as a screening technique for dehydrati on avoidance in wheat. Field Crops Research 5: 137–146.

Blum A (1988) 'Plant breeding for stress environments. (CRC Press:Boca Raton, FL).
Blum, A., B. Sinmena, J. Mayer, G. Golan & L. Shpiler, (1994) Stem reserve mobilization supports wheat grain filling under heat stress. Aust J Plant Physiol 21: 771–781.

Boyer, J.S. (1982). Plant productivity and environment. Science 218: 443-448.

Butler JM. Master's thesis. Fort Collins: Colorado State University; 2002. Quantitative trait locus evaluation for agronomic and morphological traits in a spring wheat population.

Cardon, L.R., and J.I. Bell (2001) Association study designs for complex diseases. Nat. Rev. Genet. 2: 91–99.

Carter, G. A. (1991) Primary and secondary effects of water concentration on the spectral reflectance of leaves. American Journal of Botany, 78: 916-924.

Cavanagh CR, Chao S, Wang S, Huang BE, Stephen S, Kiani S, Forrest K, Saintenac C, Brown-Guedira GL, Akhunova A (2013) Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. Proceedings of the National Academy of Sciences 110: 8057-8062.

CIMMYT. 1995. CIMMYTINARS Consultancy on MEl Bread Wheat Breeding. Wheat Special Report No. 38. Mexico, D.F.: CIMMYT.

Clark, S. G.; Snell, F. J.; Goss, L. C.; Avery, A. L.; Latta, R. A. (1993) Pasture grasses and legumes 93. Department of Agriculture, Victoria, research report 141.

Danson, F. M., Steven, M. D., Malthus, T. J., and Clark, J. A., (1992) High-spectral resolution data for determining leaf water concentration. International Journal of Remote Sensing, 13: 461-470.

Demmig-Adams B, Adams WW., III. (1992). Photoprotection and other responses of plants to high light stress. Annu Rev Plant Physiol Plant Mol Biol. 43: 599–626.

Dudal, R. (1976) Inventory of the major soils of the world with special reference to mineral stress hazards. In M.J. Wright (ed.) Plant adaptation to mineral stress problem soils Cornell Univ. Press, Ithaca, NY: 3-13.

Dvorak, J., Luo, M.-C., Yang, Z.-L. and Zhang, H.-B. (1998) The structure of the Aegilops tauschii genepool and the evolution of hexaploid wheat. Theoretical and Applied Genetics 97: 657–670.

Eberhart, S.A., and W.A. Russell. (1966) Stability parameters for comparing varieties. Crop Sci. 6: 36-40.

FAO. (1986) Production Yearbook vol. 40. Food and Agriculture Organization of the United Nations. Rome. Italy

Fischer, R.A. (1986) Physiological limitations to producing wheat in semitropical and tropical environments and possible selection criteria. Proc. Internal Symp. Wheat for tropical environments. CIMMYT/UNDP, Mexico: 209–230.

Fischer, R.A., D. Rees, K.D. Sayre, Z.M. Lu, A.G. Condon, and A. Larque Saavedra. (1998). Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. Crop Sci. 38: 1467–1475.

Fischer, RA, and Turner, NC. (1978) Plant Productivity in the Arid and Semiarid Zones. Annual Review of Plant Physiology Vol. 29: 277-317.

Fischer, R. A. (1980) Influence of water stress on crop yield in semiarid regions. In: Adaptation of Plants to Water and High Temperature Stress. Turner, N. C. and Kramer, P. J., Eds., Wiley, New York: 323–339.

Fisher, R. A. (1993) Irrigated spring wheat and timing and amount of nitrogen fertilizer. II. Physiology of grain yield response. Field Crops Research, 33: 57-80.

Fischer, R.A., D. Rees, K.D. Sayre, Z.M. Lu, G.A. Condon and A. Larque Saaverdra. (1998) Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. Crop Sci. 38: 1467-1475.

Fokar, M., H.T. Nguyen, and A. Blum. (1998) a. Heat tolerance in spring wheat. I. Estimating cellular thermotolerance and its heritability. Euphytica 104: 1–8.

