Association mapping for heat tolerance in D-genome synthetic hexaploid wheats

QUAID-I-AZAM UNIVERSITY
ISLAMABAD

A PhD dissertation in partial fulfillment for the degree of doctor of philosophy in Plant Sciences (Plant Biochemistry and Molecular Biology)

by

Abdul Aziz

Department of Plant Sciences,
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In dedication to
my loving, caring and supportive
parents,
family and to my Elder Brother their
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LIST OF ABBRREVIATIONS

AFLP	Amplified fragment length polymorphism
AM	Association mapping
ANOVA	Analysis of variance
bp	Base pair
BWP	Bahawlpur
Chr	Chromosome
Cm	Centimeter
cM	Centimorgan
CV	Coefficient of variation
DArT	Diversity array technology
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide phosphates
EDTA	Ethylenediaminotetraacetic acid
FDR	False discovery rate
GLM	General linear model
GWAS	Genome wide association studies
GY	Grain yield
h^2	Broad sense heritability
HS	Heat stress
ISD	Islamabad
LD	Linkage disequilibrium
MAF	Minor allele frequency
MAS	Marker assisted selection
MLM	Mixed linear model
NOR	Normal treatment
PCA	Principal component analysis

PCR	Polymerase chain reaction
QTL	Quantitative trait loci
RAPD	Randomly amplified polymorphic DNA
SND	Sindh
SNP	Single nucleotide polymorphism
SPAD	Soil and plant analyzer development
SSR	Simple sequence repeat
Taq	Thermus aquaticus
µL	Microlitre

Abstract

Adaptation of wheat (*Triticum aestivum* L.) to high temperature could be improvedby introducing genes from wild relatives. We evaluated the responses of 200 D genome synthetic hexaploid wheats (*T. turgidum* × *Aegilops tauschii*; 2n=6x=42;AABBD^{t}D^{t}) to high temperature to determine their potential for wheat improvement under field and controlled conditions. Under controlled conditions at grain filling stage, wheat plants were exposed to a brief heat stress (3 days, 37/27 ºC) 10 days after anthesis and the plants evaluated for a number of morphological and physiological traits. In total, 114 synthetic hexaploid wheats and 6 bread wheat genotypes were evaluated for different heat responses during the grain filling stage. Considerable genetic variation was observed among wheat genotypes for various heat responses, particularly for single grain weight, chlorophyll retention, rate and duration of grain filling.Overall, the findings suggested that more than one adaptation process contributed to heat tolerance. Generally, genotypes with more stable grain weight under heat tended to have particular traits under stress, including the ability to maintain chlorophyll content and rate and duration of grain filling. Therefore, these traits may provide appropriate selection criteria for improving heat tolerance in wheat.

Field experiments were conducted in two different temperature scenarios (normal sowing time, "NOR", and late sowing time to expose the plants to heat stress, "HS"), for two years at three different locations, to assess the effect of terminal high temperature on yield related traits. High temperature stress overall led to 39% reduction in grain yield and significant reductions of 28% in days to heading, 26% in plant height, 16% in grain number m^{-2}, and 18% in thousand kernel weight. The six most heat tolerant synthetic hexaploid wheats (SHWs) on the basis of higher grain yield across environments were AUS30284, AUS33384, AUS30288, AUS3029, AUS33409, and AUS30629. In ridge regression analysis, individual agronomic traits explained between 8.74 to 35.2% of variation in grain yield under HS treatments, with an average of 30.47%. In NOR treatments, individual agronomic traits explained between 8.85 and 45.49% of the variation in grain yield, with an average of 34.45% across all traits. Although, days to heading was negatively correlated with grain yield in heat stressed environment, but unlike adapted germplasm it did not explained significant variation in grain yield. Thousand grain weight explained more of the variation in grain yield in all environments followed by grain numbers m^{-2}.

Biochemical and physiological traits under terminal heat stress we evaluated were evaluated under field conditions using 13 Synthetic hexaploid wheats.High temperature stress overall led to 24.26% reduction in chlorophyll-a, 28.16% in chlorophyll-b, 22.16% in total chlorophyll, and 31.73% membrane stability index leakage was observed. While an increase of 29.83% soluble sugarand 41.78% proline contents in heat stress compared to normal was observed. Grain yield was positively correlatedwith the thousand-kernel weight (r= 0.67)in normal and heat stress environment and Chlorophyll a, Chlorophyll b and total chlorophyll have significant positive correlation with membrane stability index (r= 0.85, 0.63 and 0.60) in both normal and heat environments, respectively.

Association mapping (AM) was used to find out marker-trait associations (MTA) in SHW for heat tolerance using DArT markers. Data from all field experiments was used to find significant MTAs. LD and population structure discovered a high amount of genetic diversity present within SHWs. Five key sub-populations were identified using population structure within this panel of association mapping. In total, 17 MTAs for grain yield related traits in heat stress and 14 MTAsin normal environments were identified on chromosome 1A, 3B, 3D, 5B, 6B and 7B. From these 31 MTAs, 14 MTAs were common in both heat and normal conditions. These MTAs can be candidates for cloning genes linked to heat tolerancestudies.

In conclusion synthetics hexaploid showed significant variation for grain yield and physiological traits and highly tolerant synthetic hexaploids were identified that can be used further to develop high yielding verities adapted under high temperature stress.

Chapter 1

1. INTRODUCTION

Wheat is biosphere's preeminent crop in terms of productivity, nutrition and harvested area. For 8000 years, it is considered as the most essential food in Europe, North Africa and central Asia. Overall it gives 690 million tons' grain production (FAOSTAT, 2013). Furthermore, globally food need has been predicted to raise up continuously for 40 years (Godfray et al., 2010). The global requirement for cereal is calculated to increase1048 million metric tons in 2050, which is 56% higher than the demand for the year 2000 and 26% increase is predicted for wheat (Hubert et al., 2010). Hence, it is imperious to enhance the wheat productivity to keep pace with the food requirements of global population.

Wheat (*Triticum aestivum* L.) has a great buffered genotype due to polyploidy, showing huge genetic changeability having three different alleles. The productiveness of the major crops including cereals is decreased by many factors including biotic and abiotic stresses including high temperature. In many region of world approaching climate states suggest that global warming may be auspicious for wheat, but in the zones with optimal temperatures these climate changes could lower wheat productivity by lowering grain yield (Tubiello et al., 2000).

One of the major factor affecting the crop productivity across the world adversely is Climate change (Qin et al., 2002), (Farooq et al., 2011). Climate change prediction models suggested that this elevation will rise up by 1.8-5.8°C (IPCC, 2007), hence, in near future this heat elevation and stress will be alarming for wheat growing areas (Mitra and Bhatia, 2008; Semenov, 2009). Wheat faces rise in temperature at different phenological phases and is very sensitive to heat. However, reproductive phase affected by heat stress ore adversely than vegetative phase. (Wollenweber et al., 2003: Wardlaw and Wrigley, 1994).

Wheat yield is highly affected by high temperature stress followed by lower water availability, so the main aim of wheat breeding should be developing the cultivars to endure both type of stresses (Tester and Bacic, 2005). In order to support food security, genetic variability is one of the more reliable way. In order to cope with both abiotic and biotic stresses *Aegilops* species are thought to provide the genetic variability and genetic resource to raise the genetic

prospective of growing wheat. (Kihara, 1944; McFadden and Sears, 1946),). Wheat wild species are the main cause of breeding for resilience to climate change (Mujeeb-Kazi et al 2013; Trethowan and Mujeeb-Kazi 2008). Previously, there has been lot of efforts to characterize wheat wild species for heat tolerance to identify promising candidates for pre-breeding and breeding Pradhan et al. 2012; Hairat and Khurana 2015).

Various new methods are introduced by Plant breeding to improve the crops and to raise the crop productivity which will help to increase the global food productivity. Modern technologies offer support to rise the selection efficiency for agronomically valuable traits as the availability of DNA markers of different types based on hybridization, polymerase chain reaction (PCR), plant retrotransposons and single nucleotide polymorphisms (SNPs) are used successfully for characterizing germplasm and mapping experiments (Landjeva et al., 2007). Since the first wheat RFLP maps were published by (Deynze et al., 1995), the major contribution to mapping efforts has been the development of 2,200 of SSR markers (http://wheat.pw.usda.gov/GG2/), many through public-private consortia. Microsatellites or simple sequence repeats (SSRs) are easy to use, (Röder et al., 1998: Chabane et al., 2008). Diversity Array Technology (DArT) provides whole-genome fingerprints, generally with a high marker density (Jaccoud et al., 2001; Marone et al., 2012). DArT markers are biallelic dominant markers (Neumann et al., 2011), hence the homozygous and heterozygous states cannot be distinguished (http://www. diversityarrays.com/). Many genetic maps of wheat that include DArT markers have been produced (Mantovani et al., 2008) (Wittenberg et al., 2005) (Akbari et al., 2006). These also helps to understand the genetic variation in non-model organisms. (James et al., 2008) and wild species (Alsop et al., 2011). Due to next generation technologies costs of sequencing DNA has been down to the point so that sequence genotype data is easy to get high genetic variation. (Poland et al., 2012). Genotyping-by-sequencing (GBS) (15 types) and 50 SNP arrays platforms are established in over 25 crop species.

Regardless of improvements, the genetic basis of abiotic stress adaptation is not well known (Krattinger et al., 2009), (Wahid et al., 2007) and no "heat-tolerance" genes have been cloned. (Cossani and Reynold, 2012). (Reynolds and Tuberosa, 2008; Pinto et al., 2010).

Generally, Linkage mapping studies are helpful to find genetic basis of quantitative qualities in plants. GWAS have become increasingly popular and successful, since they have been successfully used in animal and human genetics (Hästbacka et al., 1992). However, genome-wide association studies (GWAS) is used to between natural diversity and phenotypic traits and has been extensively used to overwhelmed the limitations of pedigree-based (QTL) mapping. (Breseghello and Sorrells, 2006; Sorrells and Yu, 2009). 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) to study population genetics and genetic dversity in natural populations and germplasm collections, and 3). GWAS has been extensively helpful to find the genes or QTLs in Arabidopsis (Atwell et al., 2010), rice (Huang et al., 2010), maize (Kump et al., 2011), potato (Malosetti et al., 2007), and wheat (Letta et al., 2013).

The aims and goals of the study were:

1) To screen synthetic hexaploid wheats for agronomic and physiological parameters in order to improve their breeding value
2) To study genotype by environment interaction for heat tolerance in synthetic hexaploid wheats
3) To conduct genome-wide association studies in synthetic hexaploid wheats for important physiological and agronomic traits under heat stress.

Chapter 2

2. REVIEW OF LITERATURE

Our crop production and environment is affected by Global climate change. Envirotyping" is proposed, as a third "typing" technology, to interpret the ecological influences on crops, accompanying with phenotyping and genotyping. Ecological factors can have composed through evaluation of companion organisms, soil information systems and soil measurement, geographic system and canopy properties. Genotype-by-environment interaction (GEI), integrative phenotyping, both biotic and abiotic factors and genes response to environmental signals helps in crop phenotype prediction and modeling.

2.1 Origin and domestication of bread wheat

The fertile-crescent prolonged to Iraq, Israel, Turkey, Syria, and Iran is the birth place of bread wheat (Gill and Friebe 2002). The origin of bread wheat occurred in two separate amphidiploidization events. Circa 380,000 years ago, hybridization between the diploid *Triticum urartu Tumanian* ex Gandilyan (2n = 2x = 14, A^uA^u) and the closest extant of *A. speltoides Tauschii* (2n = 2x = 14, SS), followed by 6 spontaneous chromosome doubling produced emmer wheat: *T. turgidum subsp. dicoccoides* (2n = 28, genomes AABB) (Dvořák and Zhang, 1990; Gill et al., 2007). About 10,000 years ago, the wild emmer wheat was domesticated following the mutation in its inflorescence and transformed in to *T. turgidum subsp. dicoccum (*AABB). Circa 8000 years ago, at farmers 'fields in Caspian Iran, due to second hybridization between *T. turgidum subsp. dicoccum* (AABB) and *Ae. tauschii* Coss. (2n = 14, genome DD), followed the spontaneous chromosome doubling that results into bread wheat, *Triticum aestivum* subsp. *aestivum* L. (2n = 42; AABBDD) (Kihara, 1944; McFadden and Sears 1946). The haploid genome of *T. aestivum* comprises 21 chromosomes of different sizes that consist of seven homeologous groups (2n=6x=42). Due to the presence of the Ph1 gene on the long arm of Chromosome 5B pairing of homeologous chromosomes is prohibited, making the hexaploid wheat genetics close to diploid species (Akhunov et al., 2003). The genome size of hexaploid wheat is approximately 17,000 Mb, roughly 40 times the size of rice, which results majorly from large duplication of gene and intergenic sequences (Akhunov et al., 2003).

