ELUCIDATING AND MAPPING HEAT TOLERANCE IN WILD TETRAPLOID WHEAT (*TRITICUM TURGIDUM* L.)

A Dissertation

by

MOHAMED BADRY MOHAMED ALI

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

DOCTOR OF PHILOSOPHY

December 2010

Major Subject: Plant Breeding

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ABSTRACT

Elucidating and Mapping Heat Tolerance in Wild Tetraploid Wheat

(*Triticum turgidum* L.). (December 2010)

Mohamed Badry Mohamed Ali, B.A.; M.S., Assiut University, Egypt

Chair of Advisory Committee: Dr. Amir M.H. Ibrahim

Identifying reliable screening tools and characterizing tolerant germplasm sources is essential for developing wheat (*Triticum aestivum* L.) varieties suited for the hot areas of the world. Our objective was to evaluate heat tolerance of promising wild tetraploid wheat (*Triticum turgidum* L.) accessions that could be used as sources of heat tolerance in common- and durum-wheat (*Triticum durum*) breeding programs.

We screened 109 wild tetraploid wheat accessions collected by the International Center for Agriculture Research in the Dry Areas (ICARDA) from the hottest wheat growing areas in Africa and Asia, as well as, two common wheat checks for their response to heat stress by measuring damage to the thylakoid membranes, flag leaf temperature depression (FLTD), and spike temperature depression (STD) during exposure to heat stress for 16 beginning at anthesis. Measurements were taken on the day of anthesis then 4, 8, 12, and 16 days post anthesis (DPA) under controlled optimum and heat-stress conditions. Individual kernel weight (IKW) and heat susceptibility index (HSI) measurements were also obtained. Prolonged exposure to heat stress was associated with

increased damage to thylakoid membranes, as indicated by the high ratio of constant fluorescence (O) to peak variable fluorescence (P).

A positive and significant correlation was found between O/P ratio and both FLTD and STD under heat-stress conditions. A negative and significant correlation was found between FLTD and HSI and between STD and HSI based on the second and third measurements (4 and 8 DPA). Correlations obtained after the third measurement were not significant because heat-stress accelerated maturity and senescence.

For a pedigree-based mapping strategy a family approach was then developed by crossing and back-crossing heat-tolerant and heat-susceptible germplasm. A set of 800 lines resulting from the pedigree-based family approach was phenotyped using FLTD, chlorophyll content and yield and its components under heat stress. Genotyping of these lines was accomplished using simple sequence repeat (SSRs) markers. Some QTLs associated with heat stress tolerance were identified. This study identified potential heat-tolerant wild tetraploid wheat germplasm and QTL conditioning heat tolerance that can be incorporated into wheat breeding programs to improve cultivated common and durum wheat.

DEDICATION

To my parents, wife, and sons, Omar, and Kareem

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NOMENCLATURE

QTL	Quantitative Trait Loci
HSI	Heat Susceptibility Index
FLTD	Flag Leaf Temperature Depression
STD	Spike Temperature Depression
VC	Variance Component
PWR	Pedigree Wide Regression
LD	Linkage Disequilibrium
AA	Association Analysis

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CHAPTER I

INTRODUCTION: IMPORTANCE OF PHYSIOLOGICAL AND MOLECULAR GENETICS IN ELUCIDATING HEAT TOLERANCE IN WHEAT

Introduction

The world population is expected to reach 8.9 billion by 2050. Asia and Africa, where wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) are staple crops, will represent 59.1% and 19.8%, respectively, of the world population (UN, 2004). This population growth is expected to cause a food crisis. Heat stress is a major abiotic stress factor limiting wheat production not only in developing countries, but also worldwide. Expected increase in air temperatures due to global warming will even further exasperate the food crises and put pressure on wheat breeders and physiologists to improve yield potential of wheat under these heat stress conditions. Identification of newer sources of heat tolerance in wheat is paramount. Wild tetraploid wheat (e.g. *Triticum turgidum* L.) germplasm collected by the International Center for Agricultural Research in the Dry Areas (ICARDA, Syria) from the hottest places on earth where wheat is grown could potentially be used as a source of genes for improving heat tolerance in conventional wheat. Wild wheat is generally well adapted to warm and dry environments and possesses higher genetic diversity for heat tolerance than conventional wheat (Cox, 1998; Edhaie and Waines, 1992).

This dissertation follows the style of Crop Science.

Determining mechanisms associated with heat tolerance and identifying screening methods are vital for improving heat tolerance in plants (Ristic et al., 2007a). Meanwhile, fast ways to identify quantitative trait loci (QTL) using family based mapping for developing marker assisted selection (MAS) tools that will help to optimize breeders' time and resources.

The wheat genome and the importance of wild tetraploid wheat in wheat breeding

Wheat (*Triticum* spp.) is the primary food grain directly consumed by humans worldwide, and more acreage is dedicated to its commercial production than any other crop in the world (Briggle and Curtis 1987). Wheat also provides about one-fifth of the calories consumed by humans (FAOstat 2007). Durum wheat (*Triticum turgidum* L.), possessing the A- and B genome, is an important cereal crop used mainly for different food products such as pasta, couscous, and burghul (Kubaláková et al., 2005). The world's annual production of durum wheat is around 27.5 million metric tons, which is about one-tenth of the total wheat production (Kubaláková et al., 2005).

Wild emmer wheat [*Triticum turgidum* ssp. *dicoccoides* (Körn.) Thell.] (A,B genome) evolved in the Fertile Crescent area of the Middle East (Harlan and Zohary 1966) where wheat was domesticated 8,000 to 12,000 years ago and is believed to be the progenitor of both durum (AB genome) and common bread (ABD genome) wheats (Huang et al.,

2002; Nevo, 2001). Not surprisingly, the A- and B-genome chromosomes in both cultivated wheat species are > 99% identical (Kubaláková et al., 2005).

On the basis of their chromosome number, the known species of wheat can be divided into three major groups: diploids, tetraploids, and hexaploids, with 7, 14, and 21 pairs of chromosomes, respectively (McFadden and Sears, 1946).

Common hexaploid hard red spring wheat (genome: AABBDD; 2n=6x=42), and tetraploid durum wheat (genome: AABB; 2n=4x=28) are cultivated in the same area of the Northern Great Plains, while hard red winter wheat (genome: AABBDD; 2n=6x=42) is grown in the northern, central, and southern US Great Plains (Lanning et al., 2008). Durum wheat is an allotetraploid wheat that originated from hybridization and subsequent polyploidization of two diploid cereal species the *Triticum uratu* (2n = 2x =14, AA genome) and a B-genome diploid related to *Aegilops speltoides* (2n = 2x = 14, SS genome) (Kihara, 1944; McFadden and Sears, 1946). Several studies indicate that common wheat arose later from spontaneous hybridization of durum wheat and *Aegilops tauschii* about 8000 years ago (Huang et al., 2002).

Aaronsohn (1910) realized that *Triticum dicoccoides* (AB genome) possesses essential characteristics such as large grains, heat and drought tolerance, and resistance to rust; moreover, he believed that the cultivation of wheat may be revolutionized by utilization

of wild wheat and might assist in the development of new varieties adapted to dry and hot environments.

Long-term breeding programs of common wheat along with bottleneck events during wheat domestication resulted in narrowing of the genetic diversity of common wheat (Talbert et al. 1998; Bryan et al. 1999). As a result of reduced variability and crop domestication, many of the current genotypes became susceptible to both abiotic and biotic stresses due to possible loss of tolerance genes; therefore, wheat wild relatives constitute a vital source for improving biotic and abiotic stress tolerance and consequently productivity under these stress conditions (Xie and Nevo, 2008; Nevo et al., 2002). This is crucial as food demands are increasing due to an expanding world population.

Synthetic hexaploid wheat lines developed from crosses between tetraploid wheat and D genome donor *Aegilops tauschii* can be used to transfer genes from tetraploid to hexaploid wheat; therefore, some hexaploid varieties may serve as a link to transfer genes between both durum and bread wheat (Lanning et al., 2008).

Breeders constantly seek to broaden the genetic diversity of their germplasm and the use of wild relatives, as well as land races, may be ideal to achieve this goal. Langride et al. (2006) estimated that about 15% of alleles from wild wheat were utilized in current wheat varieties using classical breeding approaches (Henry and Ronalds, 1994).

Heat stress

Losses due to abiotic and biotic stresses negatively influence wheat production. Furthermore, heat and drought stresses will intensify as a result of increases in global warming arising from elevated CO_2 in the atmosphere, and could threaten the future of agriculture (Iba, 2002). Heat stress is currently a documented major limitation to wheat productivity in the drier and hotter parts of the world (Fischer, 1986). Furthermore, as the world population grows, there is a need to expand productive areas in these hot regions (Mohammadi et al., 2008). Consequently, the development of heat tolerant wheat varieties is a vital objective in wheat breeding programs (Wardlaw et al., 2002). Fokar et al. (1998a) suggested that elucidating the genetics and physiology of heat stress tolerance and using the promising germplasm as well as the proper selection approaches can help in facilitating the development of heat-tolerant wheat.

Heat stress constrains wheat yield

High temperature is a major abiotic stress factor that reduces productivity of wheat mainly during grain filling (Fokar et al., 1998 b). Wardlaw and Wrigley (1994) estimated favorable temperature for wheat during daytime to be 15°C. High temperatures, normally more than 34 °C, can decrease the final grain weight by reducing the duration of grain filling due to inhibition of photosynthesis (Al Khatib and Paulsen, 1984). Similarly, Wardlaw and Wrigley (1994) reported that wheat yield decreases by 3

to 4% when temperature increases by 1°C above 15°C under controlled conditions and kernel number declines by 12.5% by increasing temperature by 10°C from 25/20°C to 35/20°C. Under high temperature, not only yield but also quality of wheat can decline (Fokar et al., 1998 b; Wardlaw et al., 2002). Rekika et al. (2000) indicated that heat stress is one of the most important abiotic stresses affecting yield productivity in wheat, particularly, post-anthesis. Yield reductions are routinely experienced by the wheat crop in the Southern Great Plains due to higher temperature during grain filling. Heat damage in these areas is manifested in reduction in kernel number, kernel weight, and grain filling duration (Hays et al. 2007).

Under these conditions, reduction in kernel numbers might be attributed to sensitivity of pollen development to elevated temperatures. Under optimal conditions, pollen grains build up starch, storage protein, and triacylglycerides that serve as the source of energy for pollen tube growth (Clement et al. 1994). The accumulation of starch in the pollen grains can be repressed under heat stress. Heat stress can also affect the activities of key enzymes involved in starch biosynthesis and related sucrose metabolism in wheat anthers (Dorion et al. 1996). Callose plugs, β-1,3-glucan cell wall components, are regular features of normal pollen tubes in wheat (Saini et al., 1983). Saini et al. (1983) noticed poor and short pollen tubes under heat stress because of the decomposition of callose which blocks pollen tubes.

Wardlaw (1974) suggested that heat stress can affect three major components of plant systems including source, sink, and transport pathways and that grain filling in wheat can be affected by the injury of these three components.

Heat stress impairs photosynthesis

Heat stress impacts plant metabolic and physiological processes in wheat, reducing both yield and quality (Wahid et al., 2007). Demirevska-Kepova et al. (2005) reported that photosynthesis is one the most vulnerable processes to heat stress. The thylakoid membrane, in particular, is sensitive to heat stress and the level of this damage is affected by exposure time. For instance, long-term exposure to heat stress inhibits chloroplast biogenesis while short term exposure affects destacking of the grana (Takeuchi and Thornber, 1994). Heat stress increases the ratio of constant fluorescence (O) and the peak of variable fluorescence (P) (Ristic et al., 2007a). Chlorophyll fluorescence provides information on the state of photosystem II (PSII); e.g., damage to PSII is the first symptom of heat stress in a leaf (Maxwell and Johnson, 2000). Mishra and Singhal (1992) indicated that high temperature treatment of wheat leaves resulted in a decrease in the variable fluorescence to maximum fluorescence (Fv/Fm) and that the reduction in Fv/Fm ratio was mainly due to a decrease in the Fv at higher temperature, which resulted from a decrease in Fm and gradual increase in initial fluorescence (Fo). Reduction in Fv/Fm ratio as well as in Fv shows a drop in photochemical efficiency of photosystem II (PSII) by affecting energy transfer from the light-harvesting to the

reaction center (Mishra and Singhal, 1992). Heat damages photosystem II (PS II) via photo inhibition of the oxygen-evolving enhancer D1 protein in the thylakoids, while damage photosystem I (PS I) is limited (Takeuchi and Thornber, 1994). Mishra and Singhal (1992) pointed out the following possible causes of reduction in Fm: 1) structural modifications in PSII, 2) increase in the decay of excitation energy as fluorescence, 3) increase in radiation-less decay, or 4) transfer of excitation energy in favor of photosystem I (PSI).

On the other hand, moderate heat stress (e.g. 35-40°C) has a reversible effect on PSII that results from a response to downstream reactions in the Calvin cycle that are also impaired by high temperatures. Reactions downstream of PSII in the Calvin cycle are inhibited at lower temperatures than what is needed to damage PSII; therefore, the decline in photosynthesis seen at moderate heat stress cannot be due to damage to PSII (Sharkey, 2005).

Also, Pastenes and Horton (1996) indicated that the thylakoid proton conductance is increased under moderate heat stress. Also, the cyclic electron flow around PSI is increased (Egorova and Bukhov, 2002). Hence, the damage to thylakoid membrane under moderate heat stress might not be a result of damage to PSII, but may be due to pathways related to cyclic electron flow and cytochrome complex (Sharkey, 2005).

Chlorophyll biosynthesis can also be affected under heat stress in plants (Van Hasselt and Strikwerda, 1976).

Measurements of chlorophyll content with a chlorophyll meter could be useful for high throughput screening for heat tolerance in wheat (Ristic et al., 2007a). Al-Khatib and Paulsen (1984) stated that chlorophyll content of flag leaves can be used as a measure of leaf senescence and its acceleration by heat stress. Chlorophyll content was reduced with time after anthesis irrespective of treatment and cultivar (Fokar et al., 1998b). However, the mechanism by which high temperature may have caused chlorophyll loss is unclear (Ristic et al., 2007a).

Morphological adaptation to heat stress

Adaptability to heat stress can be caused by escape, avoidance or tolerance mechanisms (Blum, 1988). Plants can use these mechanisms to overcome damage due to heat stress. Leaf waxes and leaf rolling are considered mechanisms of avoidance. A waxy cuticle covers the aerial surfaces of the leaf in many plants (Chen et al., 2009). Heat stress causes the plants to lose more water through transpiration; therefore, the existence of epicuticular wax increases water use efficiency by decreasing cuticular transpiration and increasing the leaf boundary effects as well as decreasing leaf canopy temperature as a result of reflected solar radiation (Jefferson et al., 1989).

Leaf rolling is an adaptation mechanism that can decrease leaf exposure to heat stress, by decreasing the number of stomata exposed and consequently transpiration. Rolled leaves are usually cooler than the straight leaves. As a result, genotypes that posses this mechanism will be less affected by heat stress. Consequently, canopy temperature depression (CTD) can be a useful tool to distinguish between tolerant and susceptible genotypes.

The CTD trait, measured with a hand-held infrared thermometer, is calculated by subtracting the temperature of plant canopy from the ambient air temperature and can be used to process hundreds of lines in a short period of time (Ayeneh et al., 2002; Balota et al., 2007; Bilge et al., 2008). Experiments done under natural field conditions have shown a close association between grain yield of wheat and CTD in hot environments (Reynolds et al., 1994; Fischer et al., 1998). Ayeneh et al. (2002) found strong positive correlations between CTD and organ temperature depression including flag leaves and spikes on one hand and grain yield on the other hand under heat stress. Hatfield et al. (1984) indicated that the presence of awns in the spikes was not associated with heat tolerance (Hatfield et al., 1984). Other studies indicated the importance of awns in photosynthesis as well as of grain filling under heat stress in both wheat and barley (Ferguson et al., 1973; Johnson et al., 1974; Blum, 1986). In this study, individual plant flag leaf temperature depression (FLTD) and spike temperature depression (STD) were used to investigate the association with yield (Ayeneh et al., 2002).

Metabolic adaptation to heat stress

Plants have different metabolic adaptation mechanisms to defend against the negative effects of heat stress (Levitt, 1980). For example, the accumulation of certain biochemical compounds of low molecular weight such as compatible osmolytes can play a crucial role in the adaptive mechanisms in many plants grown under abiotic stresses (e.g. heat and drought stress) (Hare et al., 1998; Shakamoto and Murata, 2002). Under stress, plant species may accumulate a variety of osmolytes such as sugars, proline, tertiary and quaternary ammonium compounds, and tertiary sulphonium compounds (Sairam and Tyagi, 2004). Accumulation of these compounds can play an essential role in increasing heat stress tolerance in plants (Wahid et al. 2007).

The alternative photosynthetic attributes might be considered a different way of metabolic adaptation under heat stress. For example, in tomato and sugarcane, the increase in chlorophyll a:b ratio and the decrease in the chlorophyll : carotenoid ratio was observed in tolerant genotypes under high temperatures (Camejo et al., 2005 and Wahid and Ghazanfar, 2006). Consequently, these changes might be crucial to heat stress tolerance in plants.

Among mechanisms of heat stress tolerance, protein thermal stability (Levitt, 1980) and heat shock proteins (Feder and Hofmann, 1999; Vierling, 1991) are of paramount importance for plants. Heat shock proteins (HSPs) bind and stabilize heat-labile proteins

and protect them from aggregation under heat stress. Therefore, HSPs play an essential role in heat stress tolerance by acting as molecular chaperones (Basha et al., 2004; Feder and Hofmann, 1999; Lee and Vierling, 2000; Vierling, 1991). Wahid et al. (2007) stated that the synthesis and accumulation of HSPs under heat stress can prevent the denaturation of other proteins. Iba (2002) presented another hypothesis indicating that some members of the family of HSPs such as HSP70 participate in adenosine tri-phosphate (ATP)-dependent protein unfolding or assembly/disassembly reactions; as a result it prevents protein denaturation under heat stress. Miroshnichenko et al. (2005) hypothesized that HSPs can protect the protein biosynthesis machinery when HSPs aggregate into a granular structure in the cytoplasm under heat stress in tomatoes. Wang and Luthe (2003) noticed that heat susceptibility was related to less accumulation of chloroplastic HSPs in bent-grass. Sharkey (2005) suggested that the chloroplast HSPs can be used in protecting PSII, and Barua et al. (2003) indicated that the accumulation of low molecular weight (LMW) HSPs in the chloroplast membranes can protect PSII under heat stress. Some studies determined that HSPs are correlated with chloroplasts, ribosomes and mitochondria (Nieto-Sotelo et al., 2002; Yang et al., 2006).