Fokar, M., H.T. Nguyen and A. Blum (1998). Heat tolerance in spring wheat. II. Grain fillling. Euphytica 104: 9-15.

Gao, B.C. (1995), A normalized difference water index for remote sensing of vegetation liquid water from space, in SPIE's 1995 Symposium on OE / Aerospace Sensing and Dual Use Photonics, Vol. 2480, Orlando, FL

Gibson, L.R., and G.M. Paulsen. (1999) Yield components of wheat grown under high temperature stress during reproductive growth. Crop Sci. 39: 1841-1846.

Gregersen PL, Brinch-Pedersen H, Holm PB (2005) A microarray-based comparative analysis of gene expression profiles during grain development in transgenic and wild type wheat. Transgenic research 14: 887-905.

Hancock, James F. (2004) Plant Evolution and the Origin of Crop Species. CABI Publishing.

Hays, D.B., Do, J.H., Mason, R.E., Morgan, G. and Finlayson, S.A. (2007a) Heat stress induced ethylene production in developing wheat grains induces kernel abortion and increased maturation in a susceptible cultivar. Plant Science 172: 1113–1123.

Hays, D.B., E., M.R. and H., D.J. (2007b) Developments in plant breeding IN Buck, H.T., Nisi, J.E. and Salomón, N. (Eds.) Wheat production in stressed environments. Mar del Plata, Argentina, Springer.

Heredia A. (2003) Biophysical and biochemical characteristics of cutin, a plant barrier biopolymer. Biochim. Biophys. Acta 1620: 1–7.

Holland, J.B. (1998) EPISTACY: A SAS program for detecting two-locus epistatic interactions using genetic marker. Journal of Heredity 89: 374-375.

Holmberg, N. and L. Bülow. (1998). Improving stress tolerance by gene transfer. Trends in Plant Science 3 (2): 61-66.
.
Hossain A, Teixeira da Silva J, Lozovskaya M, Zvolinsky V, Mukhortov V (2012) High temperature combined with drought affect rainfed spring wheat and barley in south-eastern Russia: yield, relative performance and heat susceptibility index. Journal of Plant Breeding and Crop Science 4: 184-196.

Iba, K. (2002). Acclimation response to temperature stress in higher plants. Annu. Plant. Biol. 53: 225–245.

Ibrahim, A.M.H., and Quick J.S. (2001). Heritability of heat tolerance in winter and spring wheat. Crop Sci. 41: 1401-1405.

Ishag HM (2003) Genotypic differences in heat stress in wheat in the irrigated gezira scheme. In: Saunders DA, Hettel GP (eds) Wheat in heat stressed environments: irrigated, dry areas and rice wheat cropping systems. CIMMYT, Mexico: 170–174.

IPPC, (2007) the Landmark 2007 IPCC Report on Climate Change. Represents the formally agreed statement of the IPCC concerning key findings and uncertainties contained in the Working Group contributions to the Fourth Assessment Report.

Jenner, C.F., (1991b). Effects of exposure of wheat ears to high temperature on dry matter accumulation and carbohydrate metabolism in the grain of two cultivars. II. Carryovereffects. Aust. J. Plant Physiol. 18: 179-190.

Jenks MA, Rich PJ, Ashworth EN. (1994). Involvement of cork cells in the secretion of epicuticular wax filaments onSorghum bicolor (L.) Moench. International Journal of Plant Science 155: 506–518.

Jensen, L.B., Courtois, B., Shen, L., Li, Z., Olofsdotter, M. & Mauleon, R.P. (2001) Location genes controlling rice allelopathic effects against barnyardgrass in upland rice. Agron. J. 93: 21-26.

Jetter R, Riederer M. (1994). Epicuticular crystals of nonacosan–10–ol: In–vitro reconstitution and factors influencing crystal habits. Planta 195: 257–270.

Jetter R, Riederer M. (1995) In vitro reconstitution of epicuticular wax crystals: formation of tubular aggregates by long chain secondary alkendiols. Botanica Acta 108: 111–120.

Jetter R, Schäffer S. (2001). Chemical composition of the Prunus laurocerasus leaf surface. Dynamic changes of the epicuticular wax film during leaf development. Plant Physiology 126: 1725–1737.