2.2 Importance of wheat

Three important cereal crops are rice (*Oryza sativa*), maize (*Zea mays*) and Wheat (*Triticum aestivum* that renders approximately two-third of the energy to human diets and are main pillar to food production at global level (Peng et al., 1999). Wheat provides daily requirement of energy and possibly half of proteins to one-third of world population. The greater rise in worldwide wheat production resulted from greater yield per hectare.

Wheat is the basic natural fiber and staple food in Europe, West Asia and North Africa contributing 1/5th in human caloric intake (Shiferaw et al., 2013). It is used for human food consumption and for livestock feed as fodder and the largest crop in numerous abstemious countries. Its accomplishment is governed by partially on its higher potency, inherent capacity, adaptability and gluten protein that provide the elastic attribute to wheat. Wheat is also a source of essential amino acids, minerals, vitamins and other advantageous photochemical and fibres that constituents to the human diet and are specifically richer in whole-grain items.

Through the adoption of green-revolution cultivars having characteristic of semi-dwarf stature, photoperiod insensitivity, stem rust resistance along with the use of synthetic fertilizers and improved farming practices have substantially increased wheat production at the rate of about 1% per annum over the last 50 years (Trethowan et al., 2002). Based on wheat productivity from 1950, approximately 1.2 billion hectares of arable land were spared between 1950 and 1999, simultaneously lessening the impact of wheat production on the environment (Borlaug 2004). Even with these advances, nearly 2 billion people remain malnourished and continued progress will be necessary to meet the demand spurred by population growth and malnutrition in areas as in sub Saharan Africa. In addition, ecological variability produced by changing worldwide weather will force breeders to develop cultivars with higher tolerance to intermittent periods of severe and less predictable weather. With exports of wheat to developing countries predicted to double by 2025, it is vital that the major wheat producing countries work to meet these challenges (Rosegrant and Cline 2003).

2.3 Genetic diversity in bread wheat resources

Genetic variability inside bread wheat gene pool is very short due to very few events of hybridization have been occurred among *Ae. tauschii* and durum wheat (Dvorak et al., 1998). Wang et al. (2013) studied genomic associations between 477 *Ae. tauschii* and wheat accessions and revealed that hexaploid wheat created by hybridization of tetraploid *Triticum turgidum* (genomes AABB) with *Ae. tauschii* (genomes DD). Furthermore, their results suggested high variation and diversity in chromosomes 1D and 2D. Lelley et al. (2000) revealed that modern bread wheat cultivars show less genetic diversity than *Ae. tauschii*. Dwivedi et al. (2016) propose that a systematic landrace will enable to find alleles for increasing yield and productiveness and crops stability in susceptible environments.

2.3.2 Synthetic hexaploid wheat

Primary synthetic wheat is created, through the crossing between *T. turgidum* and *Ae. tauschii* that were considered as evolutionary ancestors of bread wheat, (McFadden and Sears 1946). Commonly, *T. turgidum* parent were used as female parent spikes were emasculated by the process of removing of anthers and pollinated using the anthers from a selected *Ae. tauschii* accession. Young embryos were protected by expurgation from seeds that were developing and then grown on culture media. Growth of root and shoot is initiated from the differentiated embryos making sprouts, which are observed for chromosomal composition. To induce chromosome doubling, colchicine treatment is applied in the crown of sprouts, therefore, hexaploid kernels set upon self-fertilization (Mujeeb-Kazi et al., 2008). For the production of primary synthetics, it is not always necessary to rescue the embryo and apply the colchicine treatment. Few combinations of *T. turgidum* and *Ae. tauschii* F1 hybrids formed without the assistance of human, and few hybrids can instinctively set their seeds comparatively at great rates (Matsuoka and Nasuda 2004). After creating the stable primary synthetic hexaploid wheat that can be easily crossed with bread wheat, to create a F1 synthetic derivatives that is genetically stable without the requirement of backcrossing (Lange and Jochemsen 1992; Mujeeb-Kazi et al., 2008). The genomes of both *T. turgidum* and *Ae. tauschii* can be exploited at the same time by using primary synthetic wheat, which can provide a platform to examine the trait expression from these progenitor genomes at the hexaploid level. The first attempt to form the

primary synthetic wheat were performing in the early 1900's, with 'synthetic spelta' being produced during an investigation to establish the progenitors of *Triticum aestivum* subsp. *spelta* (L.) Thell. a primitive form of bread wheat (McFadden and Sears 1946). The term 'synthetic hexaploid wheat' was used by McFadden and Sears (1946) to express the synthesis of this allopolyploid hybrid. Successful re-synthesis attempts by McFadden and Sears (1946) were only achieved using *T. turgidum subsp. dicoccoides* with *Ae. tauschii*, and not with other subspecies of *T. turgidum*. More recent efforts to create primary synthetic wheat have successfully hybridized the different accessions of *Ae. tauschii* with *T. turgidum* subspecies durum (Desf.) Husn. (Durum wheat) (Dreccer et al., 2007; Ogbonnaya et al., 2007), dicoccoides (Korn. Ex Ash. & Graebn.) Thell. ('Wild emmer' wheat) (Lange and Jochemsen 1992), dicoccon (Schrank) Thell. ('Emmer' wheat) (Lage et al., 2003) and carthlicum (Nevski) A. Love & D. Love (Persian black wheat) (Liu et al., 2006). In most primary synthetic wheats *Ae. tauschii* is the source of novel genetic material (Trethowan and Mujeeb-Kazi 2008).

2.3.3 Direct hybridization with *T. turgidum*

Desirable alleles from the genomes of A and B can be transferred. Bread wheat can be directly hybridised with *T. turgidum* and *T. turgidum* into the harmonizing A and B genomes of current bread wheat cultivars. In direct hybridization *T. turgidum* usually used as the female parent, while bread wheat is used as the male parent. Resistant genes have been introgressed into the A and B genomes of bread wheat for the stem rust, leaf rust and stripe rust, and powdery mildew (Knott et al., 2005). Though, direct hybridizations between *T. turgidum* and bread wheat can outcome into the genetically unsteady hybrids.

2.3.4 Direct hybridization with *Ae. tauschii*

Direct hybridisation between *Ae. tauschii* and bread wheat can be found, as the D genome of this species freely recombines with the D genome of bread wheat. This direct hybridization of *Ae. tauschii* with bread wheat has efficiently introgressed the important characters and resistance to abiotic stresses like, leaf rust, wheat spindle-streak and soil born mosaic virus, into bread wheat cultivars (Yan et al., 2003). Though, the direct hybridization of Ae. tauschii with bread

wheat needs human involvement, generally to rescue the embryo up to 4 cycles of crossing to recover a stable hexaploid (Cox et al., 1991).

2.3.5 Production and use of synthetics by CIMMYT and the world

CIMMYT begin examining application of synthetic wheat in the late 1980's (Mujeeb-Kazi and Hettel, 1995, Reeves et al., 1999). CIMMYT has used primary synthetic wheat in breeding programs to improve abiotic stress tolerance and yield sensitivity (Reeves et al., 1999). Over 1015 spring type and 186 winter type primary synthetics have been produced by CIMMYT since 1991, with an projected one third of the advanced lines circulated by CIMMYT to global breeding programs being synthetic derivatives (van Ginkel and Ogbonnaya 2007). Further their use as bases of genes for taming abiotic and biotic stresses, numerous wheat cultivars have been released that are derived from SHWs. These include Lalma and KT-2010 in Pakistan (CIMMYT Wheat Atlas), Maravilla in Mexico (CIMMYT Wheat Atlas), Carmona in Spain (van Ginkel and Ogbonnaya 2007) and Chuanmai-42 and its derivatives in China. Recently, Li et al. (2014) reviewed the current status of synthetic-derived wheat cultivars released in Southwestern China. They reported that 16 commercial wheat varieties including Chuanmai 28, 42, 43 and 47 have been released from using SHW. Apart from released cultivars, a significant proportion of international bread wheat screening nurseries by CIMMYT and ICARDA comprises of synthetic-derived germplasm which are distributed on annual basis worldwide.

2.4 Heat stress in wheat

Three phases of wheat growth and development include: vegetative, reproductive, and grain filling stage. During vegetative stage heat stress is not much considerable because of its sowing. In wheat, most important yield components are single kernel weight and grains number. (Satorre and Slafer 1999). Depending on intensity, time period and heat stress duration, grain filling may be disrupted affecting yield. In addition, cultivars and germplasm have been evaluated in the field or using glass or plastic house under late season or off-season heat stress to create heat stress during anthesis (Ferris et al., 1998; Khanna-Chopra and Viswanathan, 1999). Preceding to anthesis, heat stress outcome is in seed sterility, due to the sensitivity of microspore and megaspore development (Tashiro and Wardlaw, 1990). The major effect contributed to post-

anthesis heat stress is the reduction in individual kernel weight, due to reduction in the endosperm and embryo maturation (Randall and Moss, 1990). Others have also reported a considerable reduction in kernel number at post-anthesis, with the maximum reduction seen shortly after pollination, resultant from short-term high intensity heat shock (Hays et al., 2007a). Heat tolerant varieties that sustain yield components under both constant and short-term heat shock have been recognized and emphasis is to place these heat tolerant sources into current breeding programs (Hays et al., 2007b) At last, one of the main aim in breeding programs is to produce heat tolerant wheat varieties and to use the well promising germplasm and proper selection methods (Wardlaw et al., 2002, Fokar et al. (1998a).

2.4.1 Heat stress limiting wheat yield

Among abiotic stresses, intensifying temperature in crops confines the productiveness in many regions of the world (Al-Khatib and Paulsen 1984). At the same time 7 million hectares of cultivated land are affected by the frequent heat stress (Fisher and Byerlee 1990). In temperate climates terminal stress often affects about 36 million ha of wheat crop (Reynolds et al., 2001).

Wheat is very penetrating to heat stress (Slafer and Satorre 1999) and this high temperature is increasing in wheat growing areas with the passage of time (Hennessy et al., 2008). Both vegetative and reproductive phases of wheat are highly affected by heat stress but the reproductive phase faces severe change due to high and direct effect on dry weight and kernel number (Wollenweber et al., 2003). In future, the major emphasis is on reactions to high heat changes during growing and maturing phases.

The suitable temperature for reproductive and grain filling stage for wheat ranges from 12 to 22°C exposure to temperatures more than this can prominently lessen grain production (Tewolde et al., 2006). Every raise of 1°C above 15°C reduces wheat production 3 to 4% under controlled conditions and by raising from 1°C from 25/20°C to 35/20°C cause grain number to decrease by 12.5% (Wardlaw and Wrigley 1994). In many countries wheat is exposed to high temperature during grain filling duration and therefore negatively disturbing plant productivity and quality of grain. (Altenbach et al., 2003).

During heat stress an increase in floret abortion also occur during anthesis stage. (Wardlaw and Wrigley, 1994). Elevated temperature between anthesis to maturity reduces the grain yield by reducing the time to capture assimilates.

Terminal and repeated stress is occurred when temperature exceeds to 17°C in the serenest month of the season (Fischer and Byerlee 1991, Acevedo et al., 2002). Semenov and Shewry (2011) found that heat stress at flowering period as a principal harvest decreasing factor in European wheat. Heat stress, normally more than 34 °C, can decrease the final grain weight by reducing the grain filling duration because of photosynthesis inhibition (Al Khatib and Paulsen, 1984). Under these conditions, reduction in kernel numbers might be attributed to sensitivity of pollen development to elevated temperatures data gained from the study of Gibson and Paulsen (1999) exposed that high temperature 10 days after the anthesis prolonged to ripening caused reduction by 78%, 63% and 29% in grain yield, grains number and final grain weight, respectively. On the other hand, high temperature started 15 days after anthesis till ripening had no effect on grains number but 18% decrease in kernel weight.

Spikelet fertility, grain filling duration and grain size is less when at night time heat stress occur (Prasad et al., 2008b). This can be overcome by developing stress tolerant varieties (Wahid et al., 2007).