Other kinds of proteins are engaged in heat stress tolerance by acting as molecular chaperones. Examples of these proteins include the prokaryotic protein synthesis initiation factor (IF2), protein synthesis elongation factors (EF-G) (Caldas et al., 2000) and chloroplast protein synthesis elongation factor (EF-Tu) (Caldas et al., 1998; Malki et al., 2002). These kinds of proteins play a role in protecting unfolded proteins from

aggregation under high temperature (Bukovnik et al., 2009). In maize, EF-Tu acts as molecular chaperone and protect chloroplast stromal proteins from aggregation under heat stress; therefore, it can play a vital role in heat stress tolerance (Momicilovic and Ristic, 2004). Another study in spring wheat, suggested that EF-Tu plays a key role in heat stress tolerance by displaying a chaperone activity and decreasing aggregation of Calvin cycle enzyme Rubisco activase under heat stress (Ristic et al., 2007b). The accumulation of EF-Tu in considerable amounts has been noticed in heat tolerant cultivars more than heat susceptible cultivars (Bukovnik et al., 2009). Particularly, in winter wheat, the accumulation of chloroplast EF-Tu were more in heat tolerant cultivars than heat susceptible under heat stress (Ristic et al., 2008).

The cytosolic counterpart of chloroplast EF-Tu, EF-1α, may be involved in heat stress tolerance in mammalians cells under heat stress by activating the heat-shock transcription factor 1 (Shamovsky et al., 2006). This protein is expressed and accumulated in wheat during heat stress (Bukovnik et al., 2009), and it is possible that the expression of EF-Tu and EF-1α is regulated at the mRNA level (i.e. transcriptionally). Changes in the accumulated amounts of the initiation factors can be necessary during seed development to facilitate shifting the translational environment to accommodate developmental changes in translational activity (Gallie et al., 1998).

Quantitative trait loci (QTL) and their importance for breeding

Genetic maps of Quantitative trait loci (QTL) of physiological parameters are prerequisites to marker assisted selection (MAS) for heat tolerance in wheat. Tanksley (1993) suggested that molecular markers could help breeders to track genetic loci without extensive, expensive, and time consuming field trials. This will not negate the need for field work but would rather reduce cost, increase breeding efficiency, and allow selection for heat-stress tolerance and other traits as well. These QTL were used by Ottaviano et al. (1991) to understand and delineate heat stress tolerance in cereals. QTL mapping and association genetic analysis can be useful in replacing specific alleles and identifying candidate genes for traits of interest (Cardon and Bell, 2001). The high level of synteny and homology within the wheat genus can facilitate transfer of identified QTL and candidate genes from wild tetraploid wheat to conventional hexaploid wheat to improve heat stress tolerance of common and durum wheats (Peng et al., 1999). Grain yield in cereals is generally controlled by a number of QTL and is highly affected by the environment, making it hard to make large gains in yield improvement (Kato et al., 2000). Heat stress tolerance is a quantitatively inherited and normally distributed trait (Blum, 1988; Yang et al., 2002). Therefore, determining the physiological mechanisms associated with heat tolerance and finding QTL associated with these mechanisms might be vital for heat tolerance in wheat breeding program. Furthermore, it is crucial to know the association between heat tolerance associated assays and grain yield under heat stress to justify the use of these assays as selection tools.

Simple sequence repeats (SSR) and their importance for breeding

Simple sequence repeats (SSRs) or microsatellite markers have been developed by Tautz (1989) and Weber and May (1989). The SSR markers showed polymorphism between species and within species in wheat (Plaschke et al., 1995). Röder et al. (1998) listed some advantages of using SSR markers including: 1) abundance, 2) high polymorphism, 3) consistent distribution across the genome, 4) requirement of small amounts of genomic DNA for analysis, 5) convenience of mapping of agronomic traits, and 6) application of analysis of SSRs to large numbers of plants.

The SSR markers can help breeders to select genotypes carrying gene(s) of interest; therefore, molecular maps based on these markers provide the breeders efficient strategies for MAS that may optimize time and resources (Korzun et al., 1998). Röder et al. (1998) suggested that SSRs provide noticeable markers for quantitative traits and facilitate their manipulation in segregating plant breeding populations. Korzun et al., (1999) suggested that SSRs identified in hexaploid wheat provide tremendous sources of molecular markers for genetic studies and breeding of durum wheat.

Linkage map and its importance to breeding

The SSR markers have been used to develop linkage maps in hexaploid wheat (Chalmers et al., 2001; Liu et al., 2005; Somers et al., 2004; Torada et al., 2006).

Genetic linkage maps are powerful tools for many studies such as gene tagging, genome characterization, QTL analysis, evolutionary studies, and marker development for MAS (Chu et al., 2010). Construction of a genetic map can play a vital role in linkage analysis of agronomic traits and can be used to detect QTL for both abiotic and biotic stresses; and therefore, facilitate MAS (Peleg et al., 2008). Genetic maps can be valuable for hexaploid common wheat and tetraploid durum wheat that have large genomes (Chu et al., 2010). Some studies have been carried out on the construction of whole genome linkage maps in hexaploid wheat (Chalmers et al., 2001; Liu et al., 2005; Somers et al., 2004; Torada et al., 2006); on the other hand, a limited number of whole genome maps have been constructed in tetraploid wheat, including durum (Blanco et al., 1998; Elouafi and Nachit 2004; Peleg et al., 2008).

The evolution of both hexaploid and tetraploid wheat was independent, and in spite of both having the A and B genomes their two genomes vary (Chalupska et al., 2008). Therefore, the development of more linkage maps in tetraploid wheat will provide essential resources and tools for genetic studies and breeding in durum and hexaploid wheat (Chu et al., 2010). Association analysis might fill the gap between QTL analysis and MAS in plant breeding programs (Breseghello and Sorrells, 2006a).

Linkage disequilibrium (LD) and association analysis (AA)

Linkage disequilibrium (LD) can be defined as a nonrandom association of alleles at different loci located on the same chromosome (Mackay and Powell, 2007). Therefore, LD will be observed between two loci if they are tightly linked or if the haplotype is recent (Hedrick, 2005). Significant LD in random mating populations can build up LD among barely linked or even unlinked loci (Breseghello and Sorrells, 2006a). Sorkheh et al. (2008) mentioned the following uses of LD in crop plant genomics research: 1) to study marker-trait association followed by MAS, 2) in population genetics and genetic diversity in natural populations and germplasm collections, and 3) in crop improvement programs.

Association analysis (AA), also known as association mapping or linkage disequilibrium mapping, is a method that relies on linkage disequilibrium to investigate the association between phenotypic variation and genetic polymorphisms (Flint-Garcia et al., 2003). Zondervan and Cardon (2004) mentioned that AA studies can be used to detect association between genotypes and phenotypes in a sample of individuals on the basis of LD. Use of AA can also provide a strategy to apply MAS for quantitative traits in plant breeding programs (Breseghello and Sorrells, 2006a).

Mapping of plant QTL is usually carried out using a population of recombinant inbred lines (RILs) derived from a bi-parental cross of two inbred lines that possess contrasting

traits (Jansen, 2001). Breeders may need to handle small families from crosses among elite lines to create more variability and include many more genetic backgrounds than when using bi-parental population (Crepieux et al., 2005). Mapping of small breeding or family-based mapping populations can provide an alternative to RILs.

The identical-by-descent (IBD) method based on variance components (VC) can use linkage information from family based mapping in an efficient way (Crepieux et al., 2005). Breseghello and Sorrells (2006a) mentioned the following types of populations that can be used to carry out AA in plant breeding: 1) germplasm bank collection, 2) elite breeding materials, and 3) synthetic populations. The AA in family based mapping can be implemented by using transmission disequilibrium test (TDT) (Spielman et al., 1993), a family-based association method used to detect genetic linkage between a marker and a trait of interest.

Objectives of the current study

Objective 1: Elucidate mechanisms of heat tolerance and identify efficient screening assays associated with these mechanisms in wild tetraploid wheat

Determining mechanisms associated with heat tolerance and identifying efficient screening assays associated with these mechanisms are vital for improvement of heat tolerance in wheat germplasm (Ristic et al., 2007a). Furthermore, it is crucial to know

the association between these assays and grain yield under heat stress to justify their use as selection tools. Pestsova et al. (2006) argued that wheat wild relatives contain valuable sources with high potential for contributing to improving heat tolerance in cultivated wheat. In the current study, we evaluated heat tolerance of wild tetraploid wheat (*Triticum turgidum* L.) by evaluating chlorophyll a fluorescence, FTD, STD, and kernel weight.

Objective 2: Family based mapping

Family-pedigree based QTL mapping techniques have been used successfully in humans and animals for disease mapping purposes. In this study we took advantage of a previously tested family-pedigree based QTL mapping technique to map heat stress tolerance in wild tetraploid wheat. Our goal is to raise awareness among plant breeders of the practical and theoretical aspects related to the application of AA in plant breeding. Simultaneously, we evaluated two methods including linkage and association to detect marker QTL associations. Also we compared variance component based linkage analysis and pedigree-wide regression methods in terms of their ability to detect the same marker QTL.

These two objectives lead to identification of potential heat-tolerant wild wheat germplasm that can be incorporated into wheat breeding programs targeting the improvement of heat tolerance in cultivated common and durum wheat.

Some studies have used family based pedigree approaches modified from human family based mapping approaches to detect QTL and markers associated with disease resistance in common wheat. The approach of the current study is innovative in that it introduces a family based pedigree method previously used in human genetics to detect QTL markers associated with abiotic stress tolerance, namely heat, in wild tetraploid wheat.

CHAPTER II

WILD TETRAPLOID WHEAT (*TRITICUM TURGIDUM* L.) RESPONSE TO HEAT STRESS*

Introduction

Heat stress is a major abiotic stress factor limiting wheat worldwide. Different physiological traits associated with heat tolerance have been assayed, including flag leaf temperature depression (FLTD), spike temperature depression (STD), cell membrane thermostability (CMT), triphenyl tetrazolium chloride (TTC) staining, chlorophyll a fluorescence, and reflectance spectroscopy. Canopy temperature depression (CTD), measured with a hand-held infrared thermometer, is calculated by subtracting the temperature of the canopy from the 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). Experiments done under natural field conditions have shown a close association between grain yield of wheat and CTD in hot environments (Reynolds et al., 1994; Fischer et al., 1998). Ayeneh et al. (2002) found strong positive correlations between CTD and organ temperature depression including flag leaves and spikes on one hand and grain yield on the other hand under heat stress.

* Reprinted with permission from "Wild tetraploid wheat (Triticum turgidum L.) response to heat stress" by Mohamed B. Ali, Amir M.H. Ibrahim, Dirk B. Hays, Zoran Ristic, Jianming Fu, 2010, Journal of Crop Improvement, 24, 228-243, Copyright 2010 by Taylor & Francis.

The presence of awns in the spikes was not associated with heat tolerance (Hatfield et al., 1984). This contrasts with other findings that postulate a role for awns in continuation of photosynthesis and grain filling following loss of green leaf tissue under heat stress conditions in wheat and barley (Ferguson et al., 1973; Johnson et al., 1974; Blum, 1986). In the CMT assay, electrolyte leakage from leaf tissue is measured after exposure to high temperatures (Fokar et al., 1998a; Ibrahim and Quick, 2001a; Ibrahim and Quick, 2001b). Cellular injury under heat stress can also be assessed by quantifying the reduction of TTC (Porter et al., 1995; Ibrahim and Quick, 2001a) to formazan by mitochondrial dehydrogenase respiratory enzymes in wheat seedlings (Ibrahim and Quick, 2001a). As explained by Fokar et al. (1998a), the TTC assay basically evaluates the integrity of the mitochondrial electron transport chain under heat-stressed conditions and thus represents respirational activity. Photosynthesis has been reported to be one of the most sensitive processes to heat stress in plants (Demirevska-Kepova et al., 2005) due mainly to the sensitivity of the thylakoid membrane (Takeuchi and Thornber, 1994). Heat damages the nature of photosystem II (PS II) through removal of the oxygen-evolving enhancer proteins from the thylakoids with no damage to the photosystem I (PS I) complex (Takeuchi and Thornber, 1994). It is believed that damage to the thylakoid membranes caused by heat stress leads to chlorophyll loss which can be easily measured by chlorophyll meters (Ristic et al., 2007a). Chlorophyll fluorescence measurements, on the other hand, require use of fluorometers that require dark adaptation of the leaf tissue, which limits the number of the screened plants per day (Ristic et al., 2007). Although it cannot be used to process a large number of samples, chlorophyll fluorescence is one of

the most powerful techniques available to plant physiologists (Maxwell and Johnson, 2000; Sayed, 2003). The ratio of variable fluorescence (Fv), which is measured as the difference between the maximum and minimum fluorescence, to maximum fluorescence (Fm) is an estimate of PS II maximum efficiency under abiotic stress conditions (Rachmilevitch et al., 2006). Premature plant senescence and reduction in the duration of photosynthetic activity also occur under high temperatures (Al-Khatib and Paulsen, 1984). Reflectance spectroscopy is another technique that provides a rapid assessment of heat tolerance (Dobrowski et al., 2005; Babar et al., 2006a). The spectral reflectance in the visible (VIS) wavelength (400-700 nm) is a function of light absorption by leaf chlorophyll, carotenoids, and anthocyanins (Babar et al., 2006). While most or all of the aforementioned physiological approaches are reliable, closely associated with heat tolerance, and have the potential to be used as screening tools in breeding programs, they have some limitations due to speed of measurement, cost, and labor, e.g., TTC, CMT, spectral reflectance, and Chlorophyll a fluorescence. On the other hand, traits such as FLTD, STD, and reflectance spectroscopy require less labor and time and can be used to process thousands of lines by plant breeders and physiologists.

Determining mechanisms associated with heat tolerance and identifying efficient screening assays associated with these mechanisms is vital for the improvement of heat tolerance in wheat germplasm (Ristic et al., 2007). Furthermore, it is crucial to know the association between these essays and grain yield under heat stress to justify their use as selection tools. Pestsova et al. (2006) argued that wheat wild relatives contain valuable

genetic sources with high potential for contributing to improvement of heat tolerance in cultivated wheat. In the current study, we evaluated heat tolerance of wild tetraploid wheat by evaluating chlorophyll a fluorescence, FTD, STD, and kernel weight.

Materials and methods

Sixteen wild tetraploid wheat accessions and two common wheat check cultivars (Table 2.1) were screened for their response to heat stress by measuring damage to the thylakoid membranes, FLTD, STD, individual kernel weight, and HSI. Plant growth conditions and heat treatments were similar to those described by Ristic et al. (2007a). Briefly, plants of each genotype were grown in ten pots (Metro Mix 200 potting soil [Hummert Int.], three seedlings per pot) in a greenhouse and were watered daily and fertilized weekly (Miracle Gro fertilizer (24:8:16; Stern's Miracle-Gro Products, Inc., Port Washington, NY)) for the entire duration of the experiment.

At the beginning of the flowering stage (50 % of the plants at growth stage Feeks 10.5.1 [Large, 1954]), plants of each genotype were divided into control (five pots) and heat treatment (five pots) groups. In each group, ten plants were randomly selected (two plants per pot).

Table 2.1. Sixteen wild tetraploid wheat accessions and two common wheat check cultivars used in the current study along with their geographical origin.

No.	Species	Cultivar/ Subspecies	accession no.	Geographical origin
1	*T. aestivum*	Kauz	Check	Mexico
2	*T. aestivum*	Siete Cerros	Check	Mexico
3	*T. turgidum*	*cartlicum*	IG45057	Turkey
4	*T. turgidum*	*cartlicum*	IG45171	Turkey
5	*T. turgidum*	*cartlicum*	IG44999	Turkey
6	*T. turgidum*	*dicoccon*	IG45073	Oman
7	*T. turgidum*	*dicoccon*	IG45303	Ethiopia
8	*T. turgidum*	*dicoccon*	IG45393	Eritrea
9	*T. turgidum*	*dicoccon*	IG45441	Syria
10	*T. turgidum*	*dicoccon*	IG88723	Greece
11	*T. turgidum*	*dicoccon*	IG44961	Turkey
12	*T. turgidum*	*dicoccon*	IG45069	Oman
13	*T. turgidum*	*dicoccon*	IG54388	Georgia
14	*T. turgidum*	*dicoccon*	IG45413	Bulgaria
15	*T. turgidum*	*polonicum*	IG110572	Algeria
16	*T. turgidum*	*polonicum*	IG127682	ICARDA
17	*T. turgidum*	*turgidum*	IG83047	Turkey
18	*T. turgidum*	*turgidum*	IG45448	Ethiopia

One flag leaf and one spike per selected plant were randomly chosen and tagged (total of ten flag leaves and ten spikes per group were tagged). The tagged leaves were later used to measure chlorophyll a fluorescence and FLTD. The tagged spikes were used to measure STD. The treatment group was exposed to heat stress for sixteen days (day/night temperature, 36/30°C; relative humidity, 90-100%; photoperiod, 16/8 h; photosynthetic photon flux [PPF], 280 µmol m-2 s-1 [Sylvania cool white fluorescent lamps]) in a growth chamber (Conviron, Model PGW-36, Winnipeg, MB, Canada) and the control group was maintained under optimum conditions (day/night temperature, 22/18°C; relative humidity, 90-100%; photoperiod, 16/8 h; photosynthetic photon flux [PPF], 280 µmol m-2 s-1 [Sylvania cool white fluorescent lamps]) in a growth chamber (Conviron, Model PGW-36, Winnipeg, MB, Canada). For each genotype, heat treatment started when 50% of the plants reached Feeks 10.5.1 growth stage (Large, 1954).