Jetter R, Kunst L, Samuels L. (2006). Composition of plant cuticular waxes. See Ref. 111: 145–81.

Jones HG, Serraj R, Loveys BR, Xiong L, Wheaton A, Price AH (2009) Thermal infrared imaging of crop canopies for the remote diagnosis and quantification of plant responses to water stress in the field. Functional Plant Biology 36: 978–989.

Jordan WR, Shouse PJ, Blum A, Miller FR, Monk RL. (1984). Environmental physiology of sorghum. II. Epicuticular wax load and cuticular transpiration. Crop Science 24: 1168–1173.

Ji X, Shiran B, Wan J, Lewis DC, Jenkins CL, Condon AG, Richards RA, Dolferus R (2010) Importance of pre-anthesis anther sink strength for maintenance of grain number during reproductive stage water stress in wheat. Plant, Cell & Environment 33: 926-942.

Kaitao Lai, Paul J Berkman, Michal Tadeusz Lorenc, Christopher Duran, Lars Smits, Sahana Manoli, Jiri Stiller, David Edwards. (2012) Plant and Cell Physiology 53(2): e2

Kase, M., and Catsky, J. (1984). Maintenance and growth components of dark respiration rate in leaves of C3 and C4 plants as affected by leaf temperature. Biol. Plant. 26: 461–470.

Koch K, Ensikat HJ (2008) The hydrophobis coatings of plant surfaces: Epicuticular wax crystals and their morphologies, crystallinity and molecular self-assembly. Micron. 39: 759-772.

Kong L, Sun M, Xie Y, Wang F and Zhao Z (2015) Photochemical and antioxidative responses of the glume and flag leaf to seasonal senescence in wheat. Front. Plant Sci. 6: 358. doi: 10.3389/fpls.2015.00358.

Kimberly D. Cameron, Mark A. Teece and Lawrence B. Smart. (2005). Increased Accumulation of Cuticular Wax and Expression of Lipid Transfer Protein in Response to Periodic Drying Events in Leaves of Tree Tobacco. Plant Physiology January 2006 vol. 140 no. 1: 176-183.

Kirigwi, F.M., Van Ginkel, F.M., Brown-Guedira, G. Gill, B.S., Paulsen, G.M., Fritz, A.K. (2008). Markers associated with a QTL for grain yield in wheat under drought. Molecular Breeding. 20: 401-413.

Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller B. (2009). A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323: 1360-1363.

Kuo, C.G., H.M. Chen & H.C. Sun, (1992). Membrane thermostability and heat tolerance of vegetable leaves. In: C.G. Kuo (Ed), Adaptation of Food Crops to Temperature and Water Stress: 160–168.

Lemieux B (1996) Molecular genetics of epicuticular wax biosynthesis. Trends Plant Sci 1: 312–318.

Lubbers, E.L., Gill, K.S., Cox, T.S. and Gill, B.S. (1991) Variation of molecular markers among geographically diverse accessions of Triticum tauschii. Genome 34: 354–361.

Levitt (1980) responce of plants to Environmental stress. New York: Academic Press.

Macas, B., Gomes, C., and Dias, A. S. (1999). Efeito das temperaturas elevadas durante o enchimento do grao em trigo mole e rijo no Sul de Portugal. Melhoramento 36: 27–45.

Macas, B., Gomes, M. C., Dias, A. S., and Coutinho, J. (2000). The tolerance of durum wheat to high temperatures during grain filling. In: Durum Wheat Improvement in the Mediterranean Region: New Challenges: 257–261.

MapChart: Software for the graphical presentation of linkage maps and QTLs. The Journal of Heredity 93 (1): 77-78.

Maestri, E., N. Klueva, C. Perrotta, M. Gulli, H. T. Nguyen, and N. Marmiroli. (2002). Molecular genetics of heat tolerance and heat shock protein in cereals. Plant Molecular Biology 48: 667-681.

Mason RE, Mondal S, Beecher FW, Pacheco A, Jampala B, Ibrahim AM, Hays DB (2010) QTL associated with heat susceptibility index in wheat (Triticum aestivum L.) under short-term reproductive stage heat stress. Euphytica 174: 423-436.