Sharma et al. (2014) evaluate 24 synthetic hexaploid wheat duirng normal and late sown condition and they suggested that synthetic hexaploid wheat is enormously helpful cause for heat tolerance hence allowing breeders to bloc various foundations of genetic unevenness to expand heat tolerance in their program. However, very few synthetic hexaploid wheat are considered for heat tolerance out of the wide array available. (Cossani and Reynolds, 2015).

2. 5 Impact of high temperature on wheat physiological traits

2.5.1 Leaf chlorophyll

Constant rise in temperature decline leaf chlorophyll content. An imperative decrease in it was found when Yangmani-9 and Xuchou-26 at anthesis stage were exposed to extraordinary heats of 32/24°C and 34/22°C at 7 d (Zhao et al., 2007). In synthetic hexaploid wheat

chlorophyll concentration of flag leaf at 10 d after anthesis was decreased by 11% to 38% when more heat is applied (Yang et al., 2002 Djanaguiraman et al., 2010).

SPAD meter could be accurate to measure chlorophyll content to screen heat tolerance in wheat (Ristic et al., 2007a). Al-Khatib and Paulsen (1984) acknowledged that chlorophyll contents of flag leaves are useful to find leaf senescence and its increase by high temperature. Contents of chlorophyll was decreased with time after the anthesis regardless of treatment and cultivar (Fokar et al., 1998b). Though, high temperature causing decrease in chlorophyll content is undefined. (Ristic et al., 2007a).

2.5.2 Heat stress effects photosynthesis and chlorophyll content

In wheat due to heat stress physiological and biological methods are affected decreasing both productiveness and excellence (Wahid et al., 2007). Demirevska-Kepova et al. (2005) stated that photosynthesis is the furthermost susceptible methods to high temperature stress. (Takeuchi and Thornber, 1994: Ristic et al., 2007a). Chlorophyll fluorescence provides information on the condition of photosystem II (PSII); e.g., harm to PSII is the first indication of heat stress in a leaf (Maxwell and Johnson 2000). Mishra and Singhal (1992) described that high temperature treatment of wheat leaves resulted in a reduction in the variable fluorescence to maximum fluorescence (Fv/Fm) that decrease in Fv/Fm ratio was primarily due to decrease in Fv at higher temperature, which resulted from a decline in Fm and regular increase in initial fluorescence (Fo). Decline in Fv/Fm ratio as well as in Fv reveals a drop in photochemical competence of photosystem II (PSII) by affecting energy relocate 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).

2.5.3 Stay green

Stay-green may be predominantly important under stress conditions such as heat and drought (Distelfeld et al., 2014; Thomas and Ougham 2014). Hindrance in the expression of senescence-related genes allows stay green (SG) genotypes to sustain photosynthesis (Lim et al., 2007). Although SG is renowned as an adaptive physiological trait for stress conditions, under

heat stress, the optimal pattern of pigment loss in order to improve grain yield has not been identified. (Vijayalakshmi et al., 2010: Lopes and Reynolds, 2012). Even though the Normalized Difference Vegetation Index (NDVI), is reliable indicator of greenness integrating all chlorophyll, linked with heat tolerance (Keskitalo et al., 2005).

The flag leaf, substantially contribute the nitrogen uptake and carbon assimilation and remains green for longer than other leaves, has been suggested to be a good target for senescence/pigment loss investigations (Kipp et al., 2014). There are various methods for monitoring the chlorosis including visual scoring (Vijayalakshmi et al., 2010), measuring NDVI with a Green Seeker sensor (Lopes and Reynolds 2012), and estimating chlorophyll content with a portable SPAD meter (Vijayalakshmi et al., 2010; Lopes and Reynolds 2012). Indirect flag leaf chlorophyll measurement with a portable SPAD meter provides a fast, non-invasive, easy, and inexpensive way to monitor senescence over time on a single plant. The SPAD meter measures transmittance of the leaf in the red (650 nm) and infrared (940 nm) wavelengths (Minolta 1989). A strong relationship exist between SPAD readings and chlorophyll concentration in wheat leaves (Uddling et al., 2007).

2.5.4 Canopy temperature

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. (Kadioglu et al., 2012; Sirault et al., 2015:Sarieva et al., 2010). A waxy cuticle covers the aerial surfaces of the leaf in many plants (Chen et al., 2010). 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 possess 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 measured CTD trait and find by, is calculated by deducting temperature of plant canopy from air temperature (Balota et al., 2007). Close link between grain wheat productivity and CTD is found in hot environments under natural conditions (Fischer et al., 1998). Ayeneh et al. (2002) revealed strong positive associations among CTD and organ temperature depression. Hatfield et al., (1984) indicated that awns presence was not linked 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 (Blum, 1986).

2.6 Heat tolerance genetics in wheat

Some work has shown the mapped chromosomal areas at grain filing stage in wheat linked with heat easiness. Yang et al. (2002b) exhibited two quantitative trait loci (QTL), on chromosomes 1B and 5A linked with grain filling period under high temperature (30 °C). Mohammadi et al. (2008 a and b) reported QTL for GFD on chromosome 2D and on chromosomes 1B, 5B, and 7B in response to a brief severe heat stress under controlled conditions for heat susceptibility index (HSI). Mason et al. (2010) mapped QTL for HSI of main spike yield components and grain filling duration, in response to severe heat stress (3 days of 38/18 °C applied 10 DAA), on chromosomes 1A, 1B, 1D, 2A, 2B, 3B, 4A, 5A, 5B, 6D, and 7A. Mason et al. (2011) detected 14 QTL for organ temperature depression (spike and flag leaf) and HSIs of primary spike yield components on 9 chromosomes (1B, 2D, 3B, 4A, 5A, 5B, 6D, 7A, and 7B) in response to 3 days of 38/18 °C (10 DAA). At 7 of these loci, QTL for organ temperature depression co-localized with HSI QTL, with lower spike temperatures linked to higher tolerance. Seven QTL regions (on chromosomes 1B, 3B, 4A, 5A, 5B, and 6D) in their study were familiar with those reported by Mason et al. (2010). The tolerance source was similar (Halberd) but the susceptible parent differed (Cutter) in (Mason et al., 2010) and Karl 92 in (Mason et al., 2011). Kumar et al. (2010) reported three QTL for stay-green under field high temperature conditions, on chromosomes 1AS, 3BS, 7DS. Vijayalakshmi et al. (2010) showed nine QTL for senescence related traits, on chromosomes 2A, 3A, 3B, 6A, 6B, and 7A. Naruoka et al. (2012) found QTL associated with permanence of leaf greenness on chromosomes 1B, 2D, 3A, 3B, 4A, 5B, and 7B. At the QTL on chromosome 4A, longer green leaf area duration was associated with higher xylem exudation under hot, dry conditions in numerous field trials.

Paliwal et al. (2012) detected QTL for HSIs of yield and CTD, on 7B and 4B chromosomes. They observed co-localization of HSI QTL and co-localization of QTL for thousand grain weight potential (under normal conditions) and HSI of thousand grain weight on chromosome 6B. Mason et al. (2013) reported QTLs for grain yield and yield related traits, HSI of yield and also canopy temperature depression under field high temperature conditions, on 9 wheat chromosomes (2D, 3B, 3D, 4B, 5A, 5B, 5D, 7A, and 7D).

2.6.1 Quantitative trait loci mapping (QTL)

Two parents having different traits shows mapping population as heat tolerant against heat intolerant. These populations help to find chromosomal location of QTL and to find the number, influence, size and some quantitative traits of wheat. Bi-parental populations have ability to find QTL along chromosomes and requirements of very few markers for coverage of genome (Sorrells and Yu, 2009). The disadvantages of bi-parental population mapping approach are: 1) Only two alleles can be evaluated at a locus. 2) Low mapping resolution due to few recombinations. 3) Longer time required to develop mapping population.

2.6.2 Association mapping

Association mapping method involves diverse cultivar to obtain information on marker trait association. It is helpful to find polymorphism of genes which gives different phenotype. Association mapping is associated to find the QTL linked with the desired trait. It does not require bi-parental mapping population (Gupta et al., 2005). Association mapping help to find the link between traits of interest and haplotype blocks NAM (Nested association mapping) called special mapping population allow chromosomal location finding and QTL detection (Ersoz and Buckler, 2009However, NAM populations are currently available only for a limited number of crop species. The NAM population in maize was developed by crossing 25 diverse inbred lines to a common reference inbred B73 to produce 25 bi-parental recombinant inbred line families that have one parent in common (Cook et al., 2012). Association mapping analysis steps include cultivar selection with genetic variation, recording phenotypic data, genotyping, kinship assessment, to find phenotypic and genotypic linkage and using statistical method (Abdurakhmonov and Abdukarimov, 2008).

Two major classes of association mapping are GWAS and Bi-parental mapping (Zhu et al., 2008) GWAS is a favorable methodology for scanning the whole genome and Bi parental mapping target the genes with identified function (Tabor et al., 2002).

2.6.3 Genome wide linkage disequilibrium (LD) in wheat

The strength and patterns of LD in wheat vary among chromosomes and genomes. Analysis of LD for 43 U.S wheat cultivars has shown the intra-chromosome LD decay below r2< 0.2 within 10 cM (Chao et al., 2007). On the contrary, significant long range LD (over 30 cM genetic distance) has been recorded for chromosomes 3DL, 4DL and 6AL. At the genome level, the B genome exhibited largest fraction of significant LD despite fewer markers. In another study conducted on 96 soft winter wheats with SSR markers, LD decayed rapidly within 1 cM for chromosome 2D but extended up to 5 cM for chromosome 5A (Breseghello and Sorrells, 2006). Similarly, Yao et al. (2009) reported that LD decayed on average within 1 cM for chromosome 2D, within 0.5 cM for chromosome 3B, but extended up to 2.3 cM on chromosome 2A of hexaploid wheat implying the presence of large differences among wheat chromosomes in rate of LD decay. The most comprehensive analysis of LD patterns has been conducted on a total of 478 spring and winter wheats genotyped with 394 SNP markers. This study revealed that LD declined to 50% of its initial value within 6-7 cM for the A, B and D genomes (Chao et al. 2010). Genome-wide LD estimation for 251 winter wheat lines with 346 DArT makers also showed on average LD declined below r2<0.2 at 9.9 cM (Benson et al., 2012). Liu et al. (2010) genotyped 103 wheat accessions from China with 116 SSR markers on chromosome 4A and found extension of LD up to 3 cM with threshold level at r2= 0.054. The study conducted on elite durum wheat genotypes also showed the dependence of LD on different factors. For elite durum wheat (*Triticum durum* Desf.) lines genotyped with SSR markers, LD extended up to 10 cM to reach a critical threshold of r2=0.06 (Maccaferri et al., 2011). Another study on durum wheat genotyped with 58 SSR markers showed the decay of LD within 10 cM (Maccaferri et al., 2005).

2.6.4 Population structure

Structured populations may show significantly different allele frequencies because of genetic drift, genetic loci remain spuriously connected with trait when there is no actual

association. (Sneller et al., 2009). The development of a statistical model which allows accounting for population structure during association analysis has improved the application of association mapping for QTL detection in crop plants. Two steps for population structure description is to calculate the percentage of membership of each individual to population groups using unlinked random markers, and to use the percentage of membership as a covariate in the model of testing associations of markers with phenotypic traits (Ersoz et al., 2009).

Many population structure are found in wheat from highly regulated populations (Hao et al., 2010).

2.6.5 Genome wide association studies for heat tolerance

For many crops a method of QTL identification is Genome-wide association. Sukumaran et al., (2015) in their study reported SNPs associated with yield on chromosome 5A and 6A. Loci associated with maturity and plant height on 1A and 6A were found. Loci for canopy temperature (2D, 4D, 6A) were also found.

Chapter 3

MATERIAL AND METHODS

3.1 Grain filling experiment under controlled conditions

3.1.1 Plant material

In this experiment 114 synthetic hexaploid wheats and 6 Australian bread wheat genotypes as a control were used. A complete list of germplasm is given as Appendix 1. Experiment was conducted at The Plant Accelerator with the collaboration of Australian Center for Plant Functional genomics in vicinity of Waite Campus University of Adelaide.