To avoid or minimize possible dehydration of the leaf tissue during stress treatment, pots of the treatment and control group were kept in trays containing ~1 cm deep water. Chlorophyll a fluorescence, FLTD, and STD were measured after 0, 4, 8, 12, 16 d of heat stress. Chlorophyll a fluorescence was measured in the middle portion of the flag blade (half-way between the base and the tip of the blade) as described by Ristic et al. (2007a). Both FLTD and STD were measured in the middle portion of the selected flag leaves and spikes, respectively. The ratio of constant fluorescence (O) and the peak of variable fluorescence (P) (O/P) was measured to assess the stability of thylakoid membranes (Krause and Weis, 1984; Ristic and Cass, 1993). Fluorescence

measurements were recorded at room temperature (25°C) using a pulse modular fluorometer (Model OS5-FL, Opti-Sciences, Hudson, NH). Data obtained from two plants within one pot were averaged and used for statistical analysis. Both FLTD and STD were measured for two plants for each pot for each treatment using a hand-held thermometer (Model AG-42, Teletemperature Crop, Fullerton, CA). Measurements were recorded between 11:00 and 16:00 following Reynolds et al. (1998).

At maturity, all plants of each cultivar/treatment (control and heat stress) were harvested and data on yield traits (kernel weight [KW] and number of kernels [NK]) were recorded. Individual kernel weight (IKW) was calculated as the following: IKW= KW/NK. Then IKW was the used to calculate heat susceptibility index (HSI) similar to the drought susceptibility index (DSI) calculated by Fischer and Maurer (1978). Using IKW, HSI was calculated as described by Ayeneh et al. (2002). Briefly,

$HSI = {1-(Y_h / Y)} \big/ {1-(X_h / X)}$, where Y_h is the IKW of each genotype under heat stress and Y is IKW of each genotype under optimum temperatures. The variable X_h is the average IKW of all genotypes expressed under heat stress, and X is average IKW of all genotypes under optimum temperatures.

Statistical analysis

Correlation analysis was used to test the relationship between heat damage to thylakoid membranes and HSI, FLTD and STD, FLTD and HSI, and STD and HSI. The PROC CORR PEARSON procedures in the Statistical Analysis System (SAS Institute, 2003) were used to quantify the relationship between the variables.

Results and discussion

Assessment of heat tolerance in 16 wild tetraploid wheat accessions and 2 common wheat check cultivars, namely 'Kauz' and 'Siete Cerros', was carried out by evaluating damage to thylakoid membranes using chlorophyll a fluorescence. Heat stress caused damage to thylakoid membranes (Ristic et al., 2007) which could be measured by O/P ratios using a fluorometer. Genotypes responded differently to heat stress. The most heat susceptible genotypes, as indicated by the high O/P ratios, were the wild tetraploid wheat accessions IG45413, IG88723, IG127682, and IG110572 (O/P > 439% after 16 d of heat stress; Fig. 2.1). We found O/P <186% after 16 d of heat stress in the check cultivar Siete Cerros, and wild wheat accessions IG45069, IG45393, and IG45057. Heat tolerance associated with less damage to photosystem II has been attributed to elongation factors EF-Tu (Bhadula et al., 2001; Ristic et al., 2006).

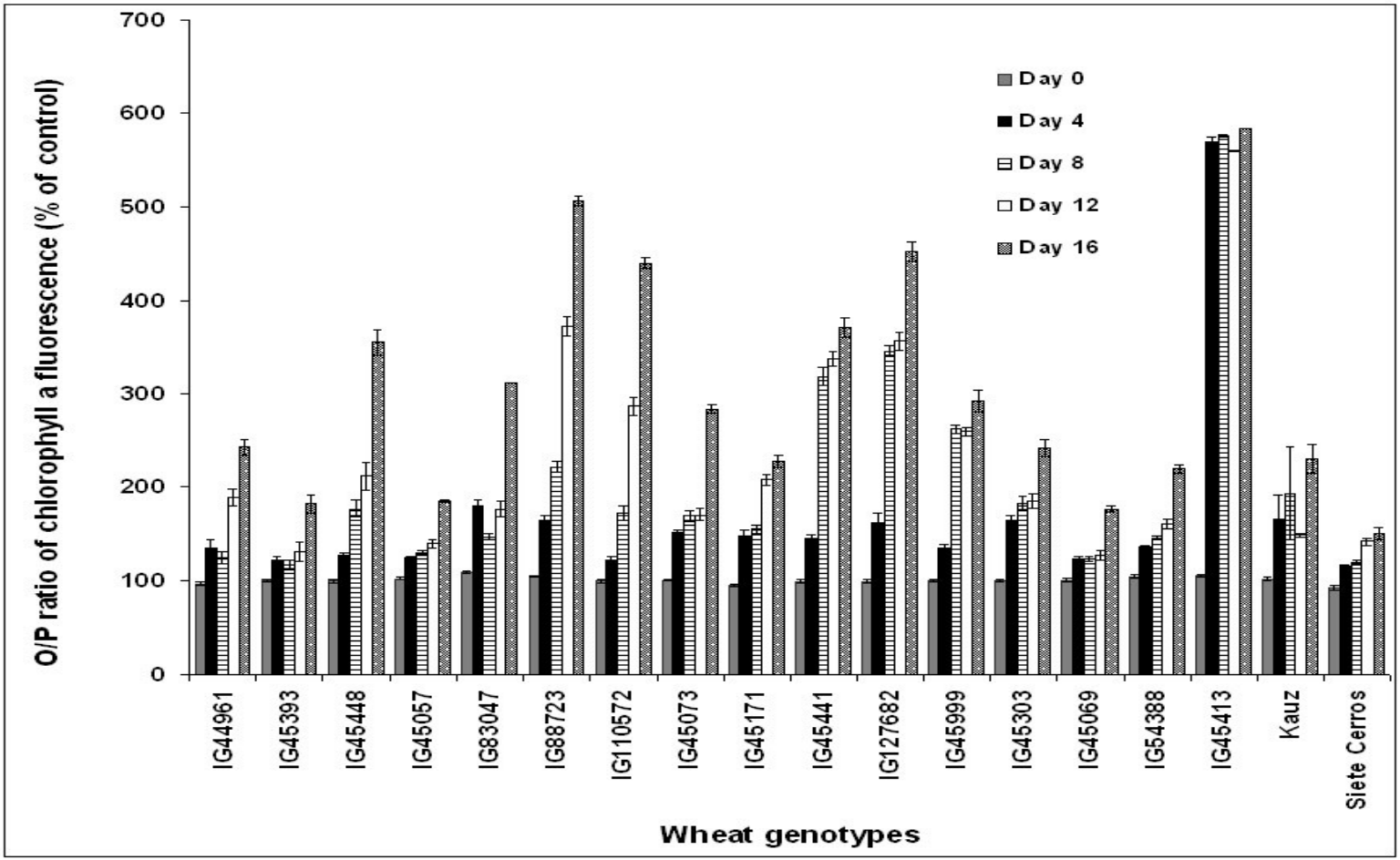

Fig. 2.1. The ratio of constant fluorescence and the peak of variable fluorescence (O/P) of 16 wild tetraploid wheats and 2 hexaploid spring wheats under heat stress. Chlorophyll a fluorescence was measured on the same flag leaves after 0, 4, 8, 12, and 16 d of exposure to heat stress. Bars indicate means ± standard errors; n=10.

In many breeding programs where heat stress is a major abiotic stress factor, grain yield and its components are used as the main selection criteria (Ehdaie et al., 1988). The HSI has been used to determine relative stress injury as it accounted for variation in both yield potential and performance under stress conditions (Bruckner and Frohberg, 1987). Low stress susceptibility (S<1) is synonymous with higher stress resistance (Fischer and Maurer, 1978). In this study, the HSI ranged from 0.353 to 1.756 for IG45069 and IG45413, respectively (Table 2.2). The HSI for the checks Siete Cerros and Kauz were 0.651 and 1.162, respectively. These results show that some of the wild tetraploid accessions were better than the heat-tolerant checks, emphasizing the potential of including these accessions in crossing blocks of breeding programs engaged in improving heat tolerance of common and durum wheat. We analyzed the relationship between HSI and O/P ratio of chlorophyll a fluorescence under heat stress as % of control at 0, 4, 8, 12, and 16 d of heat stress. A positive and significant correlation was found when data were plotted and analyzed for each single day of heat stress except for day 0 (Figs. 2.2-2.6 and Table 2.3).

Table 2.2. Individual kernel weight (IKW), under both optimum and heat stress (HS) conditions, and heat susceptibility index (HSI).

Genotypes	IKW – optimum	IKW – HS	HSI
IG83047	0.0551	0.0243	1.049
IG45073	0.0381	0.0193	0.927
IG45303	0.0297	0.0170	0.800
IG45393	0.0401	0.0300	0.472
IG45441	0.0352	0.0138	1.141
IG88723	0.0369	0.0069	1.528
IG110572	0.0478	0.0156	1.263
IG45057	0.0374	0.0233	0.706
IG45171	0.0297	0.0192	0.663
IG44961	0.0298	0.0155	0.903
IG127682	0.0532	0.0125	1.436
IG45448	0.0385	0.0151	1.139
IG45999	0.0316	0.0152	0.976
IG45069	0.0311	0.0252	0.353
IG45388	0.0337	0.0223	0.637
IG45413	0.0329	0.0021	1.756
Kauz	0.0322	0.0123	1.162
Siete Cerros	0.0330	0.0216	0.651

The correlation coefficients ranged from 0.33 (P=0.187) for day 0 to 0.93 (P <0.0001) for day 16. It is apparent from these results that the correlation coefficients and their degree of significance increased as the duration of exposure to heat stress was prolonged. The high positive correlation between HSI and O/P ratio of chlorophyll a fluorescence under heat stress in this study can be attributed to the following: i) increasing exposure to heat stress lead to more damage to thylakoid membranes as indicated by the high O/P ratios and ii) heat stress decreased both the rate and duration of photosynthesis which may have lead to decreased kernel filling.

We investigated the relationship between FLTD and STD at 0, 4, 8, 12, and 16 d of heat stress (Figs 2.7-2.11). Positive and significant correlations were found except at 0 d of heat stress (Table 2.4). The correlation ranged from 0.45 (P =0.06) to 0.99 (P =0.000) for day 0 and 8, respectively. The high positive and significant correlations between FLTD and STD under heat stress indicate that we can use either FLTD or STD to assess heat stress tolerance.

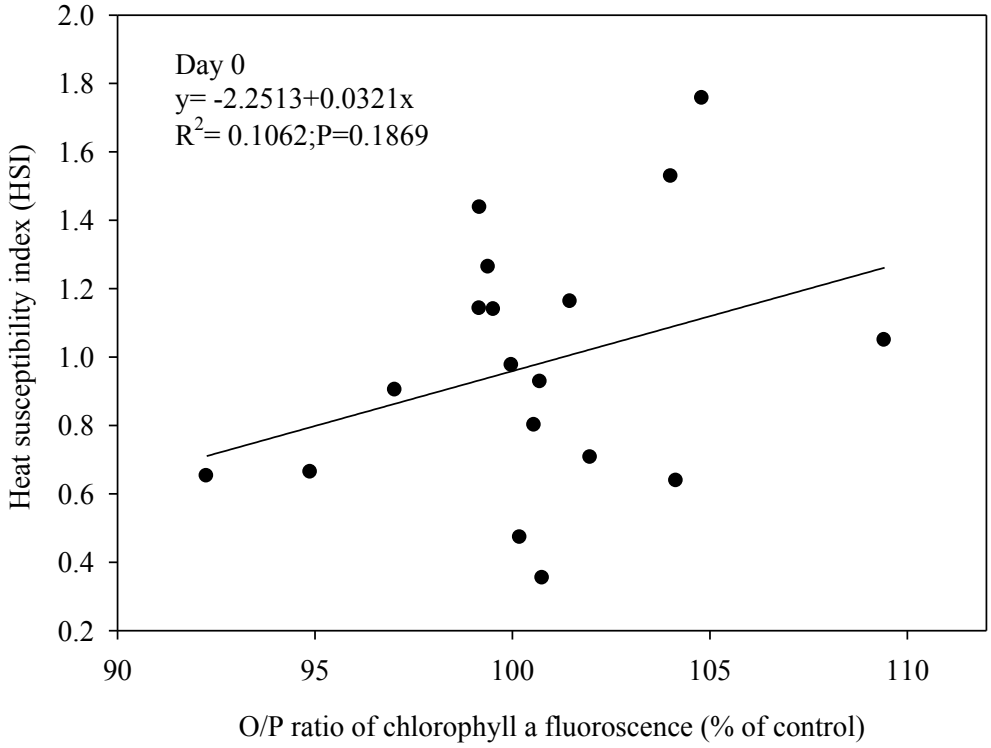

Fig. 2.2. The association between O/P ratio of chlorophyll a fluorescence (% of control) and heat susceptibility index (HSI) at 0 day (50% anthesis) before heat stress treatment.

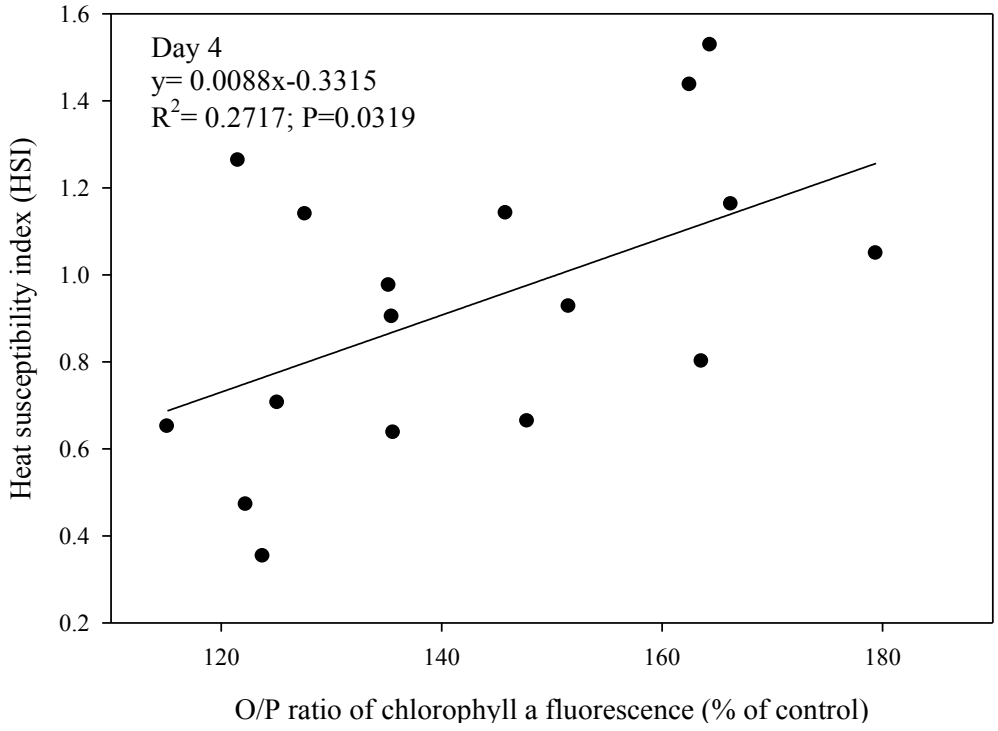

Fig. 2.3. The association between O/P ratio of chlorophyll a fluorescence (% of control) and heat susceptibility index (HSI) at 4 day of post anthesis heat treatment.

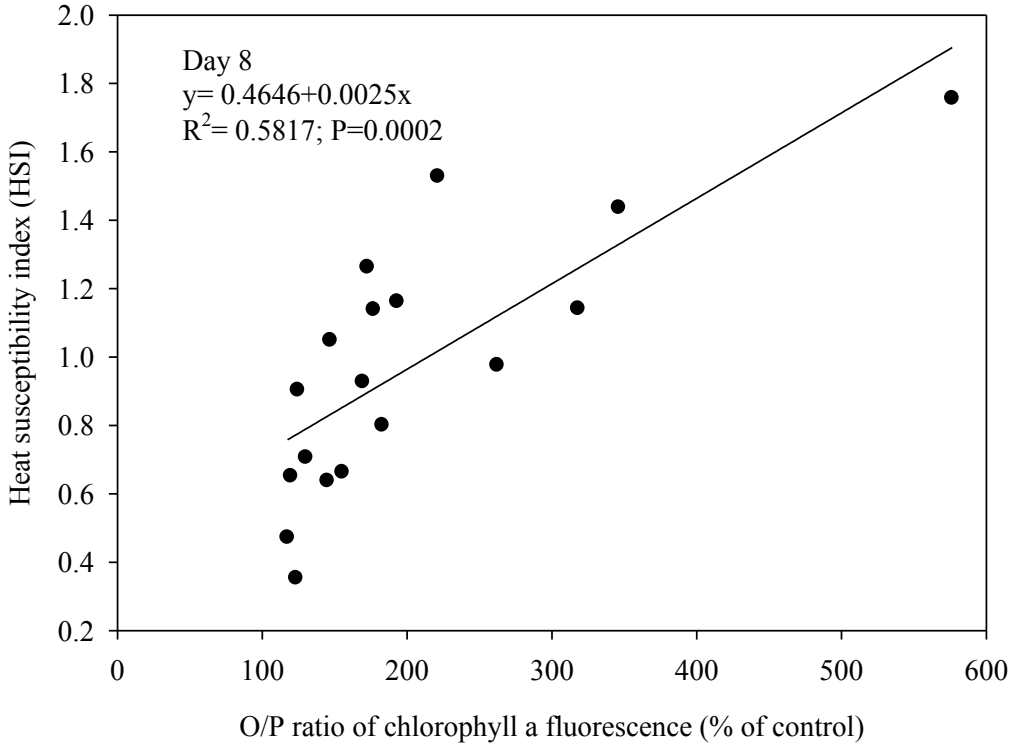

Fig. 2.4. The association between O/P ratio of chlorophyll a fluorescence (% of control) and heat susceptibility index (HSI) at 8 day of post anthesis heat treatment.