McDonald, G. K., Sutton, B. G., and Ellsion, F. W. (1983). The effect of time of sowing on the grain yield of irrigated wheat in Namoi Valley, New South Wales. Aust. J. Agric. Res. 34: 224–229.

Mohammed. S (2013) The role of leaf epicuticular wax an improved adaptation to moisture deficit environments in wheat. PhD thesis. Texas A&M University, College Station.

Mahmood S., Wahid A., Javed F, Basra S. M. A. (2010) Heat stress effects on forage quality characteristics of maize (Zea mays) cultivars. Int. J. Agric. Biol. 12: 701–706.

Mohammadi, V., A.A. Zali, and M.R. Bihamta. (2008). Mapping QTLs for heat tolerance in wheat. J. Agric. Sci. Technol. 10: 261–267.

Mondal S (2011) Defining the molecular and physiological role of leaf cuticular waxes in reproductive stage heat tolerance in wheat. PhD thesis. Texas A&M University, College Station.

Mondal S, Mason RE, Huggins T, Hays DB (2014) QTL on wheat (Triticum aestivum L.) chromosomes 1B, 3D and 5A are associated with constitutive production of leaf cuticular wax and may contribute to lower leaf temperatures under heat stress. Euphytica:1-8.

Mullan, DJ. and Reynolds, MP. (2010) Quantifying genetic effects of ground cover on soil water evaporation using digital imaging. Functional Plant Biology 37: 703–712.

Mullarkey, M., and Jones, P. (2000). Isolation and analysis of thermotolerant mutants of wheat. J. Exp. Bot. 51: 139–146.

Milthorpe FL, Moorby J. (1979). ‘An introduction to crop physiology.’ 2nd edn. (Cambridge University Press: Cambridge)

Nagarajan S., Jagadish S., Prasad A., Thomar A., Anand A., Pal M., et al. (2010) Local climate affects growth, yield and grain quality of aromatic and non-aromatic rice in northwestern India. Agric. Ecosyst. Environ. 138: 274–281 10.1016/j.agee.2010.05.012.

Nawrath C. (2006). Unraveling the complex network of cuticular structure and function. Curr. Opin. Plant Biol. 9: 281–87.

Naseem, A., M. S. Iqbal, K. Mahmood and J. Akhtar. (2001). Comparative performance of wheat (Triticum aestivum L.) genotypes under salinity stress. I: growth and yield parameters. On Line Journal of Biological Sciences 1, (1): 33-35.

Nelson JC, Deynze AEV, Sorrells ME, Autrique E, Lu YH, Merlino M, Atkinson M, Leroy P (1995) Molecular mapping of wheat. Homoeologous group 2. Genome. Savannah X. 38: 516-524.

Oshino T, Abiko M, Saito R, Ichiishi E, Endo M, Kawagishi-Kobayashi M, Higashitani A. (2007). Premature progression of anther early developmental programs accompanied by comprehensive alterations in transcription during high-temperature injury in barley plants. Mol Genet Genomics. Jul; 278(1): 31-42.

Ottaviano, E., M.S. Gorla, E. Pe, and C. Frova. (1991). Molecular markers (RFLPs and HSPs) for the genetic dissection of thermotolerance in maize. Theor. Appl. Genet. 81: 713–719.

Peñuelas J, Filella I, Biel C, Serrano L, Save R. (1993) The reflectance at the 950–970 mm region as an indicator of plant water status. International Journal of Remote Sensing. 14: 1887–1905.

Peñuelas J, Gamon JA, Fredeen AL, Merino J, Field CB (1994) Reflectance indices associated with physiological changes in nitrogen- and water-limited sunflower leaves. Remote Sensing of Environment 48: 135–146.

Peñuelas, J., F. Baret, and I. Filella. (1995). Semi-empirical indices to assess carotenoids/chlorophyll a ratio from leaf spectral reflectance. Photosynthetica 31: 221–230.

Paull J, Chalmers K, Karakousis A, Kretschmer JM, Manning S, Langridge P (1998) Genetic diversity in Australian wheat varieties and breeding material based on RFLP data. Theor Appl Genet 96: 435-446.