3.1.2 Experimental design

Pots (8 × 8 cm, 18 cm depth) were filled with a steam-sterilized mixture of 2:1 of coco peat: Waikerie sand (pH 6.0-6.5) containing the following nutrients (mg pot-1): dolomite lime, 202; agricultural lime, 561; hydrated lime, 131; gypsum, 202; superphosphate, 202; iron sulphate, 505; iron chelate, 33.7; trace elements (Micromax), 202; calcium nitrate, 505, and slow-release fertilizer pellets (Mini Osmocote, 2022). Three seeds per pot were sown on 10th of March 2015, and seven days after sowing, plants were thinned to one healthy seedling per pot. The experiment involves 2 treatments (control vs. heat) to the 2 main plots in each block. Each main plot was divided into 120 Plants were initially grown in naturally lit greenhouse compartments (The Australian Plant Accelerator, University of Adelaide, Waite Campus, Adelaide). Measured greenhouse conditions are reported and averaged 20/16 °C day/night. Plants were watered from above every 2 days. As in some previous heat tolerance studies (Tashiro and Wardlaw 1990b; Wardlaw et al. 1989b), plants were pruned back to the single main culm by removing tillers as they appeared to enable easier management and better light penetration. A liquid fertilizer was applied to the soil fortnightly at the recommended rate from one month after sowing to plant maturity. The anthesis data and date is find. Each plant destined for heat treatment was shifted to a growth chamber after 10 days of anthesis (days after anthesis, DAA), where heat treatment (37/27 °C day/night temperature) was applied for 3 days. Leaf water potential of plants in the chamber, measured using a Scholander pressure chamber, decreased to

between -11 and -15 bar by the middle of the day, indicating that there was some foliar dehydration. Turgor then recovered completely during the night sub plots to which were assigned 36 genotypes.

3.1.3 Data collection

Relative chlorophyll content of the flag leaves was monitored from about 7 to 50 days after anthesis using SPAD meter (SPAD-502; Minolta Co. Ltd., Japan)

Details of the traits measured on individual plants are as follows. The same measurements were made at corresponding developmental stages on both control and heat treated plants.

Days to anthesis (DTA): Days from sowing to the day that exerted anthers first became visible. Chlorophyll content just before the heat treatment, in SPAD units (ChlC7-10DAA): Because SPAD readings were taken on only two days per week, the exact DAA of each measurement (ranging from 7 to 10 DAA) depended on the anthesis date of the plant.

Normalized chlorophyll content just after the treatment period, in SPAD units (ChlC13-16DAA): This value was normalized to the ChlC7-10DAA reading taken on the same plant, to account for variation in starting chlorophyll content between plants and genotypes.

Area under the normalized SPAD progress curve (AUSC): For each plant, SPAD readings continued to be taken after the heat treatment, 2 times per week, for up to ~ 50 DAA, and these values were normalized to the 7-10 DAA value. AUSC for each plant was calculated using the following equation, where Xi is the chlorophyll content (normalized SPAD units) on the ith date, ti is the date on which the chlorophyll content was measured, and n is the number of dates on which chlorophyll content was recorded (t=~7 to 50 DAA).

$$\mathrm{AUSC} = \sum_{i=1}^{n-1} \left[\left(\frac{X_i + X_{(i+1)}}{2} \right) \times (t_{(i+1)} - t_i) \right]$$

Days from anthesis to complete flag leaf senescence (FLSe): Leaves were recorded as fully senesced when they appeared completely yellow. Days from anthesis to the aforementioned stage (FLSe) was considered as an indicator of stay-green duration.

Grain filling duration (GFD); time from anthesis to maturity in days: Plants were defined as mature when spikes became ~ 95% senesced and seeds became firm. Time from anthesis to the aforementioned stage was considered as an indication of GFD.

Culm length (CL): Measured at maturity, from the soil surface to 1 cm below the collar.

Shoot weight (ShW): At physiological maturity, each plant was cut off at the soil surface and the shoot (stem + leaves) separated from the spike. Shoots were oven dried at 60 °C for 3 days before being weighed.

Spikelet number spike-1 (SpNS), grain weight spike-1 (GWS), grain number spike-1 (GNS), grain number spikelet-1 (GNSp) and single grain weight (SGW): After counting all spikelets, the spike was threshed, and the grain left in the laboratory at room temperature to reach a stable moisture content before being weighed. Grains were counted. GNSp was determined by dividing GNS by SpNS and SGW was determined by dividing GWS by GNS. Grains from floret positions 1 + 2 (the two most basal positions) and floret positions >2, were initially counted and weighed separately, to check for potential differences in grain set and size between different positions on the spikelets. Basal spikelets that were underdeveloped (small spikelets without grain) were ignored.

Harvest index (HI): Determined for each plant (main tiller) by dividing GWS by above ground biomass (GWS + ShW).

3.1.4 Data analysis

DTA, GWS, SGW, GNS, GNSp, SpNS, CL, ShW and HI were recorded for each plant, while the other traits were measured on all plants in just four blocks. Statistical analyses and figures were prepared by using the R programming language (R Development Core Team 2014). Expected means were obtained using a linear mixed model with ASReml-R software (Butler et

al. 2009). Least significant difference (LSD) values were used for mean comparisons. Pearson correlation tests and principal component analyses (PCA) were used to study relationships between traits measured under control or heat conditions, and between response ratios of different traits (RH/C = Mean trait value Heat treatment / Mean trait value Control). Pearson correlation tests and PCAs were performed using Psych (Revelle 2014) and Facto Mine R (Lê et al. 2008) packages, respectively.

3.2 Field experiment

3.2.1 Plant material

In this study, an association panel comprised of 205 SHW were received from AGG, Victoria, Australia, as phenotyped for the current experiment (Appendix 1). These SHWs were derived from the groupings of 44 durum wheat cultivars and 149 *Ae. tauschii* (Rasheed et al., 2014) showing resistance to stress (Mulki et al., 2013 and Joukhadar et al., 2013) and characterized by quality of kernel (Emebiri et al., 2010).

3.2.2 Field evaluation

Sowing completed at three locations during two years (2013 and 2014), with two sowing treatments normal and heat stress treatment in each location and year. The three locations were: Regional Agricultural Research Institute (RARI) Bahawalpur referred as 'BWP', National Institute of Agriculture (NIA) Tando Jam Sind referred as 'SND', and National Agricultural Research Center (NARC), Islamabad referred as 'ISB'. Year and location prefix is given e.g. SND14NOR and SND14HS were trials in Sind during 2014 using normal and late sowing dates, respectively. Normal sowing date was 15th November and late sowing was 20th December.

Germplasm were planted using randomized complete block designs with three replications. Plot size was 2 meters by 2 rows, with 15 cm between rows and 30 cm between plots. Small plot grain drill was used for sowing. Triple superphosphate (4.3 g m^{-2} of P_2O_5),and urea was applied before planting and second irrigation (8.6 g m^{-2} of N). Irrigation was done at different growth stages during the crop season. (Acevedo et al., 2002). Weeding was performed

manually a couple of times. Data for monthly maximum minimum and mean temperature and relative humidity is presented during each of the trial during two cropping season in Appendix 3.

3.2.3 Phenotypic traits measurement

Data were recorded for plant height , days to flowering , tillers per plant ,spike length ,spikelet's per spikes were counted for three spikes from each line per entry, spike weight per spike was determined measuring the weight of three spikes from each row per entry, number of grains per spike rains weight per spike is determined by measuring the weight of grains per spike from each row per entry , GN ,TGW was measured after harvesting.Grain yield (GY) was determined as the weight of grain harvested per unit area (kg m^{-2}).

3.2.4. Biochemical and physiological parameters

This experiment was conducted on 13 selected synthetics hexaploids during 2014 and 15 at Islamabad under field condition). Data was recorded for the TKW after harvesting and Grain yield (GY) .BY was recorded before the threshing by weighing the whole plant.

3.2.5 Chlorophyll analysis

From each sample chlorophyll was extracted which was carried out by using the method of Hiscox and Israelstam (1979). Fresh leaf samples of approximately 0.5 g were harvested in a test tube contained 6000 ul Dimethylsulphoxide (DMSO) heated to 60 to 65°C in a water bath. For extraction 0.5 g of leaf sample was homogenized with the help of pastle and mortar. Then after samples were centrifuged for 20 min at (5000 rpm) after centrifugation only supernatant was taken out. After that extracted supernant was shifted to a test tube and raised up to a final volume of 10000 ul with DMSO. Absorbance was recorded with the help of UV spectrophotometer between 643 nm and 665 nm wavelength. Chlorophyll a, b and Total chlorophyll were found by the equation of Arnon (1949).

3.2.6 Soluble sugar analysis

This analysis was determined by the method of Dubois *et al.* (1956). Fresh leaves were harvested about 0.4 gram in a test tube for 60 minutes in a liquid bath at 80 ^{0}C was frenzied

with 10000 ul of 82% ethanol. After that 600 ul sample extracted and gestated at normal room temperature for 60 minutes and 1 mL of 18% phenol and 2.5 mL of conc. H2SO4 was added to it, mixed and absorbing was recorded for samples using Spectrophotometer. By using the standard glucose curve the concentration of sugar was determined.

3.2.7 Proline content

It was recorded from fresh samples using the method of Bates et al. (1973). In test tubes fresh leaf material (approx. 0.4 g) form control and heat stress plants were taken and standardized in 5000 ul of 3 to 4% sulfo-salyclic acid. Approximately 3000 uL of a supernant was taken; mixed with 3000 ul glacial acetic acid and 3000 ul of ninhydrin mixture for 60 minutes in a liquid bath at 99 0C; At the end by collecting the upper layer, absorbance read between 520 to 525 nm on Spectrophotometer was recorded by using the standard curve.

3.2.8 Membrane stability index

It was determined by Sairam et al. (2000) method. Fresh leaves 0.3 g of equal size were harvested in a falcon tube containing 10000 ul of sanitized water in 2 sets. From two sets of tubes one set for 30 minutes was held at 40 °C to record and measure(C1) using a conductivity bridge and electrical conductivity was measured again as (C2).

3.2.9 Meteorological data

Meteorological data for monthly maximum minimum and mean temperature and relative humidity is presented for three locations during two cropping season (Appendix 3).

3.2.10 Statistical analysis

According to randomized model analysis of variance (ANOVA) was planned for all characters. By using Pearson's correlation test relationship between variables are found with the help of STATISTICA software (version 7.0). Data were also moved to principal component analysis (PCA) based on correlation technique (Hammer, Harper, & Ryan, 2001). Heritability of each attribute in the population was assessed using following formula (Nyquist 1991):

$$h^2 = \frac{\sigma^2_g}{\sigma^2_g + (\sigma^2_g / t) + (\sigma^2 / rt)}$$

where, h^2 represents the heritability, σ^2_g is genetic variance, σ^2_{gt} is genotype × trial variance, σ^2 is error variance, r shows number of replications and t number of trials.

For grain yield heat susceptibility index (HSI) (Fischer and Maurer 1978, Khanna-Chopra and Viswanathan 1999) was calculated using following the formula: HSI = (1 – Y/Yp)/D, where Y is the yield of the genotype in the late planting, Yp is the mean yield of the genotypes at normal planting time and D (stress intensity) = 1 – X/Xp, where X is the mean Y of all genotypes and Xp is the mean Yp of all genotypes. Genotypes were rated as highly tolerant (HSI ≤ 0.50), moderately tolerant (0.50 < HSI ≤ 1.00) or susceptible (HSI > 1.00) to high temperature.

3.3.1 Genome-wide association studies for terminal heat stress in SHWs

DNA of total SHW was extracted and sent to Triticarte Pty. Ltd. Australia (www.triticarte.org.au) for genotyping, as a commercial service provider for DArT markers. DArT is an array-based genotyping technology which generates DNA markers that are binary and dominant. White et al., 2008). A high-density DArT array was used and 1200 DArT markers were scored. Kompetitive allele-specific PCR (KASP; LGC Genomics, London, UK; www.lgcgenomics.com) markers were used to assay SNPs that were diagnostic of wild type and dwarfing alleles of the *Rht-B1* and *Rht-D1* genes (Rasheed et al., 2016). Sequences of primers have been reported recently (Rasheed et al., 2016) Genotyping reactions were performed using 34 cycles in a MJ Research Thermocycler (Waltham MA, USA), followed by 9 cycles in a LightCycler®480Real-Time PCR System for fluorescence detection of the products (Roche Applied Science; www.roche-applied-science.com). Reactions were assembled in a final volume of ~6 µl containing 3 µl of 2× KASP Reaction Mix (LGC Genomics), 0.17 µM of each of two

allele-specific competitive forward primers, 0.42 μM of the common reverse primer and 50 ng genomic DNA. The following cycling conditions were used for the 34 initial cycles: an initial denaturing step for 15 min at 94 °C, 9 cycles of 20 s at 61 °C (decreasing by 0.6 °C every cycle), then 25 cycles of 10 s at 94 °C and 1 min at 55 °C, and a final incubation step of 2 min at 25 °C. The additional 9 cycles in the LightCycler®480 Real-Time PCR System was performed using 10 s at 94 °C and 1 min at 55 °C per cycle. Datawere analysed using the LightCycler® 480 Software, Version 1.5 (Roche Applied Science; www.roche-applied-science.com).