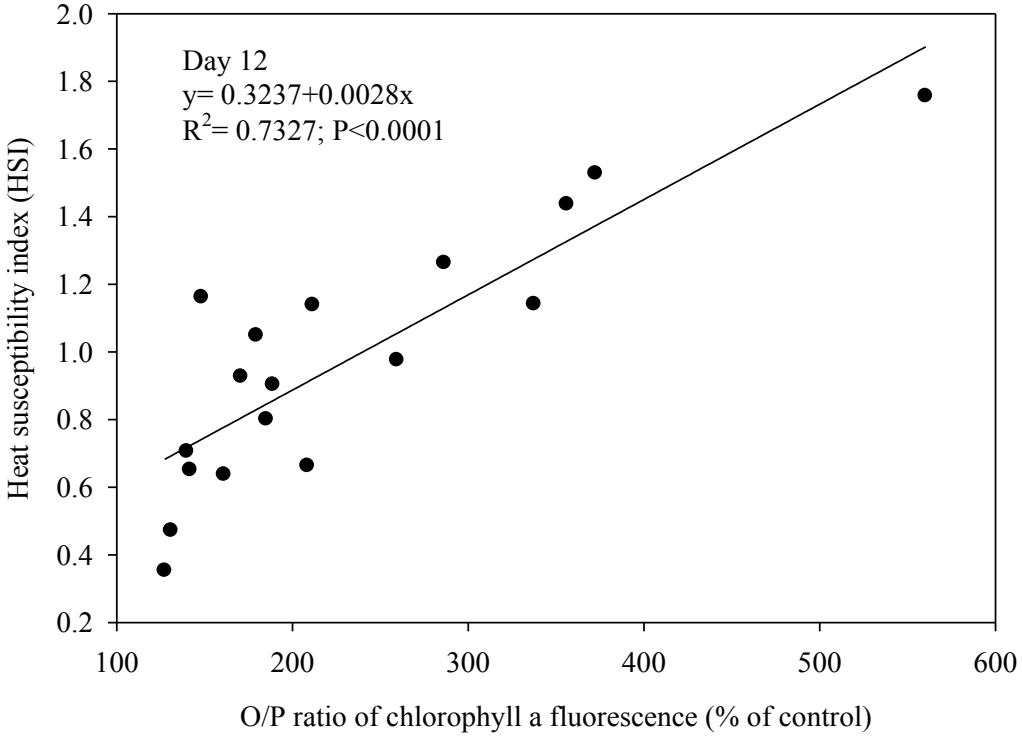

Fig. 2.5. The association between O/P ratio of chlorophyll a fluorescence (% of control) and heat susceptibility index (HSI) at 12 day of post anthesis heat treatment.

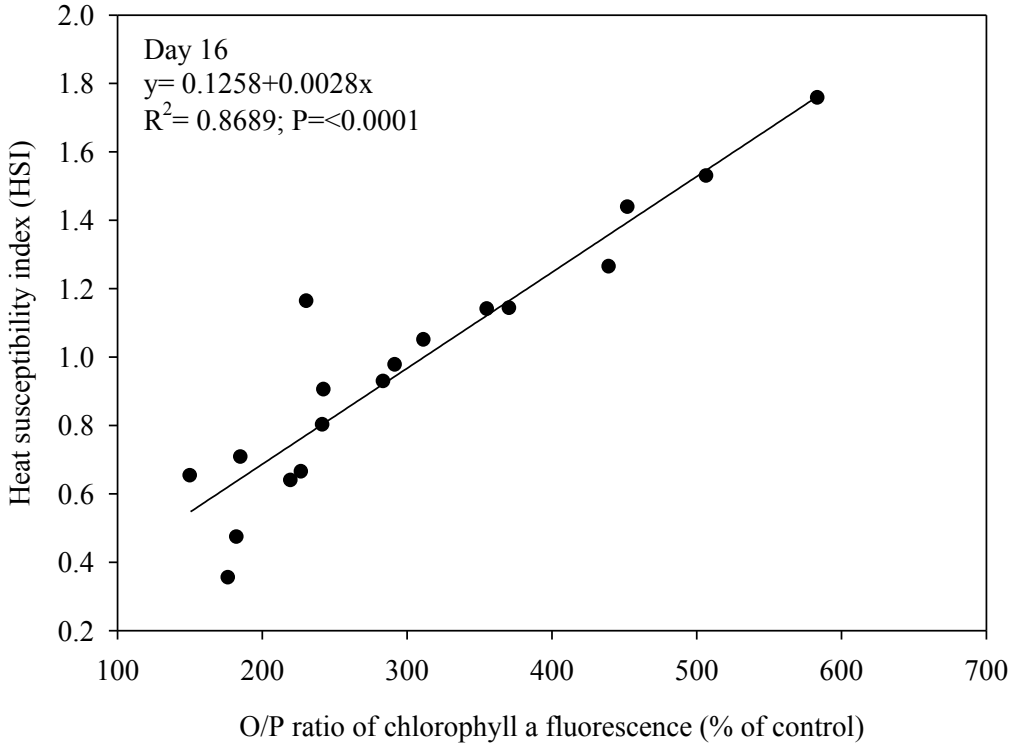

Fig. 2.6. The association between O/P ratio of chlorophyll a fluorescence (% of control) and heat susceptibility index (HSI) at 16 day of post anthesis heat treatment.

Table 2.3. Correlation coefficients between O/P ratio of chlorophyll a fluorescence (% of control) and HSI at day 0, 4, 8, 12, and 16 of post-anthesis heat treatment.

Days of heat stress	DF	R-value	P-value
Day 0	16	0.326	0.1871
Day 4	16	0.521	0.0319
Day 8	16	0.762	0.0002
Day 12	16	0.856	<0.0001
Day 16	16	0.932	<0.0001

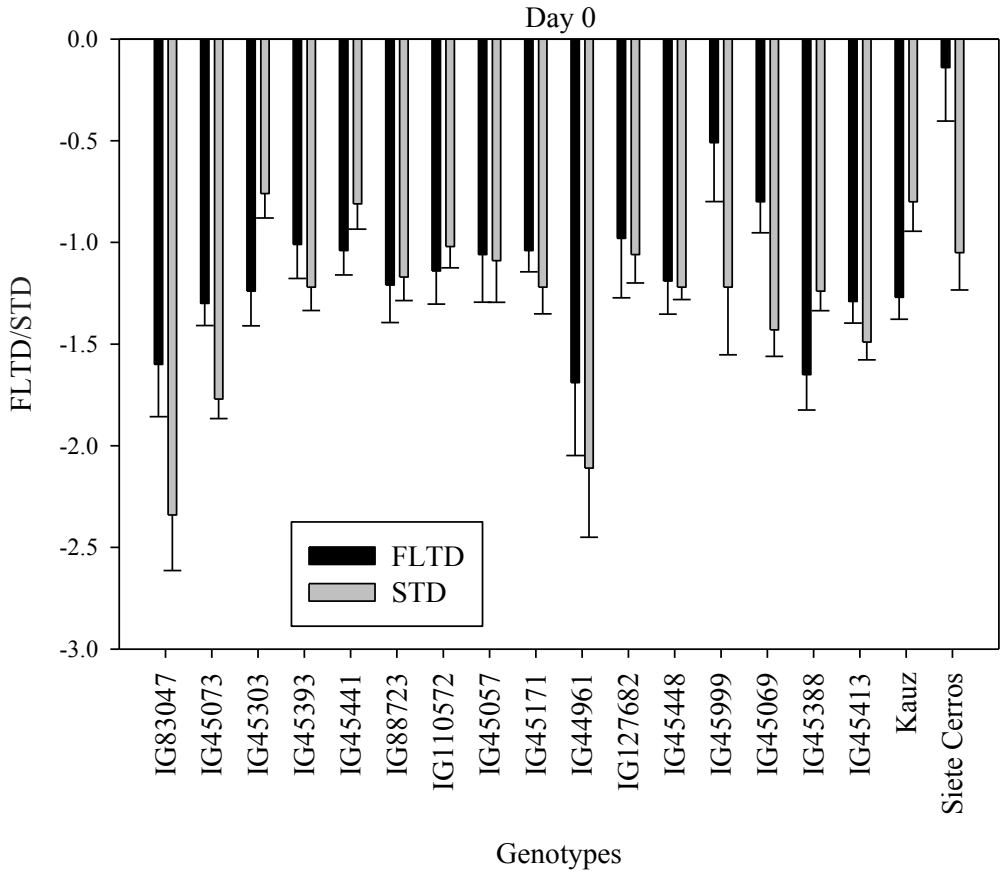

Fig. 2.7. The relationship between flag leaf temperature depression (FLTD) and spike temperature depression (STD) at 0 day (50% anthesis); before heat stress treatment. Bars indicate standard errors.

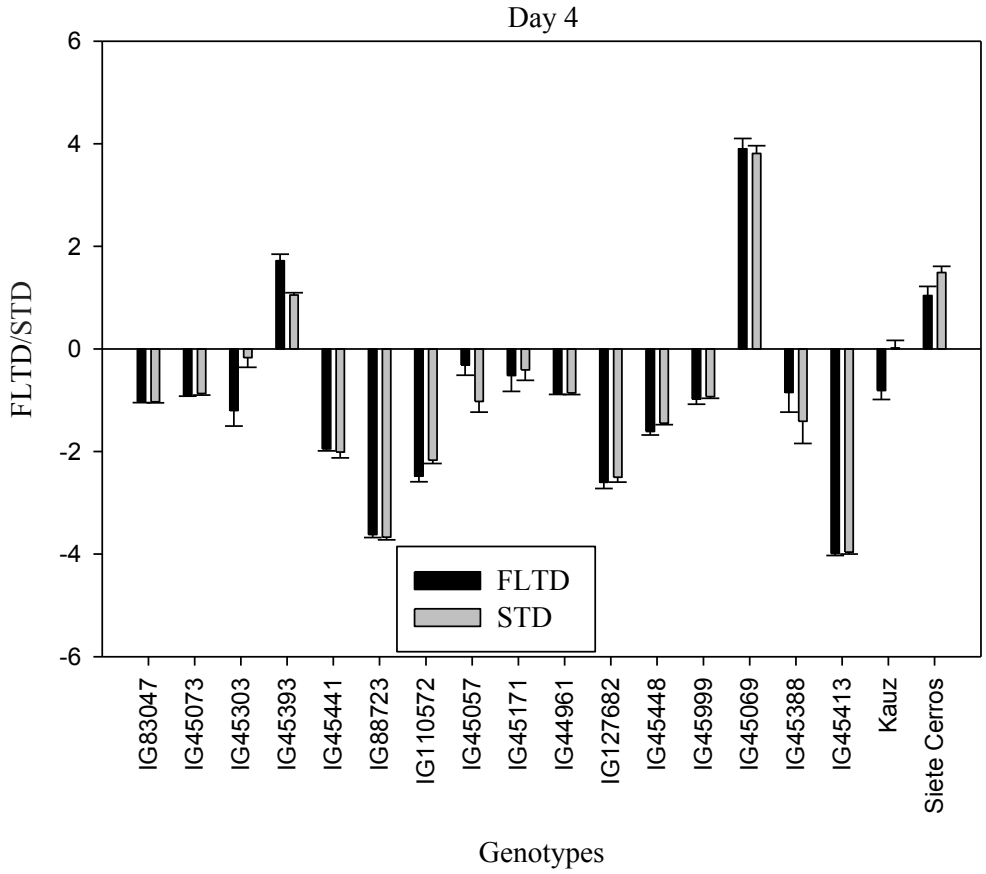

Fig. 2.8. The relationship between flag leaf temperature depression (FLTD) and spike temperature depression (STD) at 4 day of post anthesis heat treatment. Bars indicate standard errors.

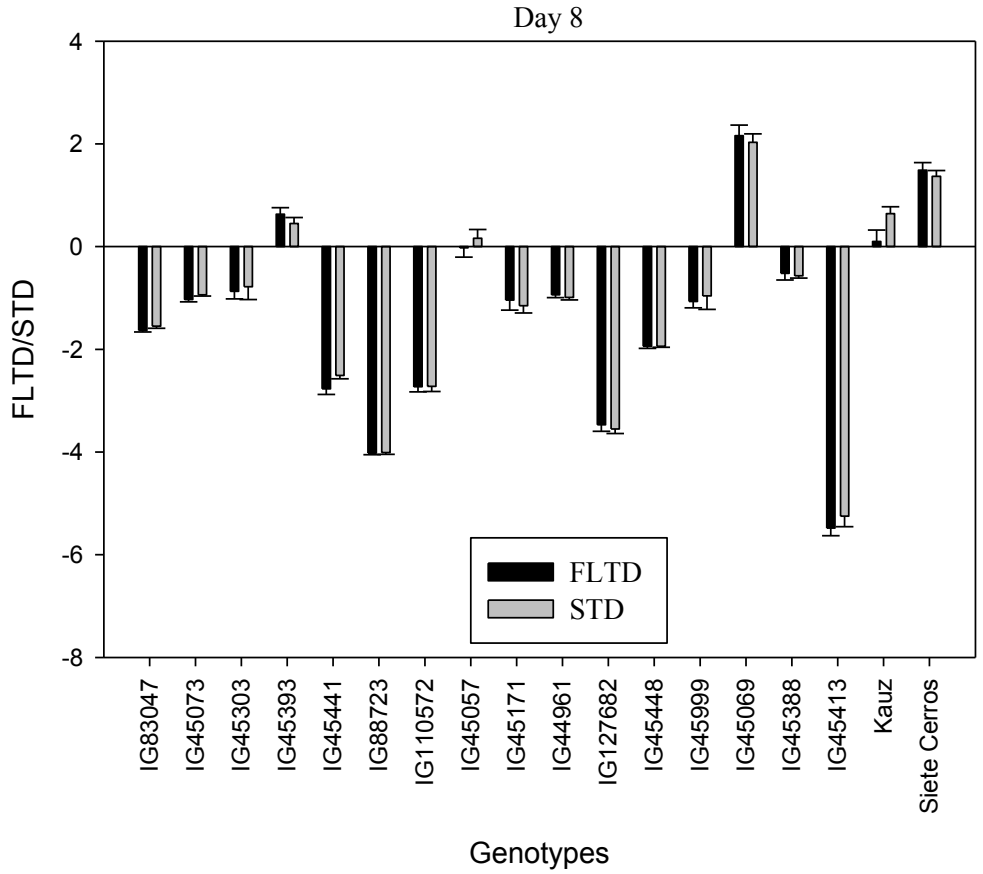

Fig. 2.9. The relationship between flag leaf temperature depression (FLTD) and spike temperature depression (STD) at 8 day of post anthesis heat treatment. Bars indicate standard errors.

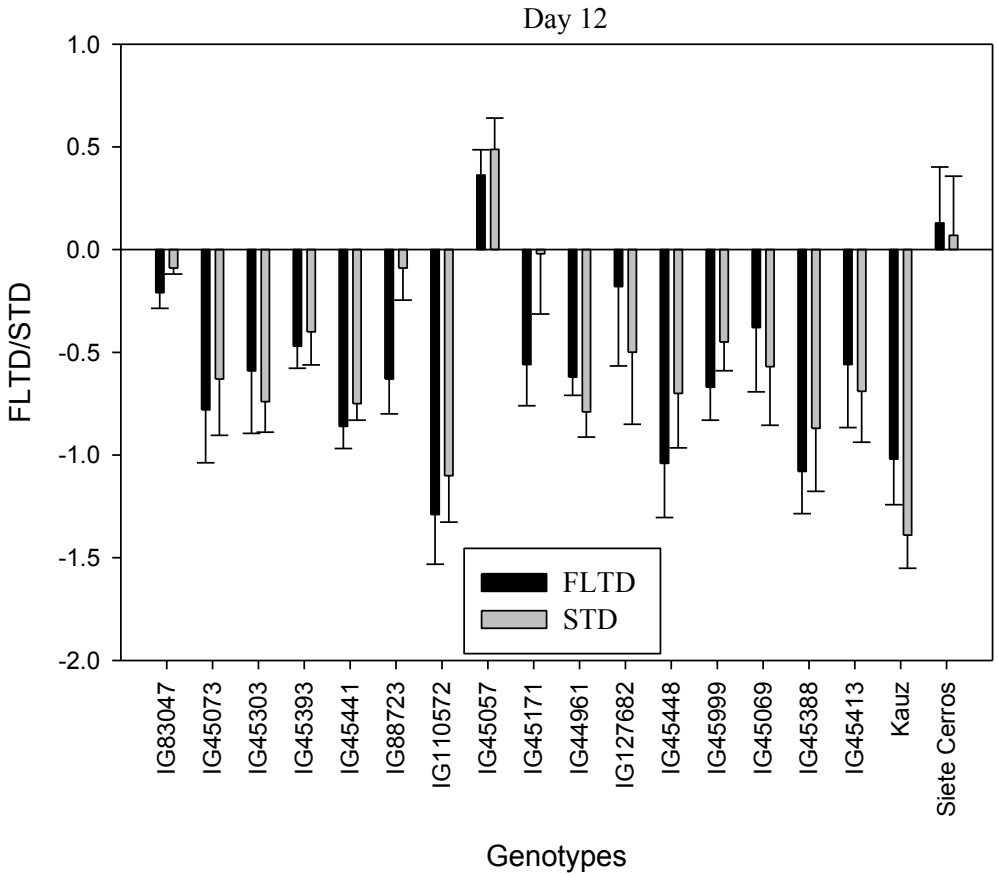

Fig. 2.10. The relationship between flag leaf temperature depression (FLTD) and spike temperature depression (STD) at 12 day of post anthesis heat treatment. Bars indicate standard errors.

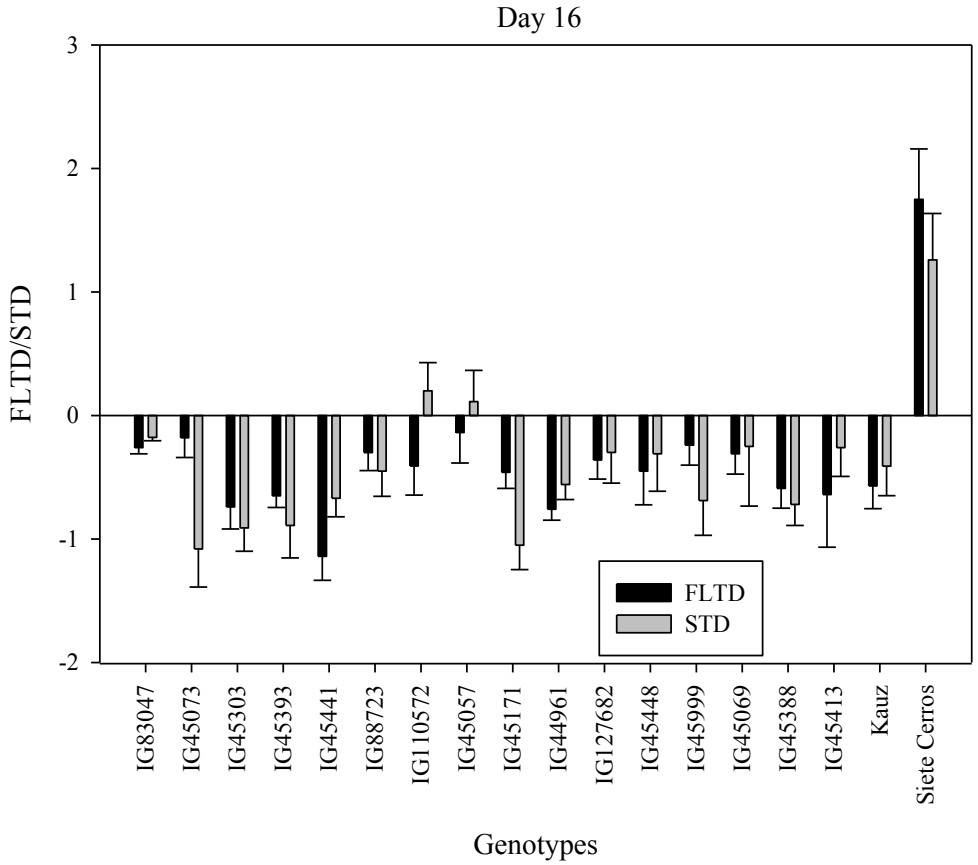

Fig. 2.11. The relationship between flag leaf temperature depression (FLTD) and spike temperature depression (STD) at 16 day of post anthesis heat treatment. Bars indicate standard errors.