Peñuelas J, Isla R, Filella I, Araus JL (1997) Visible and near-infrared reflectance assessment of salinity effects on barley. Crop Science 37: 198–202.

Pinto, RS., Reynolds, MP., Mathews, KL., McIntyre, CL., Olivares-Villegas, JJ. and Chapman, SC. (2010) Heat and drought adaptive QTL in a wheat population designed to minimize confounding agronomic effects. Theoretical and Applied Genetics 121: 1001–1021.

Porter J. R., Semenov M. A. (2005) Crop responses to climatic variation. Philos. Trans. R. Soc. B Biol. Sci. 360: 2021–2035.

Post-Beittenmiller D (1996) Biochemistry and molecular biology of wax production in plants. Annu Rev Plant Physiol Plant Mol Biol 47: 405–430.

Prasad B, Carver BF, Stone ML, Babar MA, Raun WR, Klatt AR. (2007). Genetic analysis of indirect selection for winter wheat grain yield using spectral reflectance indices. Crop Science 47: 1416-1425.

Prasad B, Babar MA, Carver BF, Raun WR, Klatt AR (2009) Association of biomass production and canopy spectral reflectance indices in winter wheat. Can J Plant Sci 89: 485–496.

Quarrie, S., S. Pekic Quarrie, R. Radosevic, D. Rancic, A. Kaminska et al., (2006). Dissecting a wheat QTL for yield present in a range of environments: from the QTL to candidate genes. J. Exp. Bot. 57: 2627–2637.

Reynolds, M.P., Balota, M., Delgado, M.I.B., Amani, I. and Fischer, R.A. (1994) Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. Australian Journal of Plant Physiology 21:717–730.

Reynolds, M.P., Nagarajan, S., Razzaque, M.A. & Ageeb, O.A.A., eds. (1997). Using canopy temperature depression to select for yield potential of wheat in heat-stressed environments. Wheat Special Report No. 42. Mexico, DF, CIMMYT.

Reynolds, M. P., Singh, R. P., Ibrahim, A., Ageeb, O. A. A., Larqué-Saavedra, A., & Quick, J. S. (1998): Evaluating physiological traits to complement empirical selection for wheat in warm environments. - Euphytica, 100 (1-3): 85-94.

Reynolds, M. P., Ortiz Monasterio J. I., McNab A. (2001). Heat tolerance. In M.P. Reynolds, I. Ortiz-Monasterio & A. McNab, eds. Application of physiology in wheat breeding. Mexico, DF, CIMMYT.

Reynolds MP, Dreccer F, Trethowan R. (2007) Drought adaptive traits derived from wheat wild relatives and landraces. Journal of Experimental Botany 2007;58: 177-186.

Reynolds, MP., Pask, AJD. and Mullan DM. (Eds.) (2012) Physiological Breeding I: Interdisciplinary Approaches to Improve Crop Adaptation. Mexico, D.F.: CIMMYT.

Rhee Y, Hlousek-Radojcic A, Ponsamuel J, Liu D, Post-Beittenmiller D. (1998) Epicuticular wax accumulation and fatty acid elongation activities are induced during leaf development of leeks. Plant Physiology 116: 901–911.

Riederer M, Schneider G. (1990). The effect of environment on the permeability and composition of citrus leaf cuticles.Planta 180: 154–165.

Richards, R.A., Rawson, H.M. and Johnson, D.A. (1986). Glaucousness in wheat: Its development and effect on water-use efficiency, gas exchange and photosynthetic tissue temperatures. Aust J Plant Physiol. 13: 465-473.

Richards, R.A. (1996). Increasing the yield potential of wheat: manipulating sources and sinks. In M.P. Reynolds, S. Rajaram & A. McNab, eds.Increasing yield potential in wheat: breaking the barriers, Mexico, DF, CIMMYT: 134-149.

Rialto Snape JW, Foulkes MJ, Simmonds J, Leverington M, Fish LJ, Wang Y, Ciavarrella M (2007) Dissecting gene× environmental effects on wheat yields via QTL and physiological analysis. Euphytica 154: 401-408.

Richards, R.A. (2006) Physiological traits used in the breeding of new cultivars for water-scarce environments. Agricultural Water Management80: 197–211.