3.3.2 Statistical analysis for GWAS

Polymorphic information content (PIC) and gene diversity and allelic frequency of markers were calculated using Power Marker v3.25 (Liu and Muse 2005). Minor allelic frequency less than 6% of DArT markers were discarded from the data set to decrease false positives. The remaining DArT markers were incorporated into a linkage map by surmising marker order and position from a consensus genetic map of wheat (Detering et al., 2010) (ordering 4,000 wheat DArT markers). DArT array was used and 1100 DArT markers were scored.

3.3.3 Population structure

For the calculation of population structure 40 unlinked markers specific to all chromosomes of A, B and D genomes in all synthetic hexaploids were selected. To avoid the physical linkage, the distance among the 2 markers were selected on the same chromosome was at least 48cM. A model based (Bayesian) cluster software STRUCTURE 2.3.3- was used to estimate the population structure (Pritchard et al., 2000). The number of subpopulations (K) was set from 2-15 based on admixture and correlated allele frequencies models. For each K, 15 runs were performed separately. Each run was carried out with 1lac iteration and 1lac burn-in period. A value of K was selected where the graph of *InPr* (X/K) peaked in the range of 2-15 subpopulation. For selected K again 10 runs were performed each with 1lac iteration and 1lac burn-in period. An ad-hoc quantity statistic (ΔK) based on the rate of change in the log probability of data between successive K values (Evanno et al., 2005) was used to predict the real number of subpopulations.

3.3.4 Linkage disequilibrium

Pairwise linkage disequilibrium (LD) pattern was measured using TASSEL 5.0.0 software (Bradbury et al. 2007). The comparison wise significance was computed using 1,000 permutations as implemented in TASSEL software. The position of DArT markers in terms of genetic distances (cM) were based on the DArT consensus map (Detering et al. 2010). LD levels and the rate of LD decay were computed by calculating r^2 for pairs of DArTs and plotting them against genetic distance. The statistical significance of individual r^2 estimates was calculated by the exact test following Weir et al., (1996). Chromosome specific r^2 values were plotted using the R package LDheatmap.

3.3.5 Association analysis

Association analysis was accomplished with the help of mixed linear model (MLM) functions of TASSEL. In MLM accounting for both Q and family structure matrix (Kinship, K matrix) to control both Type I and Type II errors (Yu et al. 2006) was performed. To correct for multiple testing, a false discovery rate (FDR) method (Benjamini and Hochberg 1995) was used to calculate marker-specific B–H critical values at the significance level of 0.05. Marker alleles with P<B–H critical value were declared to be significantly associated with relevant grain phenotype descriptor.

Chapter 4

4. RESULTS

There was a strong significant treatment (control vs. heat) effect for most of the traits except SL PH, and SSW (Table 1.1), also there was a highly significant genotypic effect for all the traits (Table 1.1). Furthermore, significant genotype by treatment interactions were observed for GWS, IGW, FLSe, GFD, BY, Chl10-13DAA- Chl13-18DAA and Chl18-23DAA which indicated genotypic variation for the responses of these traits to heat treatment (Table 1.1).

Table 1.1. Analysis of variance for genotype (G), treatment (T) and genotype × treatment (G × T) effects for studied traits in control and heat treatment P-value are shown.

Traits	G	T	G x T
Days to heading (DH)	<0.001	NA	NA
Days to anthesis (DA)	<0.001	NA	NA
Grain filling duration (GFD)	<0.001	<0.001	<0.001
Flag leaf senescence (FLSe)	<0.001	<0.001	<0.001
Spike length (SL)	<0.001	<0.233	<0.7329
Spikelets per spike (SSP)	<0.001	NA	NA
Single spike weight (SSW)	<0.001	<0.534	<0.212
Grains number per spike (GNS)	<0.001	NA	NA
Grains number per spikelet(GNSp)	<0.001	NA	NA
Grain weight per spike (GWs)	<0.001	<0.001	<0.001
Single grain weight (SGW)	<0.001	<0.001	<0.001
Culm length	<0.001	<0.803	<0.432
Shoot weight (ShW)	<0.001	<0.039	<0.021
Harvest index (HI)	<0.001	<0.541	<0.312
Chlorophyll contents after 10-13 days after anthesis(CHL10-13DAA)	<0.001	<0.039	<0.034
Chlorophyll contents after 13-18 days after anthesis(CHL13-18DAA)	<0.001	<0.082	<0.042
Chlorophyll contents after 18-23 days after anthesis(CHL18-23DAA)	<0.001	<0.001	<0.001

*NA, not applicable; trait measured before heat treatment.
*P-value significant at 0.05

4.1.1 Days to heading and days to anthesis

These traits were taken prior to heat stress so heat stress is not applicable for these traits but there is a significant genotypic effect was found for these traits (Table 1.1). Moreover, significant genotypic variation found for the days to heading within genotypes ranging from 52 days (Young) to (137) days AUS30667 and AUS30654 (Figure 1.1). While, days form sowing to

antehsis varied significantly within genotypes ranging from 56 days (Young) to 141 days AUS30667 and AUS30654 (Figure 1.1).

4.1.2 Spike related traits

There was no significant effect of heat treatment were found on the traits SL, SSP, GNS, GNSp and CL (Table 1.1). Moreover, significant variation was found within the genotypes for all traits. Minimum SL were found 7.4cm in genotype Reeves and maximum SL 24.4 cm were found genotype and AUS34420 (Figure 1.2). SSP ranging from 14.4 (AUS34235) and 26.6 in genotypes (Waagan and Kauz) (Figure 1.2). The genotypes AUS30652, AUS30654 AUS33395 have minimum number of grains (19), while the maximum number of grains (82 and 72) were found in Kauz and AUS33408 (Figure 1.3). GNSp were ranging from 1 to 3.2 and 3.1 in genotypes AUS30652, AUS30303 and Kauz (Figure 1.3). CL minimum and maximum were found in genotypes Young (52.3 cm) and AUS33385 (127 cm) (Figure 1.4). While, these traits were not significantly affected by heat, so the combined means of control and heat-treated plants are shown in their respective figures.

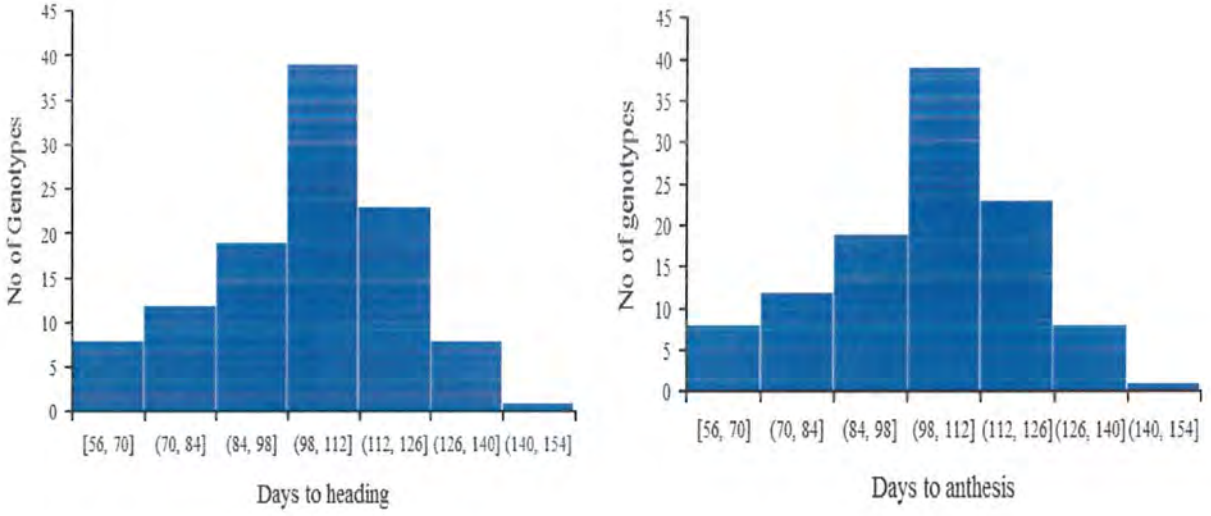


Figure 1.1. Frequency distribution plots for days to heading anthesis data averaged in normal and heat stress treatments.

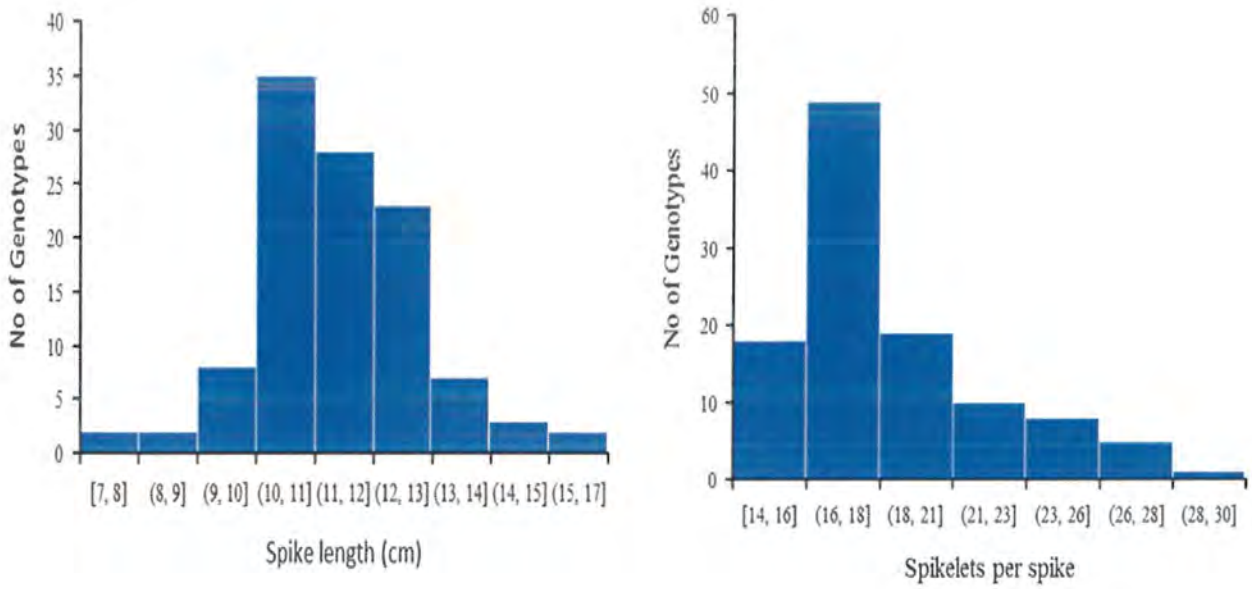


Figure 1.2. Frequency distribution plots for spike length (cm) and spikelets per spike data averaged in normal and heat stress treatments

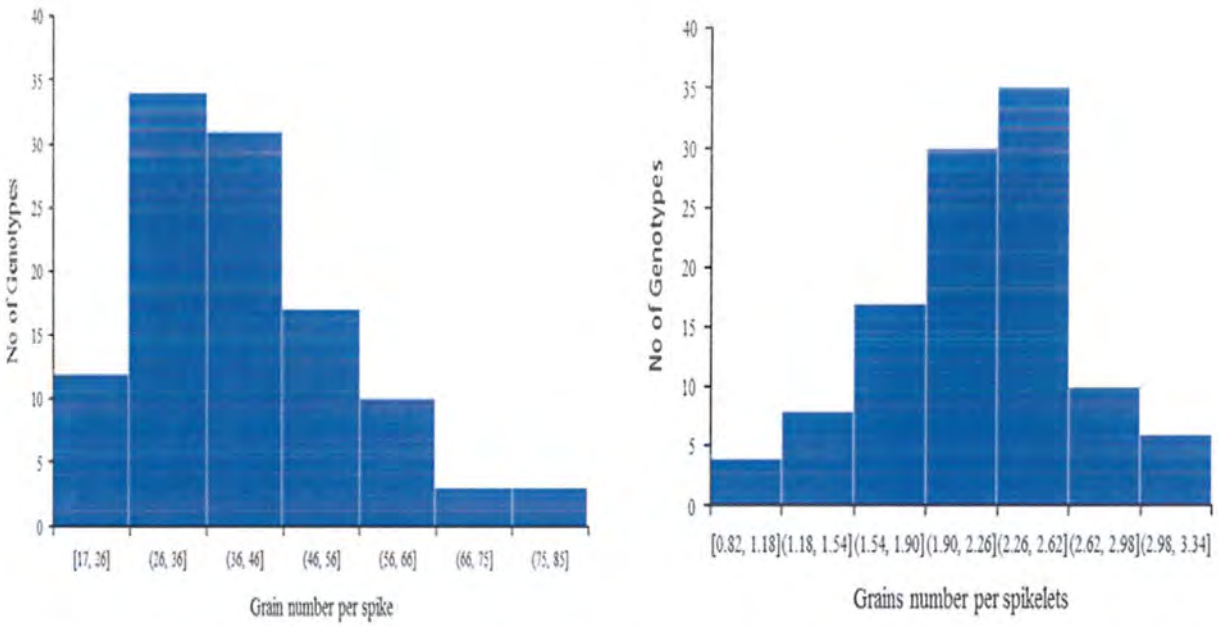


Figure 1.3. Frequency distribution plots for grains number spike and grains number per spikelets data averaged in normal and heat stress treatments.