Table 2.4. Correlation coefficients between flag leaf temperature depression (FLTD) and spike temperature depression (STD) at day 0, 4, 8, 12, and 16 of post-anthesis heat treatment.

Days of heat stress	DF	R-value	P-value
Day 0	16	0.452	0.060
Day 4	16	0.971	0.000
Day 8	16	0.996	0.000
Day 12	16	0.823	<0.00003
Day 16	16	0.763	0.0002

The correlation between each of FLTD and STD on one hand and HSI on the other hand was negative and significant for days 4 and 8 of heat stress. On the other hand, the correlations at days 0, 12, and 16 were not significant. The lack of correlation at days 12 and 16 of heat stress could be attributed to the lack of green leaf tissue starting on day 12 post-flowering onwards under the heat-stressed conditions. In general, the correlation between STD and HSI was lower than that between FLTD and HSI (Table 2.5). The positive association between grain filling rate and each of FLTD and STD in other studies indicated that cooler genotypes had longer grain filling rates (Ayeneh et al., 2002). Negative associations were found between each of STD and CTD with HSI (Ayeneh et al., 2002); however, a positive correlation was reported between HSI and CTD. Therefore, canopy temperature can be used as a tool in the selection of wheat targeted to dry production areas (Blum et al., 1989). Similarly, we can use FLTD and STD as tools for selecting wheat targeted to heat-stressed environments. The strong

correlations between either FLTD or STD and HSI at 4 and 8 d of heat stress indicate that both FLTD and STD, measured by infrared thermometers, are reliable and efficient means of assessing heat stress tolerance in wheat.

Table 2.5. Correlation coefficients between each of flag leaf temperature depression (FLTD) and spike temperature depression (STD) and heat susceptibility index (HSI) at day 0, 4, 8, 12, and 16 of post-anthesis heat treatment.

Days of heat stress	DF	FLTD		STD	
		R-value	P-value	R-value	P-value
Day 0	16	- 0.2284	0.36205	0.02578	0.9191
Day 4	16	- 0.9034	0.0000003	- 0.8562	0.0000058
Day 8	16	- 0.9134	0.0000001	- 0.8888	0.0000008
Day 12	16	- 0.2591	0.29921	- 0.25013	0.31679
Day 16	16	- 0.2190	0.382588	0.05897	0.816185

Conclusions

In conclusion, our study revealed a high significant positive correlation between damage to thylakoid membranes and HSI under heat stress. The results suggest that chlorophyll a fluorescence measured by a pulse modular fluorometer is a reliable tool for screening for heat tolerance in wheat. Our study also showed that FLTD and STD were positively and significantly associated with one another on one hand and with HSI on the other hand.

These results suggest that either FLTD or STD can be used as reliable tools for screening for heat tolerance in wheat. This study also showed that wild tetraploid wheat has excellent heat tolerance, suggesting that it can be included in crossing blocks of breeding programs targeting improving heat tolerance in common and durum wheats.

CHAPTER III

FAMILY BASED MAPPING OF QTL FOR HEAT STRESS TOLERANCE IN WILD TETRAPLOID WHEAT AS A MODEL

Introduction

Heat stress is a major abiotic stress factor for wheat (*Triticum aestivum* L.) worldwide, affecting its growth and productivity (Lobell and Asner, 2003; Wahid et al., 2007), and reducing its quality and grain yield (Stone and Nicolas, 1995). Wheat yield decreases by 3 to 4% for every increase of 1°C above 15°C under controlled conditions and kernel number declines by 12.5% by increasing temperature by 1°C from 25/20°C to 35/20°C (Wardlaw and Wrigley, 1994). We decided to utilize wild tetraploid wheat (*Triticum turgidum* L.) collected by the International Center for Agricultural Research in the Dry Areas (ICARDA, Syria) from the Fertile Crescent area in southwest Asia, because wild wheat is generally well adapted to warm and dry environments and possesses higher genetic diversity for heat tolerance than conventional wheat (Cox, 1998; Edhaie and Waines, 1992).

Identification of molecular markers associated with quantitative trait loci (QTL) for traits of interest could be useful to plant breeders engaged in marker assisted selection (MAS). Conventionally, QTL mapping approaches are applied using a population of recombinant inbred lines (RILs) derived from a bi-parental cross. The choice of parental

lines determines the power of the QTL detection (Crepieux et al., 2005). QTL analyses can be carried out using other bi-parental progenies such as backcrosses, doubled haploid, or F_2's (Crepieux et al., 2004a). Recently, diversifying of genetic backgrounds to study and map biotic and abiotic stresses has gained more popularity over traditional bi-parental populations. In many studies, multi-parental populations have been implemented for QTL mapping purposes (Christiansen et al., 2006; Jansen et al., 2003; Verhoeven et al., 2006). Family-based mapping approaches previously used in human and animal genetic studies can be applied to plant breeding populations (Crepieux et al., 2005; Jannink et al., 2001). Jannink et al. (2001) suggested that family-based approaches can be used in detecting QTL common in diverse genetic backgrounds by identifying linked polymorphic markers. Rosyara et al. (2009) applied the family-based mapping approach to wheat populations to study and map resistance to Fusarium head blight caused by *Gibberella zeae* Schw. (Petch) (*Fusarium graminearum* Schwabe). Crepieux et al. (2005) used a family-based mapping approach to map wheat kernel hardness and dough strength using 374 F_6 lines derived from 80 different parents. The QTL that were mapped by Crepieux et al. (2005), were validated successfully using a mixed model on the same population by Arbelbide and Bernardo (2006).

The family-based QTL mapping approach can be used as an early generation testing method to speed up the process of QTL mapping. Variance component (VC)-based identical-by-descent (IBD) method can be implemented by using family based mapping composed of sub-populations developed by successive crosses including either selfing or

backcrossing (Crepieux et al., 2004b). Xie et al. (1998) showed that it is feasible to use the IBD-based VC method in plant families including F_2, backcross, and full-sibs derived from crosses among multiple parents. In humans, VC-analysis is a powerful method to map unselected and normally distributed quantitative traits (Pugh et al., 1997). Cherny et al. (2004) also reported that VC-based linkage analysis was used in QTL analysis in humans. Therefore, it is possible to use it to identify QTL in plant breeding for traits of interest.

Pedigree wide regression (PWR) is calculated using trait-squared sums and differences to predict IBD shared between any non-inbred relative pairs (Sham et al., 2002). The PWR procedure was developed by Sham et al. (2002), and was found to be more efficient than the conventional method developed by Haseman and Elston (1972). Rosyara et al. (2009) stated that VC-analysis and PWR can hold complex extended family-pedigree with larger sib-ships. Abecasis et al. (2002) developed 'MERLIN software', which can be used to analyze a large number of markers and it can tolerate missing values and genotypic errors (Abecasis et al., 2002; Sham et al., 2002). This software can be used for linkage based mapping.

Linkage disequilibrium (LD), defined as the nonrandom association of alleles at different loci, has been studied comprehensively in animal science; however, modest research has been carried out regarding LD in plants (Flint-Garcia et al., 2003). Association analysis, a.k.a. linkage disequilibrium analysis, soon received considerable

attention in QTL mapping using breeding lines originating from multiple crosses or established cultivars in small or large geographical regions (Breseghello and Sorrells 2006a). Germplasm collections have been used as well for this association analysis (Breseghello and Sorrells 2006b), and suddenly seed labs started to meet needs of researcher groups interested in tapping into these pools not only for germplasm development purposes but also for the purpose of mapping genes and QTL that can later be verified by bi-parental RILS for MAS purposes. However, amidst this mapping euphoria, the use of family-based QTL mapping for association analysis has been very limited (Rosyara et al., 2009). Transmission/disequilibrium test (TDT) is a family-based method of association analysis (Spielman et al., 1993) in which a software called 'QTDT' can be used to perform quantitative transmission disequilibrium testing (QTDT) for nuclear as well as extended pedigrees (Abecasis et al., 2000a, b). Remington et al. (2001) pointed out that linkage-based analysis methods offer high power to detect QTL in genome-wide scans; whereas, association analysis increases resolution. These two aforementioned methods of analysis can help in cross-validating results and increasing the statistical power as well as identification of proper markers for MAS (Wilson et al., 2004) or marker assisted breeding (MAB). This lays the foundations for and explains the combined use of linkage and association analyses in succession in the current study.

The objectives of this study, therefore, are to 1) test the utility of using a family-based QTL mapping, commonly used in human and animal genetics and to a limited extent in mapping resistance to plant pests, for mapping heat stress tolerance in wild tetraploid

wheat, and 2) evaluate the aptitude of linkage and association analyses to detect marker-QTL linked to heat stress tolerance in the same population.

Materials and methods

Plant material

Mapping populations were derived from three way crosses among a number of wild tetraploid wheat genotypes. These genotypes have been evaluated before under heat stress (Ali et al., 2010). One heat-tolerant genotype (IG45069) was crossed to ten heat-susceptible ones (IG44999, IG44961, IG45413, IG83047, IG45441, IG127682, IG45448, IG110572, IG88723 and IG54073) (Table 3.1). The resultant F_1 from each single cross was either test-crossed or back-crossed to the same heat-susceptible genotypes. The three-way F_1 families with backcross or testcross-like structures were derived. Founder plants in the current study were defined based on Rosyara et al. (2009) as those that gave arise to their progenies. An example of how each family was developed is shown in Fig. 3.1. Nineteen families were selected for mapping analysis. This approach of crossing schemes for each family is shown in Table 3. 2. In the present study the term "family-pedigree" is defined as the description of the progenitors of any descendants in a particular family while taking into account that the breeding history of the parents is not included.

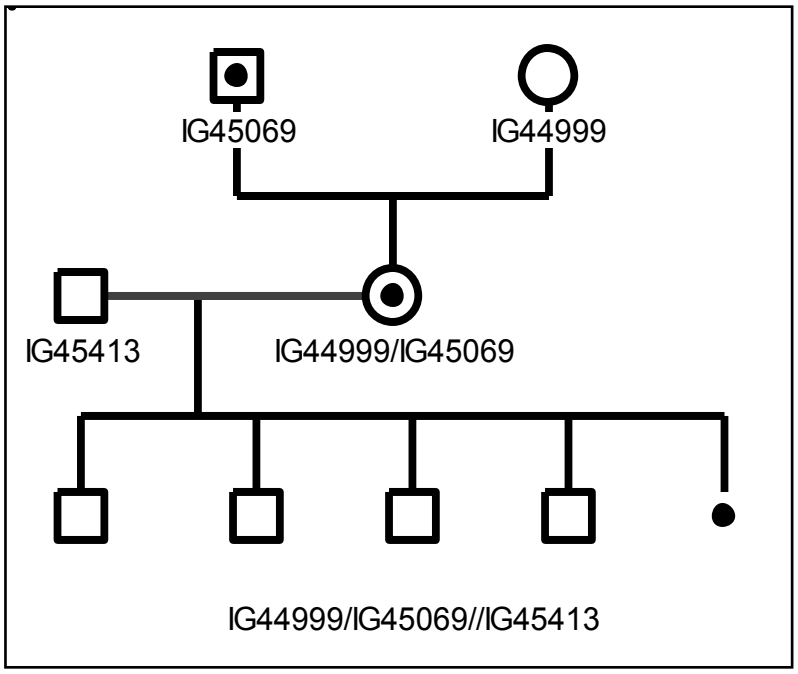

Fig. 3.1. An illustration of how each family was developed.

There were 19 families consisting of 384 individual plants. Each family has three founder parents, and the descendants in addition to the intermediate cross between the grand-parents are non-founders. The family size ranged from 12 to 31 with an average of 20. The common family structure was derived from three-way cross, which are similar to a three generation (grand-parent, parents, and descendants) human pedigree. To develop informative families for heat stress tolerance, each individual plant has a heat-tolerant grandparent, a heat-susceptible grandparent and a heat susceptible parent. This structure was used to generate a wide background of heat susceptibility with a small effect of heat tolerance. The structure developed here was based on a previous study conducted by Ali

et al. (2010) which would enable the identification of QTL/markers that are associated with heat tolerance.

Table 3.1. Eleven wild tetraploid wheat accessions used in the current study to develop families along with their geographical origin.

No.	Species	Cultivar/ Subspecies	Accession no.	Geographical origin
1	*T. turgidum*	*cartlicum*	IG44999	Turkey
2	*T. turgidum*	*dicoccon*	IG45073	Oman
3	*T. turgidum*	*dicoccon*	IG45441	Syria
4	*T. turgidum*	*dicoccon*	IG88723	Greece
5	*T. turgidum*	*dicoccon*	IG44961	Turkey
6	***T. turgidum***	***dicoccon***	**IG45069**	**Oman**
7	*T. turgidum*	*dicoccon*	IG45413	Bulgaria
8	*T. turgidum*	*polonicum*	IG110572	Algeria
9	*T. turgidum*	*polonicum*	IG127682	ICARDA
10	*T. turgidum*	*turgidum*	IG83047	Turkey
11	*T. turgidum*	*turgidum*	IG45448	Ethiopia

Tolerant parent is presented in bold

Table 3.2. Description of the families used in this study.

SN	Parent 1	Parent 2	Parent 3	Family size	Crossing Scheme
1	**IG45069**	IG44999	IG45413	20	**IG45069**/ IG44999// IG45413
2			IG83047	20	**IG45069**/ IG44999//IG83047
3		IG88723	IG45413	16	**IG45069**/IG88723//IG45413
4			IG127682	16	**IG45069**/IG88723//IG127682
5			IG45448	23	**IG45069**/IG88723//IG45448
6			IG44999	23	**IG45069**/IG88723//IG44999
7		IG44961	IG45448	19	**IG45069**/ IG44961//IG45448
8		IG45413	IG44999	31	**IG45069**/ IG45413//IG44999
9			IG110572	13	**IG45069**/IG45413// IG110572
10		IG110572	IG44999	27	**IG45069**/IG110572// IG44999
11			IG127682	14	**IG45069**/IG110572// IG127682
12		IG45441	IG127682	21	**IG45069**/IG45441// IG127682
13			IG45413	21	**IG45069**/IG45441// IG45413
14			IG110572	13	**IG45069**/IG45441//IG110572
15			IG45448	12	**IG45069**/IG45441//IG45448
16			IG44999	23	**IG45069**/IG45441//IG83047
17		IG45448	IG44999	24	**IG45069**/IG45448// IG44999
18		IG83047	IG45448	22	**IG45069**/ IG83047// IG45448
19		IG127682	IG44999	26	**IG45069**/ IG127682// IG44999

Tolerant parent is presented in bold

Phenotypic evaluation

The plants were evaluated for their response to heat stress by measuring chlorophyll content, flag leaf temperature depression (FLTD) and yield and its components including number of tillers, number of fertile spikes, number of kernels, kernel weight and individual kernel weigh. Plant growth conditions and heat stress treatment were similar to those described by Ristic et al. (2007a) with some modifications. Briefly, each individual plant was grown in one pot (Metro Mix 200 potting soil [Hummert Int]) in the greenhouse and was watered daily and fertilized weekly [Miracle Gro fertilizer (24:8:16; Stern's Miracle-Gro Products, Inc., Port Washington, NY)] for the entire duration of the experiment. In the greenhouse, data on air temperatures were measured at hourly intervals (the average daily temperature in the greenhouse was 22.7 ± 2.8°C). At the beginning of the flowering stage (50 % of the plants at growth stage Feeks 10.5.1 (Large, 1954)), one flag leaf per individual plant was tagged. This tagged leaf was later used to measure chlorophyll content and FLTD. Each individual plant was exposed to heat stress for 8 days (day/night temperature: 36/30°C; relative humidity: 90%–100%; photoperiod: 16/8 h; photosynthetic photon 130 flux [PPF]: 280 µmol m-2 s-1 [Sylvania cool white fluorescent lamps]) in a growth chamber (Conviron, Model PGW-36, Winnipeg, MB, Canada). For each individual plant, heat treatment started at Feeks 10.5.1 growth stage (Large, 1954). To avoid or minimize possible dehydration of the leaf tissue during heat stress treatment, the pots were kept in saucers containing ~ 1 cm deep water. Chlorophyll content and FLTD were measured after 0, 4, and 8 d of heat stress.

Chlorophyll content and FLTD were measured in the middle portion of the flag blade (half way between the base and the tip of the blade), as described by Ristic et al (2007). Chlorophyll content was measured using a self-calibrating SPAD chlorophyll meter (Model 502, Spectrum Technologies, Plainfield, IL), and FLTD was measured in the same blade area that was used for chlorophyll content using a handheld thermometer (Model AG-42, Teletemperature Crop, Fullerton, CA). Measurements were recorded between 11:00 and 16:00 following Reynolds et al. (1998).