Richardson, R.A., Wojciechowski, T., Franke, R., Schreiber, L., Kerstiens, G., Jarvis, M., Wieland, F. (2007). Cuticular permeance in relation to wax and cutin development along the growing barley (Hordeum vulgare) leaf. Planta. 225: 1471-1481.

Roberts M, Reader S, Dalgliesh C, Miller T, Foote T, Fish L, Snape J, Moore G (1999) Induction and characterisation of Ph1 wheat mutants. Genetics 153: 1909-1918.

Romagosa, I. & Fox, P.N. (1993). Genotype x environment interaction and adaptation. In M.D. Hayward, N.O. Bosemark & I. Romagosa, eds. Plant breeding: principles and prospects, London, Chapman & Hall : 373-390.

Röder, M.S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M.H. Leroy, P., Ganal, M.W. (1998). A microsatellite map of wheat. Genetics. 119: 2007-2023.

Satorre EH, Slafer GA (1999). Wheat: Ecology and Physiology of Yield Determination,. The Hawthorn Press ISBN 1-56022-874-1, Stroud, Gloucestershire, UK.

Sayre KD, Rajaram S, Fischer RA. (1997) Yield potential progress in short bread wheat in Northern Mexico. Crop Science 37: 36-42.

Sax, K. (1923). The association of size differences with seed coat pattern and pigmentation in Phaseolus vulgaris. Genetics 8: 552-560.

Sears, E. R., (1952) Homeologous chromosomes in Triticum aestivum. Genetics 37: 624.

Shepherd T, Griffiths DW (2006) The effects of stress on plant cuticular waxes. New Phytologist 171: 469–499.

Shi J, Li R, Qiu D, et al.(2009) Unraveling the Complex Trait of Crop Yield With Quantitative Trait Loci Mapping in Brassica napus. Genetics.182(3): 851-861. doi:10.1534/genetics.109.101642.

Simpson, D. and P. Von Wettstein-Knowless (1980) structure of epicuticular waxes on spikes and leaf sheaths of barley as revealed by a direct platinum replica technique. Carlsberg Res. Commun. 45: 465-481.

Sims, Daniel A., Gamon John A. (2003) Estimation of vegetation water content and photosynthetic tissue area from spectral reflectance: a comparison of indices based on liquid water and chlorophyll absorption features Remote Sensing of Environment 84: 526–537.

Slafer, G. A., and Satorre, E. H. (1999). Wheat: Ecology and physiology of Yield Determination. Haworth Press Technology and Industrial, ISBN 1560228741.

Spiertz, J.H.J., R.J. Hamer, H. Xu, C. Primo-Martin, C. Don and P.E.L. van der Putten. (2006). Heat stress in wheat (Triticum aestivum L.): Effects on grain growth and quality traits. European Journal of Agronomy 25: 89-95.

Suchismita Mondal, Richard Esten Mason, Trevis Huggins, Dirk B. Hays. (2015). QTL on wheat (Triticum aestivum L.) chromosomes 1B, 3D and 5A are associated with constitutive production of leaf cuticular wax and may contribute to lower leaf temperatures under heat stress. Euphytica 201, Issue 1: 123-130.

Sutter, E., Langhans, R. W. (1979) Epicuticular wax formation on carnation plantlets regenerated from shoot tip culture. J. Am. Sci. Hort. Sci. 104: 493–496.

Sutter, E., Langhans, R. W. (1982) Formation of epicuticular wax and its effect on water loss in cabbage plants regenerated from shoot-tip culture. Can. J. Bot. 60: 2896–2902.

Sutter, E., Langhans, R. W. (1979) Epicuticular wax formation on carnation plantlets regenerated from shoot tip culture. J. Am. Sci. Hort. Sci. 104: 493–496.

Stark RE, Tian S. (2006). The cutin biopolymer matrix. See Ref. 111: 126–41.

Tewolde, H., Fernandez, C. J., and Erickson, C. A. (2006). Wheat cultivars adapted to post-heading high temperature stress. J. Agron. Crop Sci. 192: 111–120.