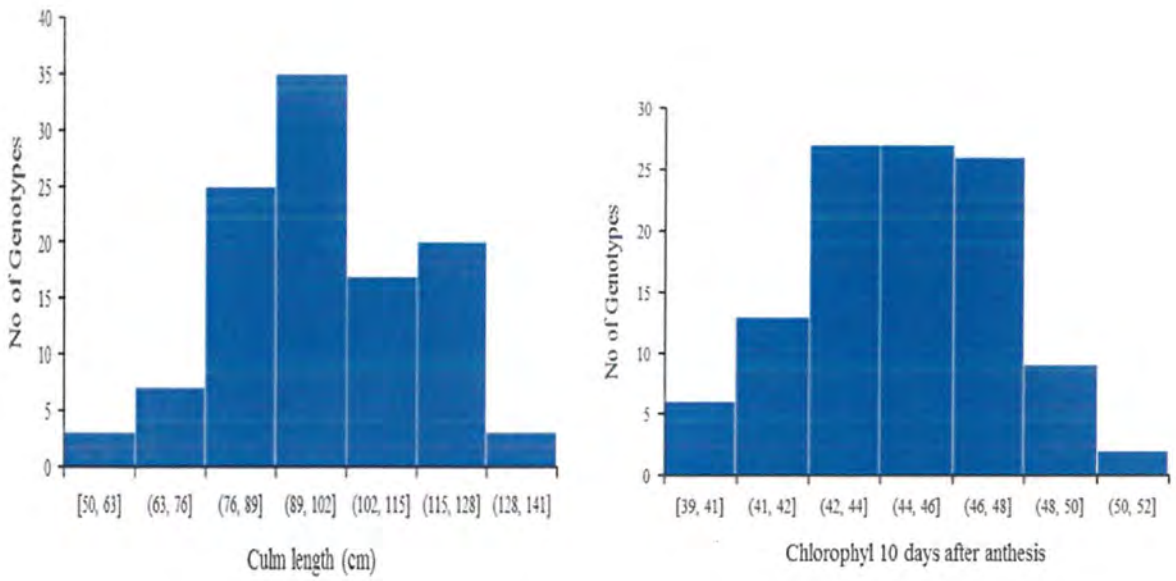


Figure 1.4. Frequency distribution plots for culm length and chlorophyll 10 days after anthesis data averaged in normal and heat stress treatments.

4.1.3 Grain yield related traits

Single grain weight (SGW) was reduced in the heat-treated plants relative to the control plants, and the effect was significant (Figure 1.5). Overall, heat reduced SGW by an average of 18.0%. Genotypes AUS30645, Young, AUS30667 AUS34405, AUS33404, AUS30632, AUS30280, AUS33416 andAUS34408 showed the least response (less than 3.0%) and were therefore the most tolerant of the varieties, while genotypes AUS33400, AUS34242, AUS34448, AUS33393 and AUS33408 showed the greatest responses and were therefore the most intolerant (more than 40.0% reduction in heat-treated plants relative to control; Figure 1.8). AUS30300, AUS34436 and AUS34233 had the highest SGW under control conditions (0.08 mg), while AUS30632, AUS34435 and AUS30300 had the highest SGW in heat stress condition (0.077, 0.069 and 0.067 g). Kauz, Young and AUS33402 had the lowest SGW under control condition (0.032, 0.036 and 0.039 g), while genotypes Reeves, AUS33408 and Kauz had lowest SGW in heat conditions respectively (0.029, 0.030 and 0.030 mg, respectively). The trend in response of grains weight per spike (GWS) across the genotypes was also very similar to that of SGW, which was expected because there was no detectable effect of heat treatment on grain number (Figure 1.6). AUS33408, AUS33407 and AUS30303 appeared to have the highest GWS under control condition (4.02, 3.98 and 3.92 g) and genotypes AUS33395, AUS30654 and AUS34230 have the lowest SGW in control condition respectively, (1.15, 1.16 and 1.24 g). Furthermore, in heat condition genotype AUS33407, AUS34455 and AUS33406 had highest GWS, respectively (3.71, 3.41 and 3.35 g), while AUS33394, AUS34238 and AUS30654 showed the lowest GWS under heat condition (0.72, 0.72 and 0.82, respectively). There was no significant effect of heat treatment on culm length (CL) (Figure 1.4). However, shoot weight (ShW) was significantly reduced by the treatment (by an average of 6.0%; Table 3.3). ShW showed no genotype by treatment interaction, indicating the ranking of genotypes held very similar under both control and heat conditions for this trait. AUS33410 AUS34233, AUS33407 and AUS34435 had the highest and the lowest ShW, respectively, under both control and heat conditions (Fig. 1.7). Overall, heat stress significantly reduced harvest index (HI) (by 4.0%; Figure 1.8) - a result of heat causing a greater reduction in grain weight than in shoot weight (18.0 vs. 4.0% overall reduction). Seventy-five genotypes showed a reduction in HI while 35 genotypes showed an increase. The effect was significant in 7 genotypes (Figure 1.8).

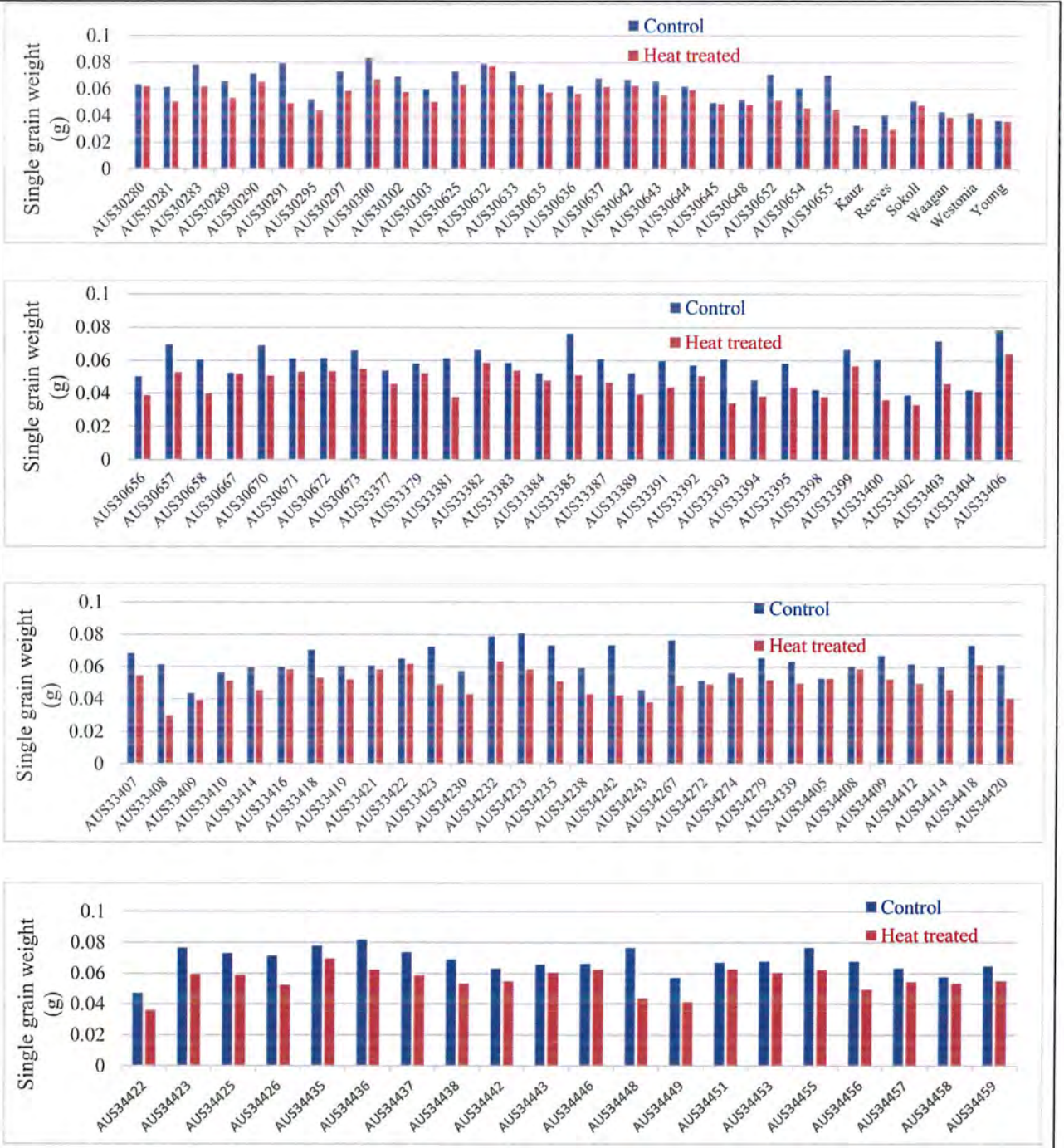


Figure 1.5. Single grain weight (g) in control and heat-treated plants for mean comparisons between genotypes within control (blue bar) or heat (red bar) treatment.

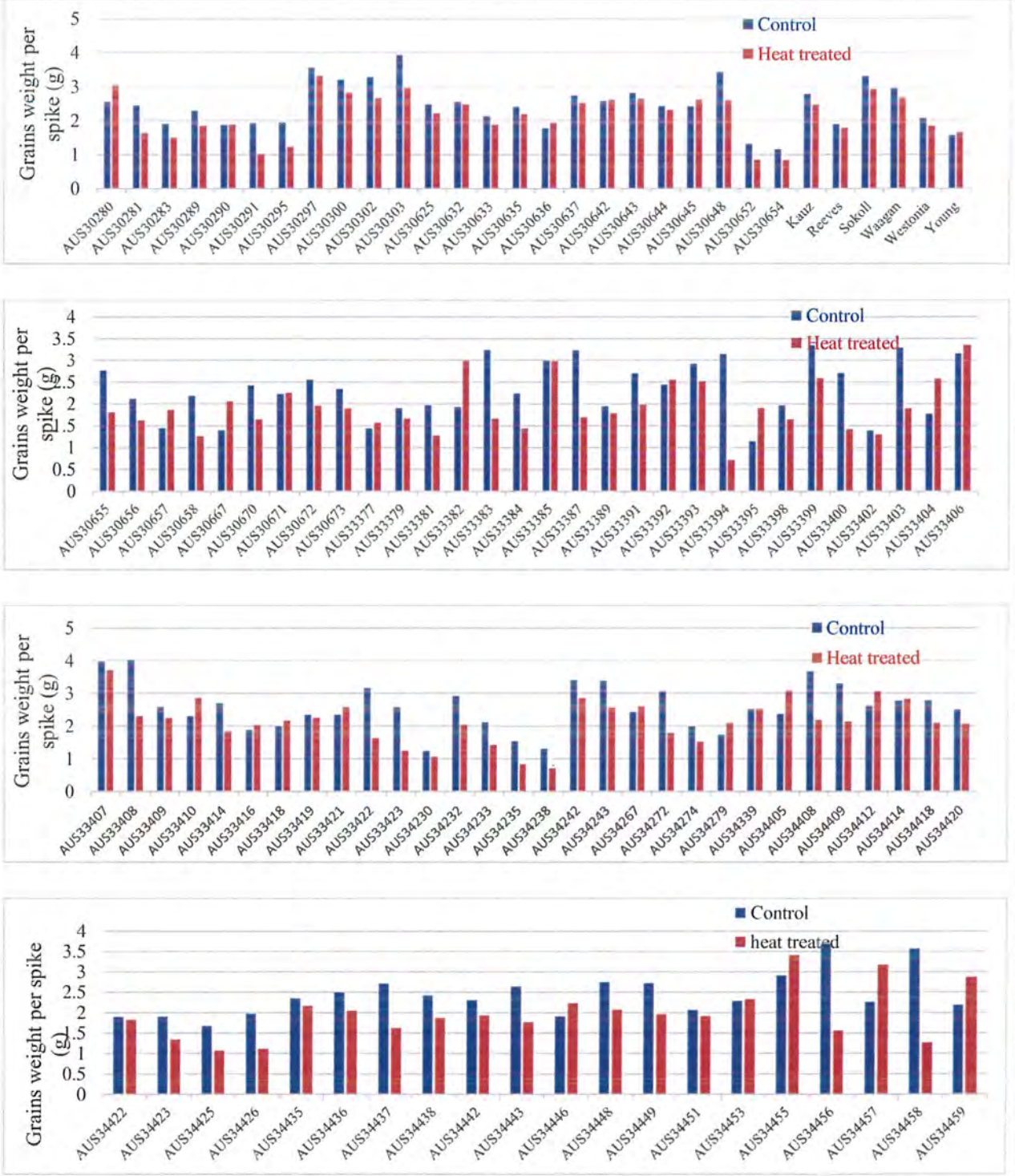


Figure 1.6. Grain weight per spike (g) in control and heat-treated plants for mean comparisons between genotypes within control (blue bar) or heat (red bar) treatment.