Genotyping

DNA was extracted from leaf samples following Saghai-Maroof et al. (1984) with minor modifications. Genotyping included previously mapped simple sequences repeats (SSR) markers (Paillard et al., 2003; Elouafi and Nachit, 2004; Somers et al., 2004; Sourdille et al., 2004; Liu et al., 2005; Singh et al., 2007; Peleg et al., 2008; Zhang et al., 2008; Carter et al., 2009). A total of 252 SSR markers were first used to screen the parents, and only 40 were polymorphic. Consequently, these 40 polymorphic markers, which represented the entire tetraploid wheat genome, were used to screen the 384 descendants. Polymerase chain reaction (PCR) was performed as per Malla et al. (2010) with minor modifications as the following: the PCR mixture (10 µL) contained 0.1 µM of forward-tailed primer (5' to 3', GTT TTC CCA GTC ACG AC), 0.1 µM 6-FAM/VIC/NED/PET-labeled M13 primer (5' to 3', GTT TTC CCA GTC ACG AC, Applied Biosystems), 0.2 µM reverse primer, 200 µM of deoxynucleotide, 1.5 mM $MgCl_2$, 0.166 unit Taq

polymerase, 200 ng of template DNA and 1X Ammonium Sulfate Buffer. After heating the mixture to 95°C for 5 min, PCR reaction was obtained following 35 cycles. The first five cycles consisted of denaturing at 96°C for 1 min, 68°C (-2°C/cycle) for 5 min, 72°C for 1 min, followed by five cycles of 96°C for 1 min, 58°C (-2°C/cycle) for 2 min, 72°C for 1 min and the remaining 30 cycles consisted of 96°C for 1 min, 45°C for 1 min, 72°C for 1 min with a final extension step of 72°C for 5 min. PCR products were scanned with GeneScan-500 LIZ as an internal size standard in an ABI 3130XL (Applied Biosystems, Inc.). The results were analyzed with GeneMapper v4.1 software (Applied Biosystems, Inc.). The parents and third generation offspring from 19 family-pedigree were genotyped. The genotype of the second generation was predicted based on that of their homozygous parents.

Statistical analysis

The Mendelian errors were tested using PEDSTATS (Wigginton and Abecasis, 2005). The analysis requires preparing the following three files: 1) a pedigree file (*.ped) which includes all the information that is necessary to construct individual relationships consisting of the following categories of columns a) a family identifier, b) an individual identifier, c) a link to each parent, d) an indicator of each individual sex, e) a set quantitative traits identifiers, and f) a set of genetic markers, 2) a data file (*.dat) which includes description of the pedigree file; i.e. indicating the data type (encoded as M-marker, T- quantitative trait), and 3) a map file (*.map) including columns for

chromosome name, marker name, and position in cM. Examples of how the pedigree file, data file, and map file were built from the original data are shown in Tables 3.3 to 3.6. To check the Mendelian errors, the following command was used: *pedstats –d filename.dat –p filename.ped*

Table 3.3. An example of the original data.

Family	Person	Father	Mother	Sex	Trait	Marker1*	Marker2*
1	Grandpa	Unknown	Unknown	M	3.5	150	110
1	Grandma	Unknown	Unknown	F	1.4	150	110
1	Father	Granpa	Granny	M	2.3	150	110
1	Mother	Granpa	Granny	F	1.5	150	110
1	Sister	Father	Mother	F	2.1	150	110
1	Brother	Father	Mother	M	1.7	150	110

*band size in bp

Table 3.4. An example of pedigree file.

1	1	0	0	1	3.5	3 3	2 2
1	2	0	0	1	1.4	3 3	2 2
1	3	0	0	1	2.3	3 3	2 2
1	4	1	2	1	1.5	3 3	2 2
1	5	3	4	2	2.1	3 3	2 2
1	6	3	4	2	1.7	3 3	2 2

Table 3.5. An example of data file.

T	Quantitative trait
M	Marker1
M	Marker2

Table 3.6. An example of map file.

Chromosome	Marker	Position (CM)
1A	Marker1	120.2
1A	Marker2	125.2

Sex of descendants was randomly assigned as males because it will not affect the analysis. We assumed that none of the traits were considered as covariates. In the current study, we used three methods of the family-based approach including VC analysis, PWR, and QTDT. Both VC and PWR make use of linkage information, while QTDT is an association-based method.

Linkage analysis -variance component (VC) method

The VC-based linkage analysis was implemented using MERLIN v.1.1-alpha 3 (Abecasis et al., 2002). The role of MERLIN in analyzing family based pedigree mapping is to divide the total variation of a trait of interest into its components, including contribution from the chromosome segment where the QTL is located, contribution due to the rest of genome, and contribution due to environmental factors. (Rosyara et al., 2009). The phenotypic variation from the trait of interest can be divided to the following components:

$$Y_i = \mu + Q_i + A_i + F_i + \varepsilon_i$$

where (Y_i) is the phenotypic value, (μ) is the mean of the population, (Q_i) is the contribution to the phenotypic trait from the loci residing on the same chromosome, (A_i) is the contribution from the remainder of the genome not accounted for in (Q_i), (F_i) is the contribution due to common family environment, and (ε_i) is the experimental error. Moreover, the variances related to these components are the following:

$$\sigma^2 y = \sigma^2 q + \sigma^2 a + \sigma^2 f + \sigma^2 e$$

Where $\sigma^2 y$ is the phenotypic variation, $\sigma^2 q$ is the variation attributed to the chromosome loci controlling, $\sigma^2 a$ is the variation due to rest of genome, $\sigma^2 f$ is the variation explained by the common family environment, and $\sigma^2 e$ is the experimental error.

Merlin-based linkage analysis approach which is most commonly known as VC analysis has been used in human genetics studies for identifying QTL related to human diseases as per Aissani et al. (2006) and Farbrother et al. (2004).

For this analysis, the following assumptions were considered: 1) absence of a relationship among the original parents, and 2) marker positions were assigned based on the consensus map distances of Somers et al. (2004). Markers which are not available in consensus maps were assigned to positions based on other studies (Paillard et al, 2003; Elouafi and Nachit 2004; Sourdille et al., 2004; Liu et al., 2005; Singh et al., 2007; Peleg et al., 2008; Zhang et al., 2008; Carter et al., 2009).

The following command was used in the software package MERLIN (Abecasis et al., 2002):

merlin –d filename.dat –p filename.ped –m filename.map –vc

Linkage method analysis -Pedigree-wide regression (PWR) method

The PWR method was estimated by MERLIN-REGRESS, a procedure of MERLIN 1.1-alpha 3, based on the regression of IBD sharing between relative pairs on the square sums and squared differences of trait (Abecasis et al., 2002).

Sham et al. (2002) described a regression-based procedure for linkage analysis which uses trait-squared sums and differences to predict IBD sharing between any non-inbred relative pairs. The following calculations of this method were described in Sham et al. (2002), and a brief description of these computations is mentioned below:
In a family pedigree with a certain number (n) of related individuals' descendants, consider the values of a particular trait of interest (X) of the descendant family members $X_1, X_2, ..., X_n$, respectively. The mean and the variance for these values of $X_1, X_2, ..., X_n$, were standardized to mean 0 and variance 1, while the joint multivariate distribution was considered to be normal. For a pair of pedigree individuals, $S_{ij} = (X_i + X_j)^2$ used to calculate the squared sum, and $D_{ij} = (X_i - X_j)^2$ where $i \neq j$ used to calculate the squared differences. Moreover, the part of alleles IBD for pedigree individuals i and j (π_{ij}) was

estimated from the genotypic data and given as $\hat{\pi}_{ij}$. These computations were carried out using Lander-Green algorithm (Lander and Green, 1987) using the MERLIN software package (Abecasis et al., 2002). The arrays $[S_{ij}],[D_{ij}]$, and $[\hat{\pi}_{ij}]$ of the whole family pedigree was implemented into the vectors S, D, and $\hat{\Pi}$ whose dimension each was $n(n-1)/2$. This approach regresses IBD sharing alleles on squared sums and squared differences (D). Therefore, $\hat{\Pi}$ is regressed on S and D.

The PWR analysis was implemented using the following command:

merlin-regress –d filename.dat –p filename.ped –m filename.map –mean 0.0 --var 1.5 --her 0.8

Quantitative transmission disequilibrium test (QTDT) (Association analysis method)

The association analysis was carried out using the software QTDT v 2.6.0 (Abecasis et al., 2000a, b). The QTDT is considered as an appropriate way to test family-based pedigree of LD (Rosyara et al., 2009). Abecasis et al. (2002) used the IBD coefficients resulted from analysis using MERLIN software to calculate QTDT or association through QTDT software.

The following command was used to produce IBD coefficients:

merlin –d filename.dat –p filename.ped –m filename.map --markerNames –ibd

Using QTDT software, within and between family components of association can be calculated (Rosyara et al., 2009). Abecasis et al. (2000b) described the association model to test the association of individual alleles of any locus with the trait of interest. In the QTDT analysis, the following hypotheses were used to test the association components:

Null hypothesis $(H_0): \overline{X} = \mu + B,$

Alternative hypothesis $(H_A): \overline{X} = \mu + B + W,$

Where B is between component of association and W is within component of association.

By default QTDT was used to test association by fitting a simple linear model to the data.

The following command was used for QTDT:

qtdt –p filename.ped –d filename.dat

The QTL locations were mapped using MapChart© 2.2 (Voorrips, 2002).

Results and discussion

The frequency distribution of chlorophyll content at 50% anthesis (0 DPA) is shown in Fig. 3.2. The plot indicates normal distribution. Frequency of chlorophyll content at 4 days post anthesis (4 DPA) approximated a normal distribution (Fig. 3.3); whereas content at 8 DPA did not (Fig. 3.4). Frequency distribution of flag leaf temperature

depression (FLTD) at 50 % anthesis followed a normal distribution (Fig. 3.5); whereas that at 4 and 8 DPA did not (Figs. 3.6 and 3.7). Similarly, individual kernel weight (IKW) did not follow a normal distribution (Fig. 3.8).

All the polymorphic markers tested along with their informative alleles for the pedigree founders were presented in Table 3.7. The number of alleles produced ranged from 2 to 8.

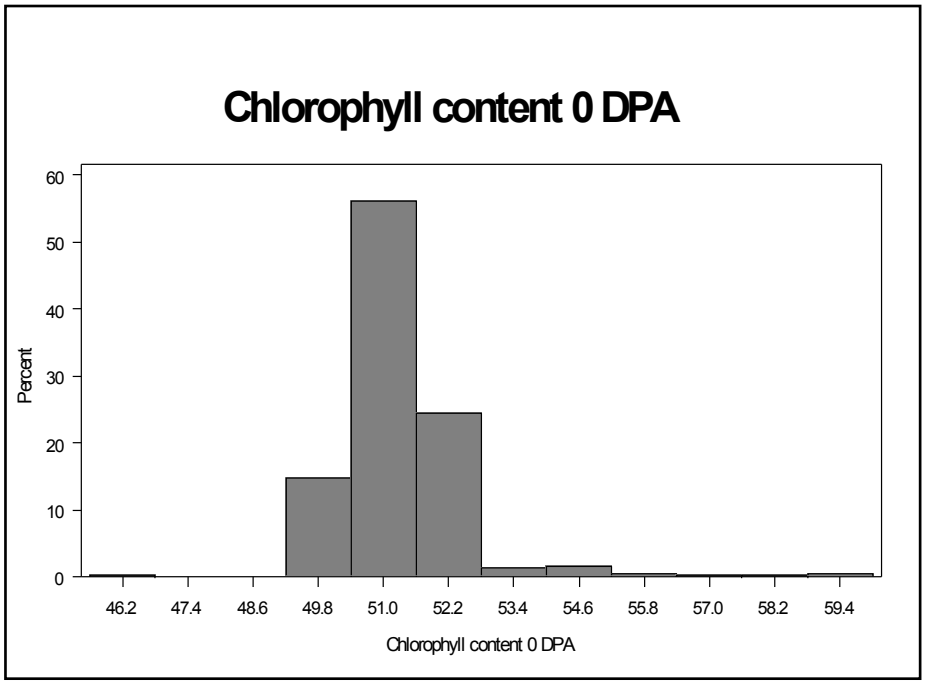

Fig. 3.2. Frequency distribution of chlorophyll content right at anthesis (0 DPA).

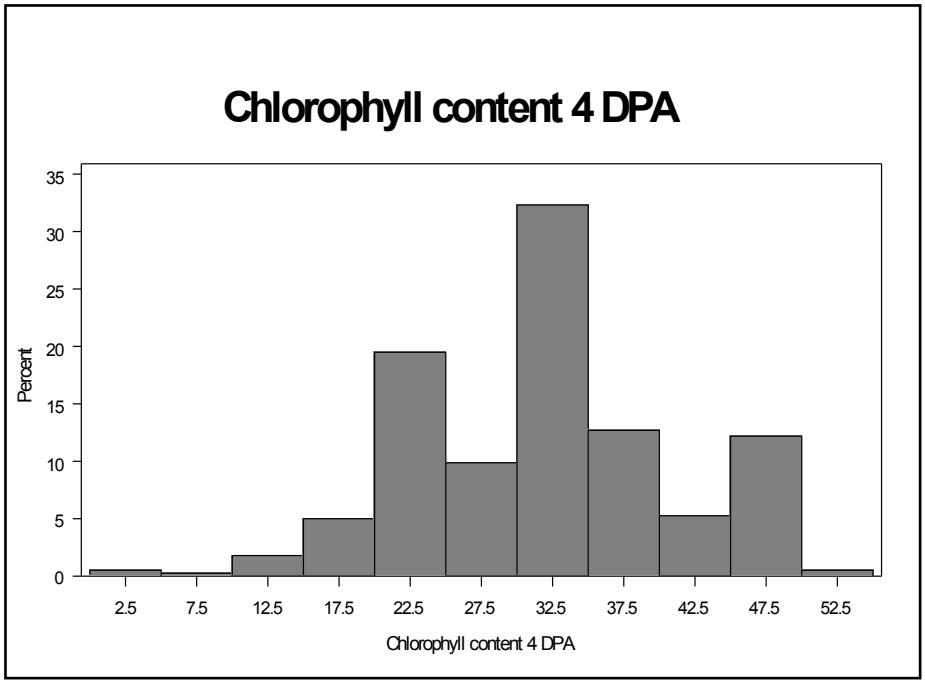

Fig. 3.3. Frequency distribution of chlorophyll content at four days post anthesis (4 DPA).

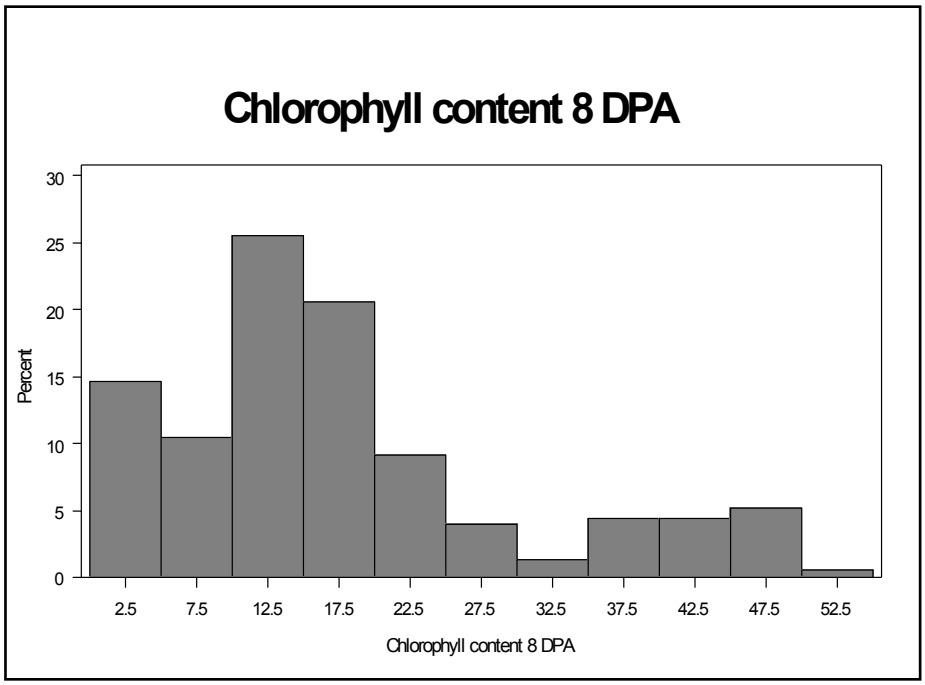

Fig. 3.4. Frequency distribution of chlorophyll content at eight days post anthesis (8 DPA).

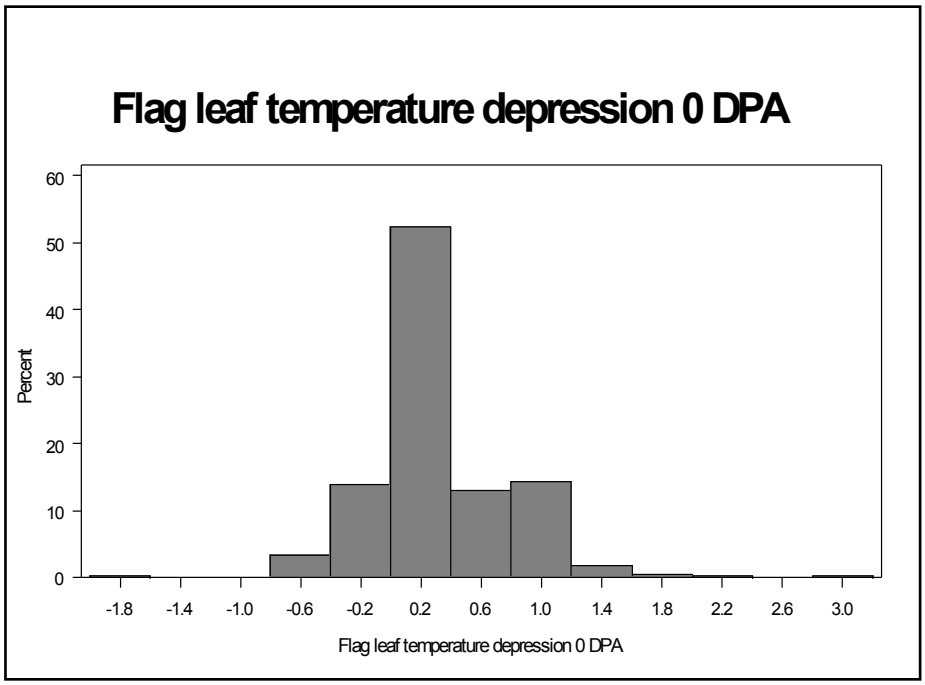

Fig. 3.5. Frequency distribution of flag leaf temperature depression right at anthesis (0 DPA).