Tester, R.F., W.R. Morrison, R.H. Ellis, J.R. Piggo, G.R. Batts, T.R. Wheeler(1995). Effects of elevated growth temperature and carbon dioxide levels on some physicochemical properties of wheat starch. Journal of Cereal Science 22: 63-71. doi:10.1016/s0733-5210(05)80008-6.

Trethowan, R.M., Ginkel, M.V. and Sanjaya, R. (2002) Progress in breeding wheat for yield and adaptation in global drought affected environments. Crop Science 42: 1441–1446.

Uddin, M.Nizam, Marshall, M.Nizam (1988) Variation in epicuticular wax content in wheat Euphytica, Vol.38(1): 3-9.

Tulloch A, Hoffman L (1971) Leaf wax of durum wheat. Phytochemistry 10: 871-876 Tullocha,. P. (1973) Composition of leaf surface waxes of Triticlrm species: variation with age and tissue. Phytochemistry, 12: 2225-2232.

Van Ooijen J (2004) MapQTL® 5. Software for the mapping of quantitative trait loci in experimental populations Kyazma BV, Wageningen.

Van Ooijen J (2006) JoinMap 4. Software for the calculation of genetic linkage maps in experimental populations Kyazma BV, Wageningen, Netherlands Voorrips, R.E., 2002.

Viswanathan, C. and R. Khanna-Chopra. (2001). Effect of heat stress on grain growth, starch synthesis and protein synthesis in grains of wheat (Triticum aestivum L.) varieties differing in grain weight stability. Journal of Agronomy and Crop Science 186: 1-7. doi:10.1046/j.1439-037x.2001.00432.x.

Von Wettstein-Knowles P, Avato P, Mikkelsen JD (1980) Light promotes synthesis of the very long fatty acyl chains in maize wax. In P Mazliak, P Benveniste, C Costes, R Douce, eds, Biogenesis and Function of Plant Lipids, Elsevier/North-Holland Biomedical Press: 271–274.

Von Wettstein-Knowles P (1995) Biosynthesis and genetics of waxes. In RJ Hamilton, eds, Waxes: Chemistry, Molecular Biology and Functions. Oily Press, Dundee, Scotland: 91–130.

Wahid, A., Gelani, S., Ashraf, M., and Foolad, M. R. (2007). Heat tolerance in plants: an overview. Environ. Exp. Bot. 61: 199–223.

Wardlaw, I.F., Dawson, I.A., Munibi, P. and Fewster, R. (1989). The tolerance of wheat to high temperatures during reproductive growth. I. Survey procedures and general response patterns. Australian Journal of Agricultural Research 40: 1–13.

Wardlaw, I.F., and C.W. Wrigley. (1994). Heat tolerance in temperate cereals- an overview. Aust. J. Plant Physiol. 21: 695–703.

Wardlaw, I. F., and Wrigley, C. W. (1994). Heat tolerance in temperate cereals: an overview. Aust. J. Plant Physiol. 21: 695–703.

Wardlaw, I.F., C. Blumenthal, O. Larroque, and C. Wrigley. (2002). Contrasting effects of heat stress and heat shock on kernel weight and flour quality in wheat. Funct. Plant Biol. 29: 25–34.

Wollenweber, B., Porter, J. R., and Schellberg, J. (2003). Lack of interaction between extreme high-temperature events at vegetative and reproductive growth stages in wheat. J. Agron. Crop Sci. 189: 142–150.

Whitecross MI. ,and Armstrong DJ. (1972) Environmental Effects on Epicuticular Waxes of Brassica napus L .Australian Journal of Botany 20: 87-95. http://dx.doi.org/10.1071/BT9720087

Yang, J., R.G. Sears, Gill B.S., and Paulsen G.M., (2002). Quantitative and molecular characterization of heat tolerance in hexaploid wheat. Euphytica. 126: 275288.

Yin, X., Guo, W., and Spiertz, J. H. (2009). A quantitative approach to characterize sink–source relationships during grain filling in contrasting wheat genotypes.Field Crops Res. 114: 119–126.

Zahedi M, Sharma R, Jenner CF (2003) Effects of high temperature on grain growth and on the metabolites and enzymes in the pathway of starch synthesis in the grains of two wheat cultivars differing in their responses to temperature. Functional Plant Biology 30: 291–300.