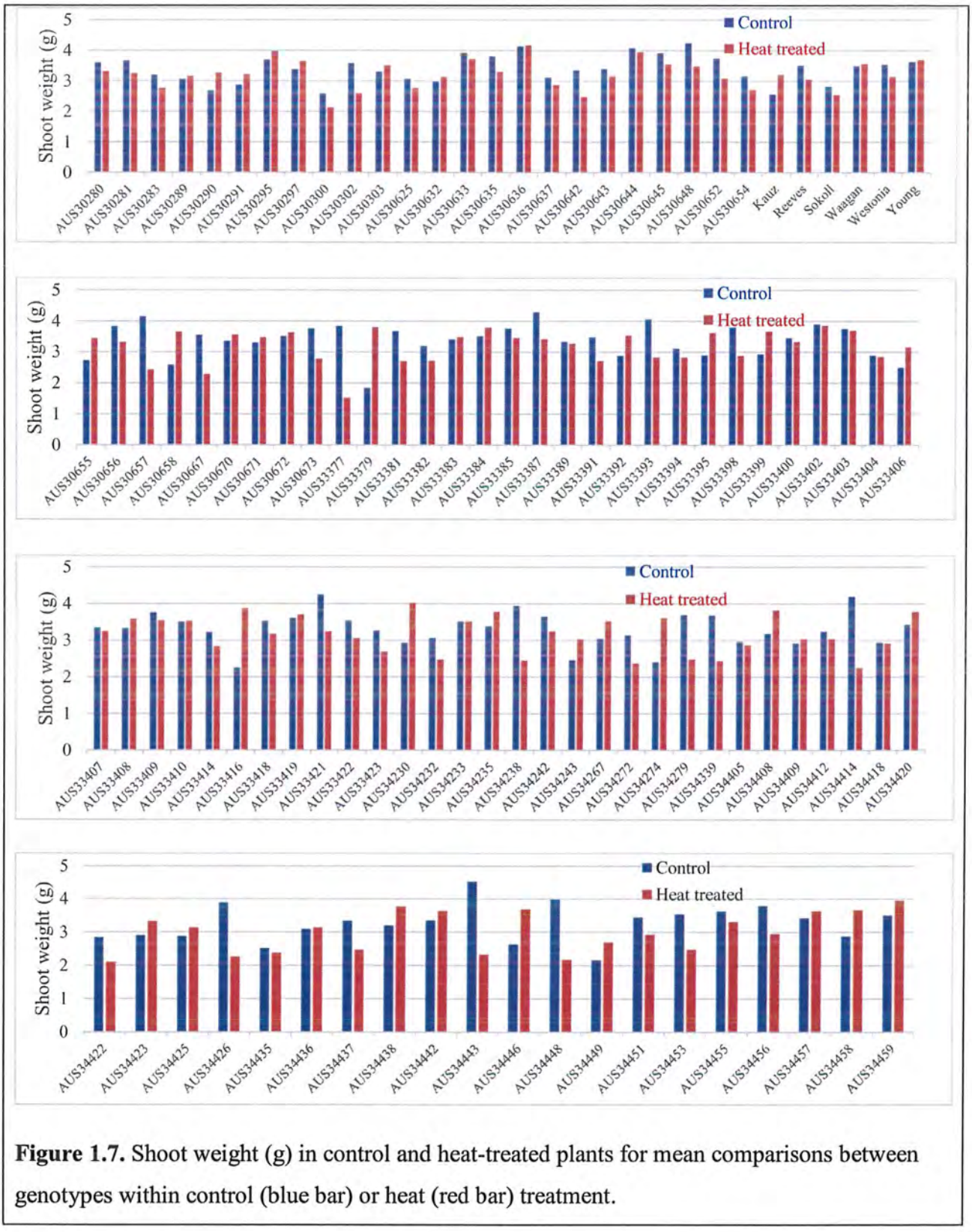


Figure 1.7. Shoot weight (g) in control and heat-treated plants for mean comparisons between genotypes within control (blue bar) or heat (red bar) treatment.

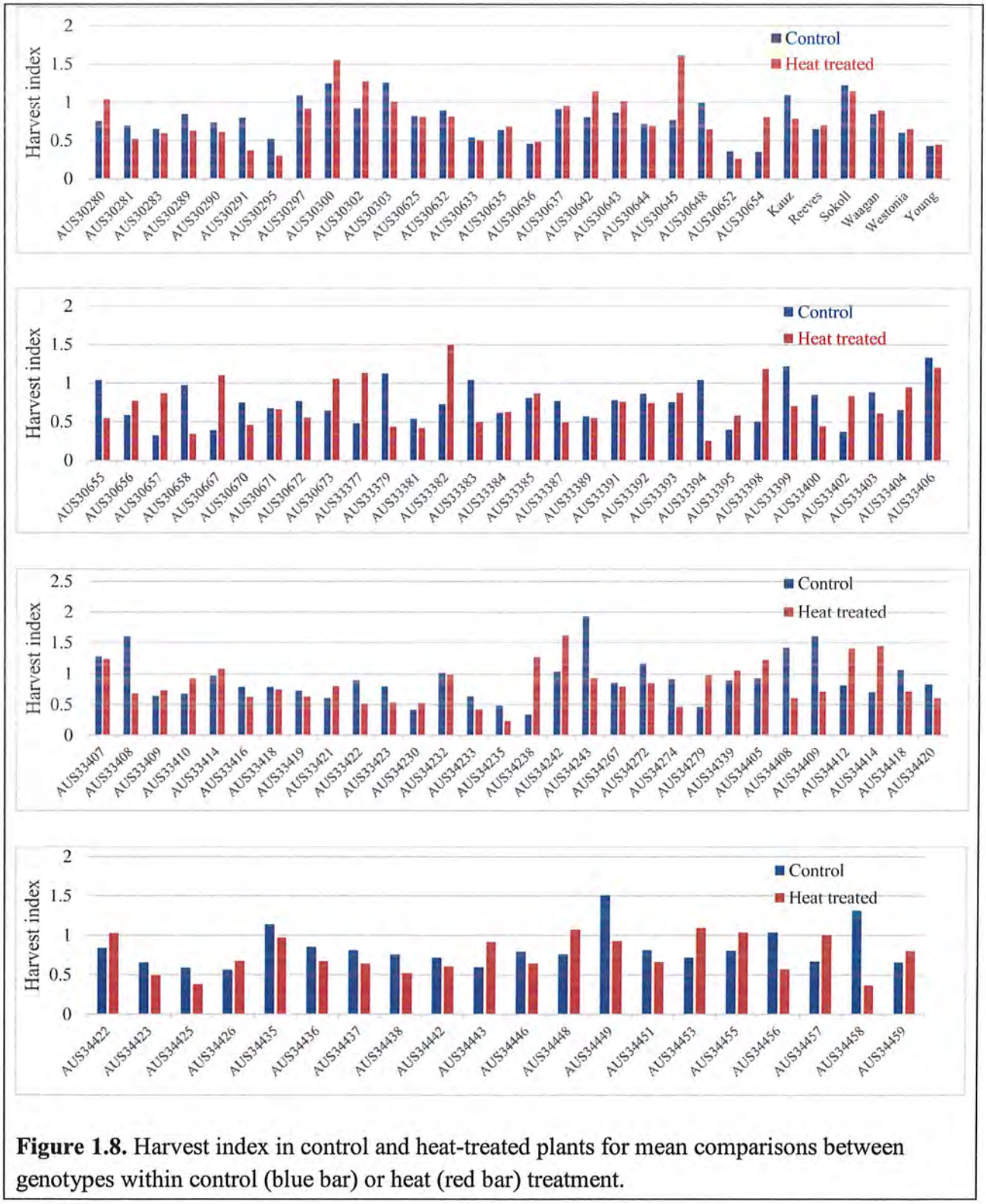


Figure 1.8. Harvest index in control and heat-treated plants for mean comparisons between genotypes within control (blue bar) or heat (red bar) treatment.

4.1.4 Grain filling duration

Overall, heat significantly shortened grain filling duration (GFD), and the average reduction was 13.0%. However, GFD showed significant genotype by treatment interaction, indicating that genotypes vary significantly in this heat response (Table 1.1). Under control conditions AUS33392, AUS33408 and AUS34436 had the longest, while the genotypes AUS33391, AUS30652 and AUS33406 had shortest grain filling duration. Furthermore, under heat-stress conditions AUS33407, AUS33382 and AUS30281appeared to have the longest, while the AUS30656, AUS33406 and AUS34420 have shortest grain filling duration, respectively (Figure 1.9).

4.1.5 Chlorophyll responses

Before the heat treatment significant variation among the genotypes were observed for chlorophyll content after 10DAA, while genotypes varying by up to ten SPAD units (Figure 1.4). The heat treatment accelerated the rate of chlorophyll loss in the flag leaves beyond the rate observed in the control plants undergoing natural senescence. Chlorophyll content (SPAD units) decreased rapidly during the treatment. Then after the treatment, it decreased at a slower rate, although generally more rapidly than in the control plants at the corresponding developmental stage, indicating that some of the effect of heat persisted after the stress was relieved. The trait 'ChlC13-18DAA' represents the first phase of the response, and is the proportion of chlorophyll retained during the treatment period relative to just before the treatment. It was reduced in the heat-treated plants relative to control in all genotypes except the genotypes Young, AUS30670, AUS33377, AUS33381, AUS33383, AUS33391, AUS33394, AUS33407, AUS33409, AUS33423, AUS34235, AUS34339, AUS34408, AUS34420, AUS34451, AUS34458 and AUS34459, they showed a different response after heat treatment they have increased the chlorophyll contents as compared to control plants the maximum increase were found for the genotypes AUS34235 by an average of (60.0%). While, the minimum losses were found for the genotypes AUS34422 (2.0%), AUS30667 (2.0%), AUS30673 (3.0%) and Reeves (3.0%) and maximum losses were found for the genotypes AUS34233 (57.0%), AUS34279 (52.0%), AUS30281 (51.0%) and AUS33379 (50.0%) (Figure 1.10). Over all heat stress losses the Chl13-18DAA by an average of 15.0%. The trait AUSC summarized the amount of chlorophyll retained during the treatment period plus the time up to ~60 days after the end of the treatment. AUSC

was decreased by heat in all genotypes except AUS34455, AUS34446 and AUS33389. While the genotypes AUS30670, AUS33377, AUS33381, AUS33383, AUS33391, AUS33394, AUS33407, AUS33409, AUS34408, AUS34423, AUS33423, AUS34235, AUS34339, AUS34408, AUS34420, AUS34451, AUS34458 had increased the AUSC under during heat as compared to control plants (Figure 1.11). On average, heat decreased AUSC by 14.0%, maximum decrease were found for the genotype by average AUS34233 (57.0%) AUS34279 (52.0%) AUS30281 (51.0%) and AUS33379 (50.0%), while the minimum decrease were found for the genotypes AUS34422 (2.0%), AUS30667 (2.0%), AUS30673 (3.0%) and Reeves (3.0%). The period from anthesis to complete flag leaf senescence (FLSe) was also reduced by heat, by an average of 9.0% days, consistent with a phenomenon of heat-accelerated chlorophyll loss. A shortening of this interval under heat conditions was observed in all genotypes except AUS30670, AUS33383, AUS33391, AUS33394, AUS33407, AUS33409, AUS33419, AUS33422, AUS33423, AUS34235, AUS34243, AUS34339, AUS34408, AUS34420, AUS34425, AUS34451, AUS34455 and AUS34459 they tend to retain their chlorophyll more days as compared the control plants (Figure 1.12). While, maximum days of Chlorophyll retention under control condition were found in the genotypes Reeves, Westonia, Young and AUS33395 respectively, (85, 84, 80, and 80 days) and minimum days for FLSe under control conditions were found for the genotypes AUS33389, AUS33409, AUS34235 and AUS34425 respectively (37, 39, 40 and 41 days). Moreover, under stress condition the maximum days to FLSe were found for the genotypes Reeves, AUS33394 Westonia and Young respectively (81, 81, 75 and 70 days), while under stress condition the genotypes AUS30289, AUS33406, AUS30632 and AUS30648 had minimum days (29, 36, 37 and 37 days) to FLSe were found (Figure 1.12).