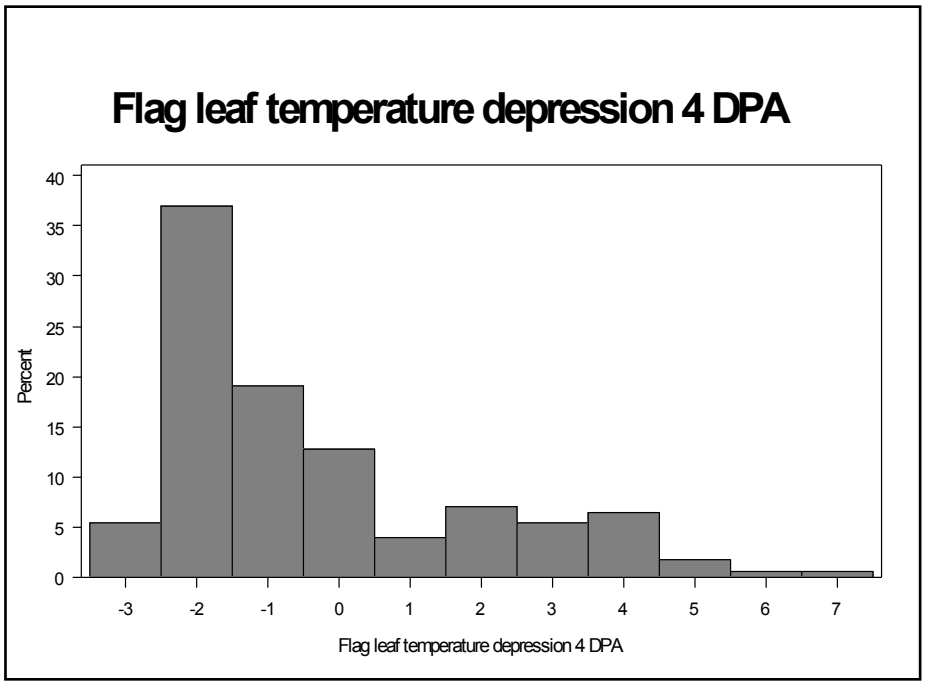

Fig. 3.6. Frequency distribution of flag leaf temperature depression at four days post anthesis (4 DPA).

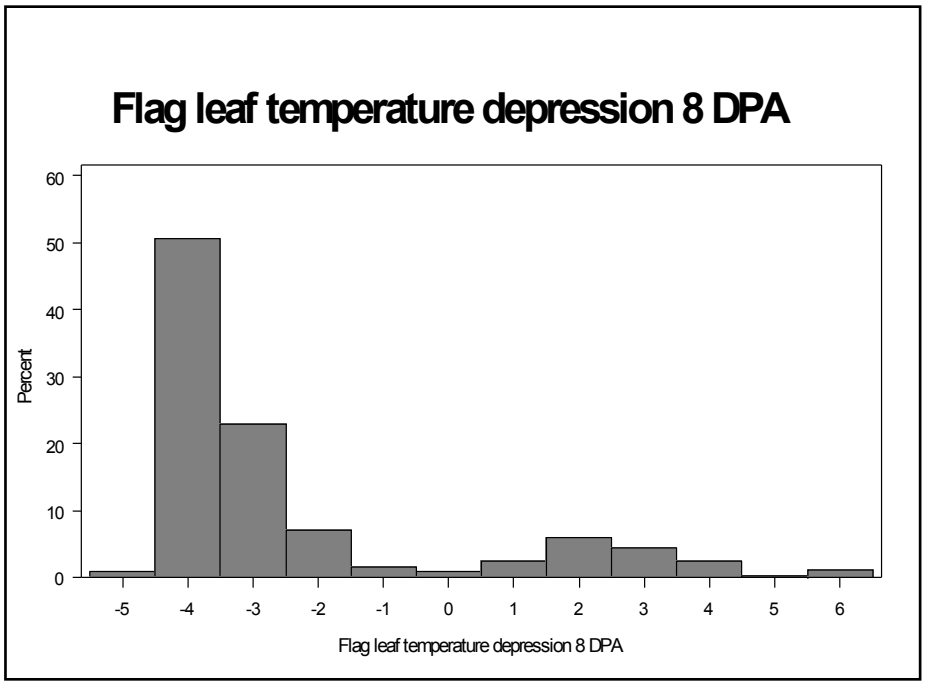

Fig. 3.7. Frequency distribution of flag leaf temperature depression at eight days post anthesis (8 DPA).

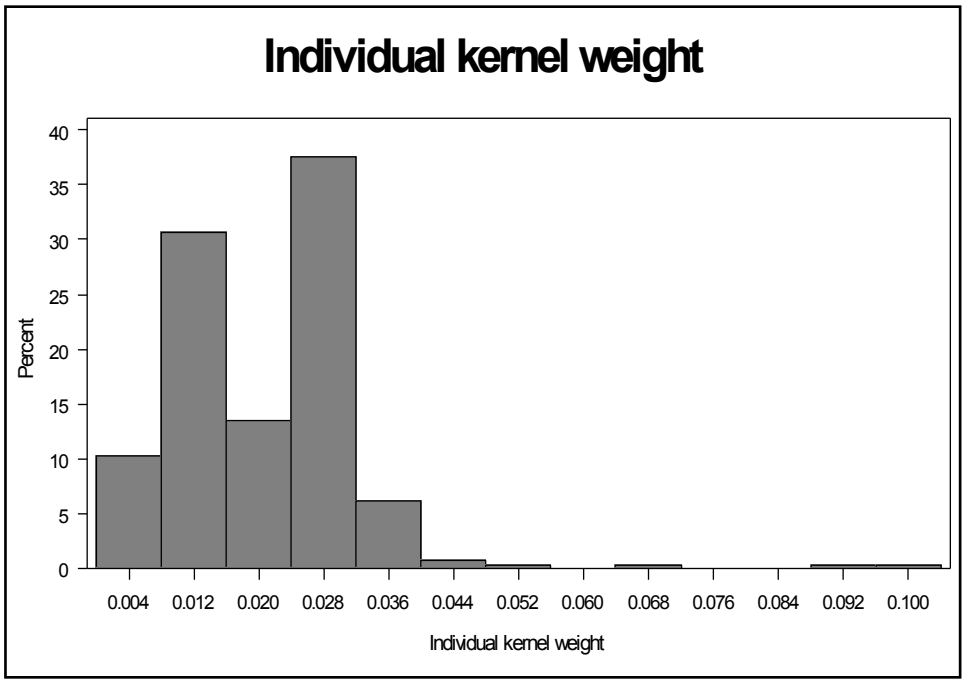

Fig. 3.8. Frequency distribution of individual kernel weight.

Table 3.7. Marker loci and their informative allele size for the pedigree founders.

SN	Markers	size (bp)	Parents
1	*Xgwm169*	201	IG83047
		203	IG44999, IG45413, IG88723, IG45448, IG45441
		205	IG127682, IG11572
		207	IG44961
		224	IG45069
2	*Xwmc388*	163	IG83047, IG45441
		173	IG44999,IG45413, IG45448
		175	IG88723
		177	IG127682, IG44961, IG110572
		179	IG45069
3	*Xwmc479*	205	IG45069
		216	IG45413
		219	IG44999, IG83047, IG88723, IG127682, IG45448, IG45441
		221	IG44961
		225	IG110572
4	*Xwmc179*	110	IG44999, IG45413, IG83047, IG88723, IG127682, IG45448, IG44961, IG110572, IG45441, IG45441
		261	IG45069
5	*Xwmc527*	391	IG44999, IG45413, IG88723, IG44961
		393	IG127682
		397	IG110572
		399	IG83047
		401	IG45441
		404	IG45069
		414	IG45448
6	*Xbarc200*	157	IG45069
		186	IG44999, IG45413, IG127682, IG44961, IG110572, IG45441, IG88723, IG83047, IG45448
7	*Xbarc55*	135	IG45069
		140	IG44999, IG45413, IG83047, IG45448, IG44961, IG110572
		149	IG45441
		154	IG127682
		172	IG88723

Table 3.7. continued.

SN	Marker	Size (bp)	Parents
8	*Xgwm210*	182	IG44999, IG45413, IG83047, IG88723, IG127682, IG45448, IG44961, IG110572, IG45441
		186	IG45069
9	*Xgwm219*	160	IG83047
		171	IG127682, IG45448, IG110572, IG45441
		179	IG45413
		188	IG44961
		192	IG88723
		198	IG44999
		200	IG45069
10	*Xgwm18*	196	IG127682, IG11572
		198	IG44999, IG45413, IG44961, IG45441
		202	IG45069
		204	IG83047, IG88723
		206	IG45448
11	*Xbarc197*	157	IG44999, IG45413, IG83047, IG88723, IG127682, IG45448, IG44961, IG110572, IG45441
		199	IG45069
12	*Xwmc79*	140	IG45448
		146	IG45441
		150	IG127682, IG110572
		156	IG45069
		168	IG44999, IG45413, IG83047, IG88723, IG44961
13	*Xwmc361*	235	IG44999, IG45413, IG83047, IG88723, IG45448, IG44961, IG45441
		237	IG45069
		246	IG127682, IG110572
14	*Xbarc23*	238	IG44999, IG45413, IG88723, IG45448, IG44961
		241	IG45069
		247	IG83047
		250	IG127682
		264	IG45441
		276	IG110572

Table 3.7. continued.

SN	Marker	Size (bp)	Parents
15	*Xbarc32*	132	IG45448
		173	IG44961
		179	IG44999, IG127682
		185	IG45069
		188	IG83047
		194	IG45413, IG45441
		197	IG88723
		204	IG110572
16	***Xbarc10***	296	IG44999, IG45413, IG83047, IG88723, IG127682, IG45448, IG44961, IG110572, IG45441
		298	**IG45069**
17	*Xbarc178*	287	IG45448
		291	IG83047, IG11057, IG45441
		294	IG45069
		302	IG88723
		306	IG44999, IG45413, IG127682, IG44961
18	*Xbarc56*	122	IG88723, IG127682, IG45448, IG44961, IG110572, IG45441
		130	IG45069
		134	IG44999, IG45413, IG83047
19	*Xbarc78*	173	IG44999, IG45413, IG88723, IG127682, IG44961, IG127682
		180	IG45069
		156	IG83047
		164	IG45448
		184	IG110572
		177	IG45441
20	*Xbarc20*	204	IG45069
		206	IG44999, IG45413, IG83047, IG12782, IG45448, IG44961, IG110572, IG45441
		216	IG88723
21	*Xbarc186*	220	IG45413, IG83047, IG88723, IG44961, IG110572, IG45441
		229	IG45069
		231	IG44999, IG127682, IG45448

Table 3.7. continued.

SN	Markers	Size (bp)	Parents
22	*Xbarc70*	217	IG44999, IG83047, IG45448, IG44961
		221	IG45441
		240	IG127682
		244	IG45413, IG88723, IG110572
		246	IG45069
23	*Xgwm234*	205	IG45448
		219	IG44999, IG83047, IG44961, IG110572, IG127682
		235	IG45441
		246	IG45413, IG88723, IG127682
		267	IG45069
24	*Xbarc163*	169	IG110572, IG45441
		175	IG45069
		178	IG83047, IG88723, IG127682
		181	IG45448
		184	IG44999, IG45413, IG44961
25	*Xbarc183*	154	IG88723
		167	IG44999, IG83047, IG44961, IG45441
		169	IG45413
		176	IG127682
		184	IG45069
		189	IG110572
		197	IG45448
26	***Xbarc128***	215	IG45413, IG44961, IG110572, IG44999, IG83047, IG45441, IG45448, IG127682, IG88723
		231	**IG45069**
27	*Xbarc60*	255	IG45069
		259	IG44999, IG45413, IG45448, IG83047, IG88723, IG45441, IG127682, IG44961, IG110572
28	***Xgwm205***	151	IG88723, IG45413, IG44999, IG83047, IG127682, IG45448, IG44961, IG110572, IG45441
		157	**IG45069**

Table 3.7. continued.

SN	Marker	Size (bp)	Parents
29	*Xgwm382*	102	IG83047
		106	IG44999, IG127682, IG44961
		115	IG45069
		137	IG45413
		139	IG45448
		141	IG45441
		145	IG88723, IG110572
30	*Xwmc661*	175	IG45448
		181	IG127682, IG45441
		185	IG44999, IG45413, IG83047, IG88723, IG44961, IG110572
		207	IG45069
31	*Xwmc245*	140	IG45069
		143	IG44999, IG83047, IG88723, IG127682, IG45448, IG45441, IG44961, IG110572
		154	IG45413
32	*Xgdm136*	103	IG45069
		107	IG44961
		110	IG44999, IG45413, IG83047, IG88723, IG127682, IG45448, IG110572, IG45441
33	*Xgwm495*	173	IG45448
		179	IG45069
		185	IG45413, IG88723
		192	IG44999, IG127682, IG44961, IG110572, IG45441
		194	IG83047
34	*Xwmc235*	245	IG110572
		248	IG44999, IG83047, IG88723
		253	IG45069
		255	IG44961
		258	IG45441
		260	IG45448
		264	IG45413
		298	IG127682

Table 3.7. continued.

SN	Markers	Size (bp)	Parents
35	*Xgwm601*	139	IG44999, IG45413, IG88723, IG127682, IG45448, IG44961, IG45448, IG110572, IG45441
		142	IG45069
		144	IG82047
36	*Xbarc35*	340	IG45069
		351	IG83047
		360	IG88723
		363	IG44999, IG45413, IG127682, IG45448, IG44961, IG45441
		368	IG1105072
37	*Xbarc180*	175	IG88723
		201	IG83047
		205	IG110572
		208	IG44999, IG45413, IG44961
		210	IG45441
		217	IG45448
		220	IG127682
		224	IG45069
38	*Xgwm162*	221	IG44999, IG45413, IG83047, IG88723, IG45448, IG44961
		223	IG12682, IG110572, IG45441
		227	IG45069
39	*Xwmc500*	164	IG45441
		166	IG44999, IG45413, IG127682, IG45448, IG44961, IG110572
		176	IG88723
		180	IG83047
		183	IG45069
40	*Xbarc40*	101	IG44999, IG45413, IG83047, IG88723, IG127682, IG45448, IG44961
		198	IG110572, IG45441
		220	IG45069

Linkage analysis -variance component (VC) and pedigree wide regression (PWR) methods

Similar results for chlorophyll content at 4 DPA were found using VC-based (Fig. 3.9a) and PWR-based linkage analyses methods (Fig. 3.9b). A QTL was found at the *Xbarc128* marker locus. This marker is located on chromosome 1B. At 8 DPA, a similar QTL was found at *Xbarc128* using VC (Fig. 3.10a) and PWR (Fig. 3.10b) methods on the same chromosome with slight differences in LOD score values. For FLTD, a QTL was found at *Xbarc10* on chromosome 2B for 4 DPA and 8 DPA using both VC and PWR methods (Figs 3.11a,b and 3.12a,b). For IKW, a QTL was located at *Xgwm205* on chromosome 5A using VC (Fig. 3.13a) and PWR methods (Fig. 3.13b). The other markers on the rest of the genome showed low LOD scores, which was indicative of absence of association with heat tolerance in this population. For example, *Xgwm18* on chromosome 1B (LOD = 1.8 and 1.9 for 4 and 8 DPA, respectively, for chlorophyll content; LOD = 1.79 and 1.8 for 4 and 8 DPA, respectively, for FLTD; LOD = 1.9 for IKW) was not significant.

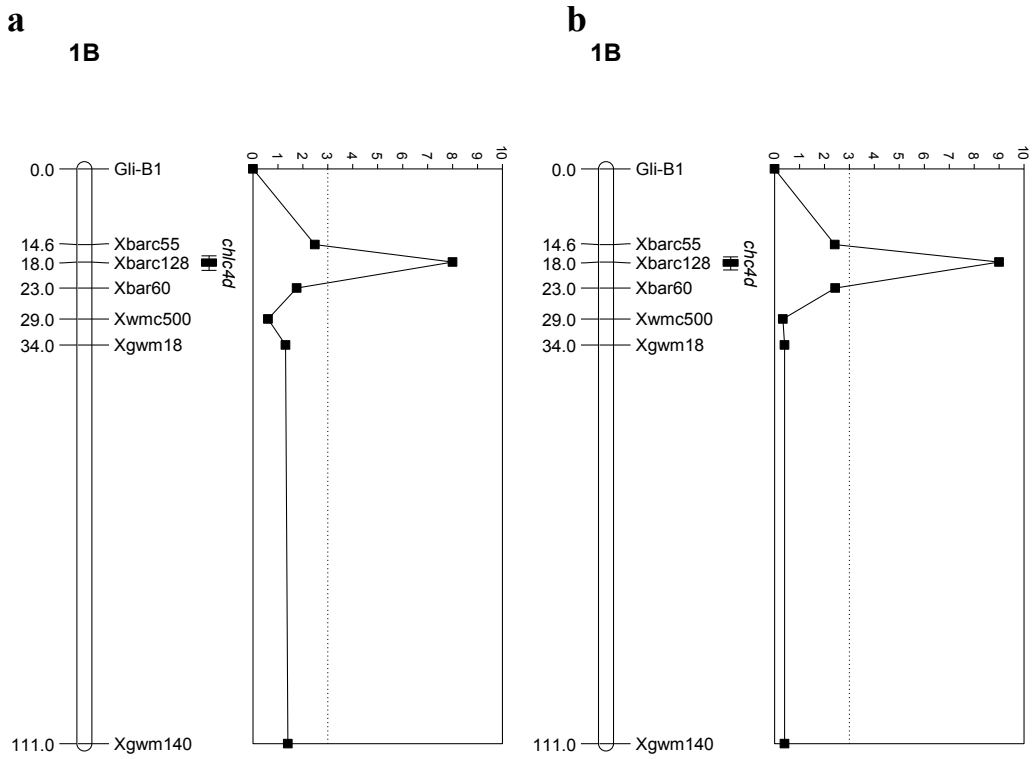

Fig. 3.9. Position of chlorophyll content (*chlc*) QTL based on **a** variance components (VC) based linkage analysis and **b** pedigree wide regression (PWR) linkage analysis at four days post anthesis (4 DPA) using MERLIN. QTL locations are indicated with 1 and 2 LOD confidence interval.

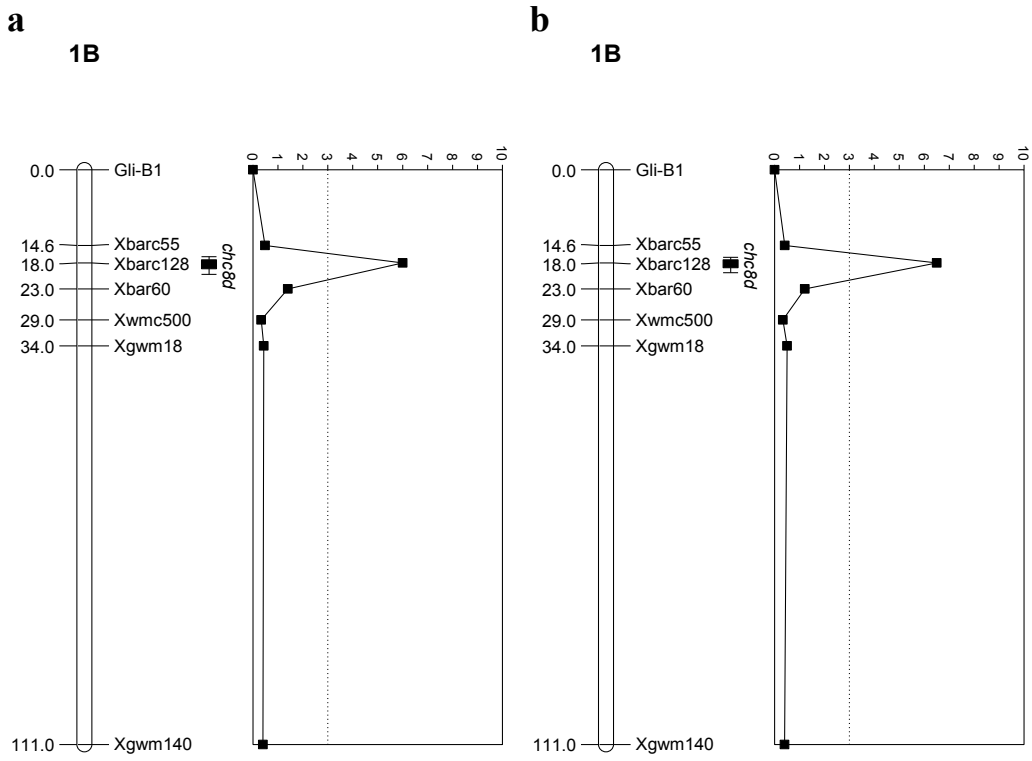

Fig. 3.10. Position of chlorophyll content (*chlc*) QTL based on **a** variance components (VC) based linkage analysis and **b** pedigree wide regression (PWR) linkage analysis at eight days post anthesis (8 DPA) using MERLIN. QTL locations are indicated with 1 and 2 LOD confidence interval.

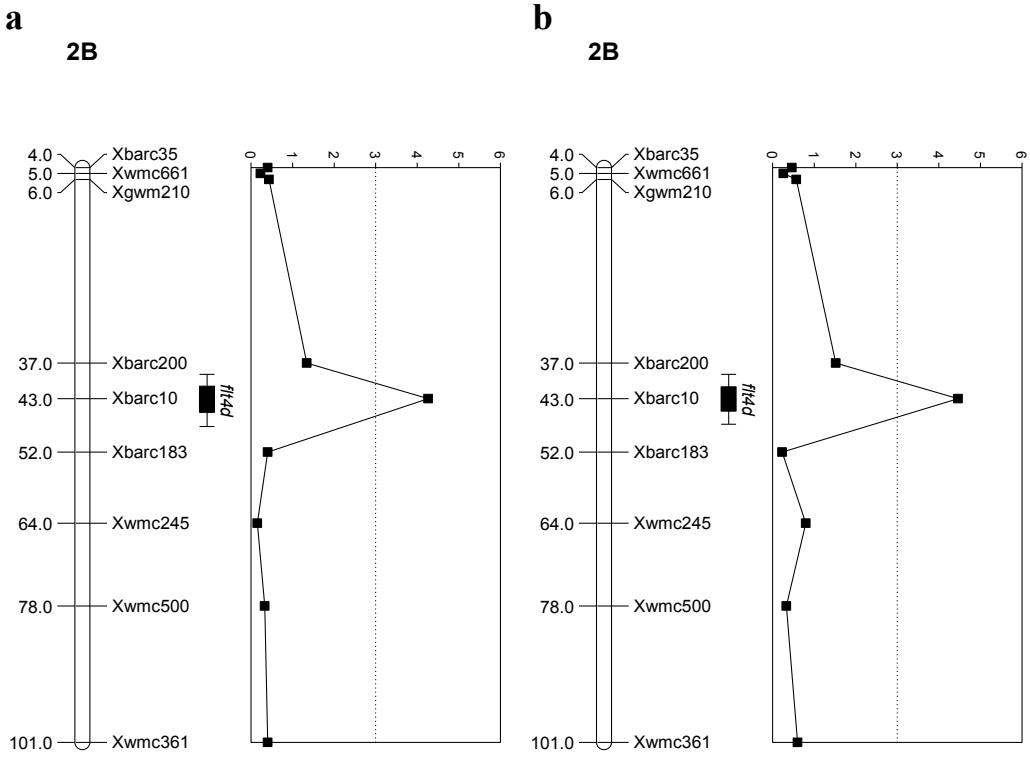

Fig. 3.11. Position of flag leaf temperature depression (*flt*) QTL based on **a** variance components (VC) based linkage analysis and **b** pedigree wide regression (PWR) linkage analysis at four days post anthesis (4 DPA) using MERLIN. QTL locations are indicated with 1 and 2 LOD confidence interval.

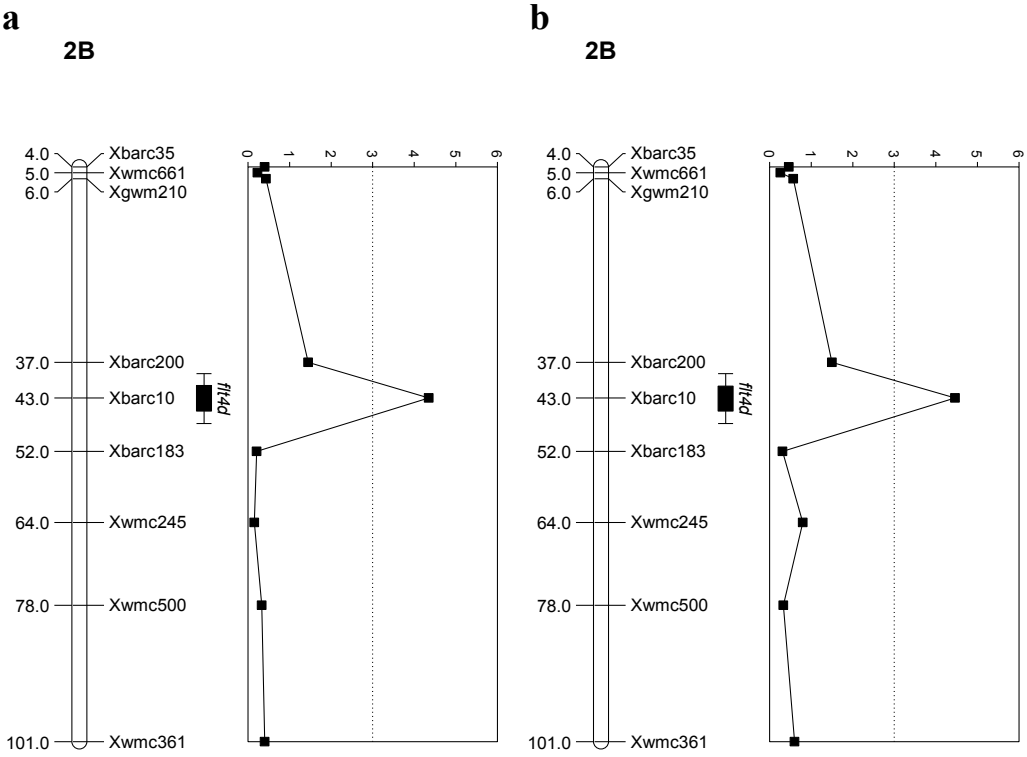

Fig. 3.12. Position of flag leaf temperature depression (*flt*) QTL based on **a** variance components (VC) based linkage analysis and **b** pedigree wide regression (PWR) linkage analysis at eight days post anthesis (8 DPA) using MERLIN. QTL locations are indicated with 1 and 2 LOD confidence interval.

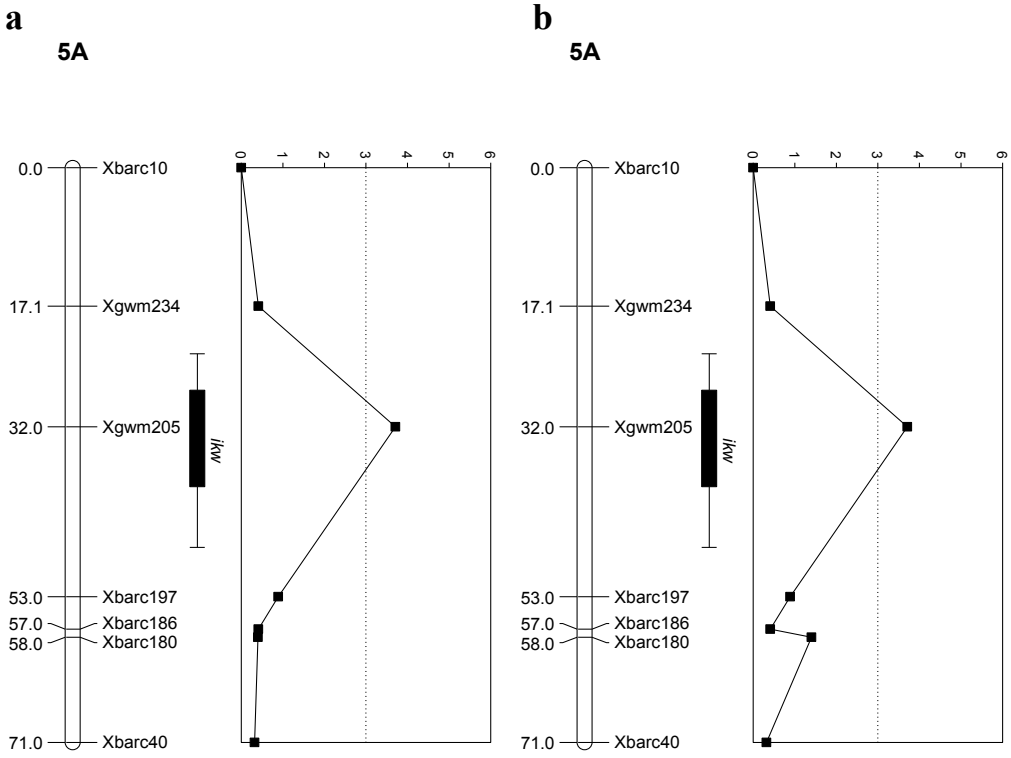

Fig. 3.13. Position of individual kernel weight (*ikw*) QTL based on **a** variance components (VC) based linkage analysis and **b** pedigree wide regression (PWR) linkage analysis using MERLIN. QTL locations are indicated with 1 and 2 LOD confidence interval.

Association method

Association analysis was conducted using QTDT v 2.6.0 (Abecasis et al., 2000a,b). The results revealed that *Xbarc128*, *Xbarc10*, and *Xgwm205* were linked to heat stress

tolerance as measured by chlorophyll content, FLTD, and IKW, respectively. For the aforementioned marker loci, the allele transmitted from the heat-tolerant parent was correlated with the respective QTL. Furthermore, based on association analysis, the following alleles had the highest significant association with the phenotypic data: *Xbarc128-231* ($\chi^2 = 36.4$, $P = 1\times10^{-7}$), and *Xbarc10-298* ($\chi^2 = 19$, $P = 2\times10^{-6}$) for chlorophyll content and FLTD, respectively, at 4 DPA. For 8 DPA, the same alleles, *Xbarc128-231* ($\chi^2 = 25$, $P = 2\times10^{-7}$) and *Xbarc10-298* ($\chi^2 = 20$, $P = 2\times10^{-6}$), revealed association with chlorophyll content, and FLTD, respectively. The allele showing the strongest association with the phenotypic data for IKW was *Xgwm205-157* ($\chi^2 = 17$, $P = 2\times10^{-5}$). All these alleles were transmitted from the heat-tolerant parent (IG45069).

Evaluation of the breeding populations for heat stress tolerance measured through chlorophyll content, FLTD, and IKW followed a normal distribution only before applying heat stress treatment and under heat stress treatment only for chlorophyll content at 4 DPA. This could be due to a smaller than optimal population size (<1,000). VC-based linkage analysis and PWR-based linkage analysis revealed that the marker locus of *Xbarc128* was significantly linked to heat stress tolerance and had LOD scores > 3.0 (threshold level) for chlorophyll content measured at both 4 DPA and 8 DPA for chlorophyll content. For FLTD, the marker *Xbarc10* was significantly associated with heat stress tolerance with LOD score > 3.0 for FLTD measured at both 4 DPA and 8 DPA. For VC-based and PWR-based linkage analyses, marker *Xgwm205* was

significantly linked to heat stress tolerance and had LOD score > 3.0 based on individual kernel weight.

Mason et al. (2010) detected several QTL associated with heat tolerance in common wheat. These include QTL controlling HSI on chromosome 2B associated with marker *Xgwm111* (36.9 cM) which maps very close to marker *Xbarc10* (43cM) that is associated with FLTD in our study. Furthermore, in the current study, we detected a QTL associated with IKW linked with marker *Xgwm205* (32 cM) on chromosome 5A. Mason et al. (2010) detected QTL associated with HSI linked to *Xwmc150* (28.4 cM) and *Xbarc197* (45.5 cM) on the same chromosome, 5A. The slight differences in the location of the QTL in the two studies might be attributed to the following reasons: 1) the current study used wild tetraploid wheat whereas Mason et al. (2010) used common hexaploid wheat. Although the two species share the same A and B genomes, they have evolved and developed separately over many years under old and modern agriculture practices, 2) the wild tetraploid wheat genotypes used in the current study have not been manipulated by breeding, 3) differences in the consensus maps used to identify marker positions were slightly different. For instance, *Xbarc10* was based on Somers et al. (2004) whereas *Xgwm111 was based on* Röder et al. (1998).

Plants have an inherent advantage compared to humans due to the contrasting phenotype of the parents for traits measured in this study which the development of adequate family-based mapping structures. In the current study, heat tolerant and susceptible

parents were selected and progeny formation was manipulated to fit the objectives of this study which is seldom achievable in human populations.

Sourdille et al. (2001) showed that SSR markers from the A and D genomes always amplified on the A and D diploid genomes; therefore, they suggested that SSRs developed from these diploid species should be exploitable in wheat. Consequently, we suggest that SSR markers associated with heat stress tolerance in wild tetraploid wheat (including genomes A, and B) might be utilizable in both cultivated durum and common wheat to help in MAS or MAB and to improve the heat stress tolerance.

The outcomes from QTDT to detect association between marker loci and phenotype were consistent with the results from VC- and PWR-based linkage analyses. The high probability values suggested that alleles of these markers were associated with heat stress tolerance. In each marker locus the alleles originating from the heat-tolerant parent were highly significantly associated with heat stress tolerance. Rosyara et al. (2009) declared that QTDT focus on transmission of particular alleles from different locus; consequently, it can be a great tool to recognize useful markers and their alleles for MAS or MAB.

Our findings are consistent with previous studies (Glazier et al., 2002; Mackay, 2001; Rosyara et al., 2009) revealing that linkage analysis is more useful for a genome-wide scan for QTL while association analysis gives more precise location of an individual

QTL. Our study is consistent with Rosyara et al. (2009) showing that MERLIN and QDTD software packages were convenient for plant studies.

Inclusion of breeding pedigrees in the family-pedigree method is not appropriate (Rosyara et al., 2009). The family-pedigree approach based on single plant phenotyping and genotyping, equivalent to single individuals in human or animal studies has been used in a family-based population with resistance to Fusarium head blight in wheat (Rosyara et al., 2009). Consequently, we suggest that the family-pedigree method be exploited in heat stress tolerance studies in wheat based on single plant phenotyping and genotyping. Therefore, family-pedigree would be a useful method in early generation testing when multiple parents are used to create diverse background for selection.

CHAPTER IV

CONCLUSION

Our results showed significant positive association between damage to thylakoid membranes and heat susceptibility index under heat stress (36/30 °C; day/night). Meanwhile, a pulse modular fluorometer, that measures chlorophyll a fluorescence, is a reliable tool for screening for heat stress tolerance in wheat. Furthermore, our results revealed that either flag leaf temperature depression (FLTD) or spike temperature depression (STD) can be used to assess heat stress tolerance in wheat due to positive and significant association with one another on one hand and with heat susceptibility index on the other hand.

The current study proved that wild tetraploid wheat possesses excellent sources of heat tolerance which warrants its inclusion in crossing blocks of breeding programs aimed at improving heat tolerance in both common and durum wheats.

Using linkage analysis methods (variance component [VC] and pedigree wide regression [PWR]) enabled the identification of the same QTL at *Xbarc128* for chlorophyll content at 4 DPA and 8 DPA with slightly differences in LOD score on chromosome 1B. For FLTD, both linkage methods (VC and PWR) led to identifying the same QTL at *Xbarc10* on chromosome 2B with minor differences in LOD score. For IKW, a QTL was found at *Xgwm205* marker locus using both methods of linkage analysis.

The association analysis method revealed that alleles which were transmitted from the heat-tolerant parent (*Xbarc128-231*, *Xbarc10-298*, and *Xgwm205-157)* showed the strongest association for chlorophyll content, FLTD, and IKW at both 4 and 8 DPA. Also, our results showed that the outcomes from QTDT to identify the association between marker loci and phenotype were consistent with the results from both VC- and PWR-based linkage analyses. Our results revealed that linkage analysis is more useful for a genome-wide scan for QTL; whereas association analysis showed the precise location of an individual QTL.

MERLIN and QTDT can be suitable for data analysis of family-pedigree approach for heat stress tolerance based on single plant phenotyping and genotyping.
Our results suggested that SSR markers associated with heat stress tolerance in wild tetraploid wheat can be utilized as a tool for MAS and MAB in improving heat stress tolerance in both common and durum wheat.

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