Figure 1.9. Grain filling duration in control and heat-treated plants for mean comparisons between genotypes within control (blue bar) or heat (red bar) treatment.

Figure 1.10. Chlorophyll 13-18 days after anthesis (normalized SPAD units) in control and heat-treated plants for mean comparisons between genotypes within control (blue bar) or heat (red bar) treatment.

Figure 1.11. Area under SPAD curve (AUSC) in control and heat-treated plants for mean comparisons between genotypes within control (blue bar) or heat (red bar) treatment.

Figure 1.12. Flag leaf senescence in control and heat-treated plants for mean comparisons between genotypes within control (blue bar) or heat (red bar) treatment.

4.1.6 Correlation of traits and principle component analysis

To find out the relationships between the traits within each treatment, (PCA) and pairwise correlation tests were done (Figure 1.13). The first two axes, i.e. PC1 and PC2, explained up to 55.92% of total unevenness under control condition, and stress (Figure 1.13).

In both control and heat treatment same trend of correlation were found between the traits. DH had significant positive correlation with DA, SGW, SL, and CL and significant negative correlation to all other traits and same trend of correlation were found for the DA respectively in both treatments (Table 1.2). Therefore, later flowering was associated with greater vegetative and grain biomass including a greater single grain weight, but was (perhaps unexpectedly) associated with faster flag leaf senescence and a shorter grain filling period. GFD had positive correlation with FLSe, Chl13-18DAA and AUSC. GNS, GNSp, GWS, SSP, SSW, CL and HI, had positive correlation with each other while significant negative correlation was found between GNS and SGW. SGW had significant negative correlation with SSP, FLSe, Chl13-18DAA and AUSC and positive significant correlation with CL. CL had Significant negative correlation with FLSe, Chl13-18DAA and AUSC. ShW is significantly negatively correlated with HI indicating that variation in HI was driven mainly by variation in GWS. While significant positive correlation was found between the FLSe, Chl13-18DAA and AUSC.

Table 1.2. Pearson's co-efficient of correlation between traits under control and heat treatment. Upper triangle represents correlations from heat treatment, and lower triangle represents correlations from control treatment.

Variables	DH	DA	GFD	GNS	GNSp	GWS	SGW	SL	SSP	SSW	CL	ShW	HI	FLSe	Chl13-18DAA	AUSC
DH	**1**	**0.99**	**-0.23**	**-0.44**	**-0.40**	**-0.23**	**0.29**	**0.27**	**-0.27**	**-0.21**	**0.32**	-0.01	**-0.24**	**-0.47**	**-0.56**	**-0.56**
DA	**0.99**	**1**	**-0.22**	**-0.44**	**-0.41**	**-0.22**	**0.30**	**0.28**	**-0.26**	**-0.20**	**0.32**	-0.01	**-0.23**	**-0.47**	**-0.56**	**-0.56**
GFD	**-0.27**	**-0.28**	**1**	0.07	0.03	0.08	0.10	-0.09	0.09	0.12	-0.10	0.04	0.05	**0.32**	**0.37**	**0.37**
GNS	**-0.49**	**-0.48**	0.09	**1**	**0.82**	**0.70**	**-0.35**	0.14	**0.75**	**0.64**	-0.09	0.07	**0.56**	0.05	0.09	0.09
GNSp	**-0.45**	**-0.44**	0.07	**0.81**	**1**	**0.70**	-0.13	-0.02	**0.24**	**0.60**	-0.04	0.05	**0.56**	0.01	0.03	0.03
GWS	**-0.25**	**-0.24**	0.03	**0.78**	**0.74**	**1**	**0.24**	0.09	**0.38**	**0.76**	0.17	0.01	**0.65**	-0.13	-0.04	-0.04
SGW	**0.46**	**0.44**	-0.09	**-0.47**	**-0.28**	0.13	**1**	-0.05	**-0.43**	0.13	**0.38**	-0.06	0.09	**-0.29**	**-0.23**	**-0.23**
SL	**0.26**	**0.27**	0.02	0.14	-0.04	**0.30**	0.13	**1**	**0.30**	0.18	0.17	0.05	0.10	0.01	0.06	0.06
SSP	**-0.24**	**-0.23**	0.086	**0.73**	**0.21**	**0.47**	**-0.46**	**0.36**	**1**	**0.41**	-0.11	0.09	**0.30**	0.07	0.12	0.12
SSW	-0.13	-0.12	0.02	**0.66**	**0.63**	**0.95**	**0.25**	**0.43**	**0.42**	**1**	**0.29**	-0.04	**0.72**	-0.15	-0.02	-0.02
CL	**0.32**	**0.32**	-0.13	-0.01	0.08	**0.36**	**0.51**	**0.39**	-0.07	**0.41**	**1**	-0.08	**0.25**	**-0.43**	**-0.38**	**-0.38**
ShW	0.08	0.09	0.04	-0.01	0.07	-0.02	-0.06	-0.01	-0.08	-0.04	0.07	**1**	**-0.49**	0.03	0.08	0.08
HI	**-0.26**	**-0.25**	0.04	**0.65**	**0.57**	**0.80**	0.07	**0.26**	**0.43**	**0.77**	**0.21**	**-0.51**	**1**	-0.12	-0.07	-0.06
FLSe	**-0.45**	**-0.46**	**0.29**	0.14	0.07	-0.09	**-0.38**	-0.08	0.16	-0.13	**-0.37**	-0.01	-0.06	**1**	**0.91**	**0.80**
Chl13-18DAA	**-0.54**	**-0.55**	**0.31**	**0.23**	0.13	0.03	**-0.31**	-0.04	**0.21**	0.03	**-0.30**	-0.11	0.12	**0.83**	**1**	**0.80**
AUSC	**-0.57**	**-0.57**	**0.30**	**0.20**	0.11	0.01	**-0.31**	-0.05	**0.20**	0.01	**-0.33**	-0.09	0.07	**0.87**	**0.97**	**1**

Values in bold are different from 0 with a significance level alpha=0.05

DH: Days to heading, DA: Days to anthesis, GFD: Grain fill duration, GNS: Grains number per spike, GNSp: Grains number spikelets, GWS: grains weight per spike (g), SGW: single grain weight (mg), SL: Spike length (cm), SSP: Spikelets per spike, SSW: Single spike weight (g), CL: Culm length (cm), ShW: Shoot weight (g), HI: Harvest index, FLSe: Flag leaf senescence, Chl13-18DAA: Chlorophyll 13-18 days after anthesis, AUSC: Area under SPAD curve.

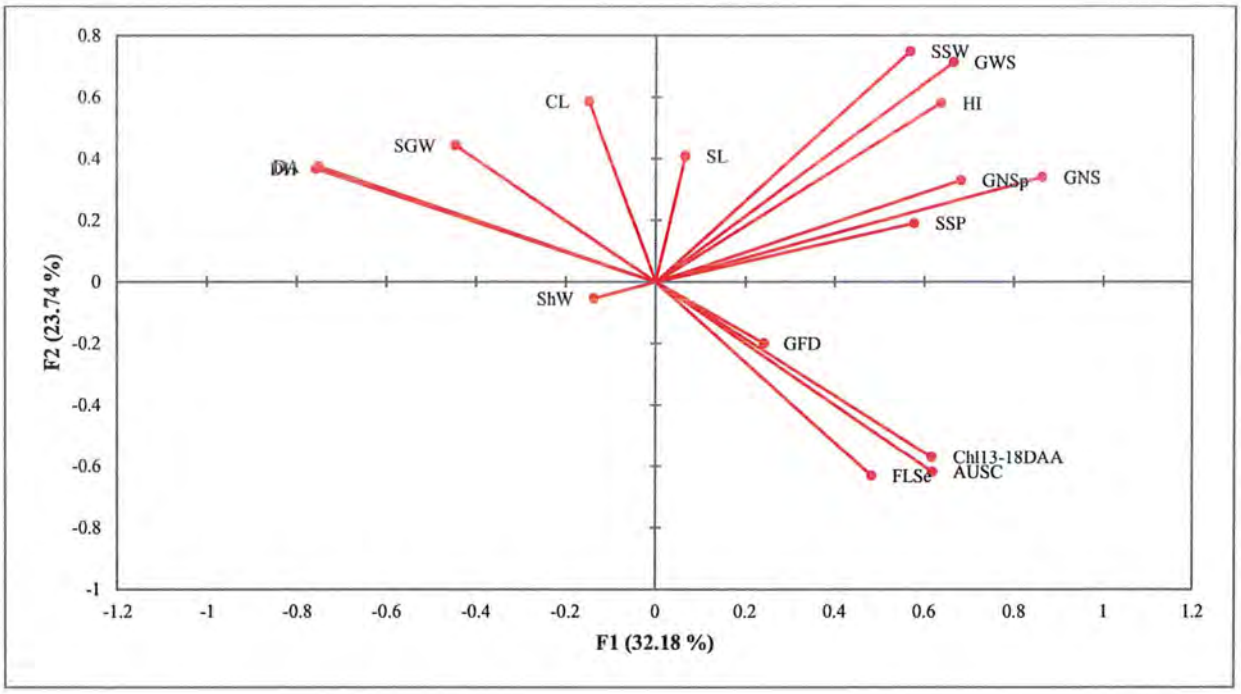


Figure 1.13. Principal component analysis (PCA), showing traits averaged in control and heat stress plants.

4.2 Analysis of variance

ANOVA showed significant genotypic, location, treatments and year effects and their interactions for all the studied parameters except DH, DM, TP and SSP and SWS which remained non-significant for year and treatment interaction (Table 2.2).

4.2.1 Effect of environmental variables on agronomic traits

Minimum and maximum daily temperature tended to increase after 15th March once the crop was at the stage of grain filling (Appendix 3). This rise in temperature was biggest for the HS environment at site Sindh (SND-15HS and SND14HS), followed by Bahawalpur (BWP15HS and BWP14HS) and Islamabad (ISB14HS and ISB15HS) (Appendix 3). Under NOR treatment, the lowest maximum day to day rise in temperature was observed at the site Islamabad (ISB14NOR and ISB15NOR) followed by Bahawalpur (BWP15NOR and BWP14NOR) and Sindh (SND-15NOR and SND14NOR). The average rise in temperature was observed during 2015 (36°C) than 2014 (34°C). The average daily maximum temperature (T_{max}) across all trials was significantly higher (3-5 °C) in the HS vs. NOR treatments.

4.2.2 Mean performance of SHWs and analysis of variance

Basic trial statistics including means and ranges of trait values in each environment are presented in (Table 2.1). The DH under NOR and HS treatments was 117.6 days and 88.4 days (28% difference). The mean DM under NOR and HS was 154.05 days and 114.21 days (29% difference) The mean PH under NOR and HS treatments were 118.7 cm and 88.4 cm (26.6 % difference). The mean TP under NOR and HS treatments were 12.52 and 9.38 (26.7% difference). The mean SL under NOR and HS was 13.68 and 10.14 cm (27% difference). The mean SSP under NOR and HS were 18.60 and 15.10 (19% difference). The mean SWS under NOR and HS was 2.65 and 1.92 (28.6% difference). The mean GNS were under NOR and HS 29.54 and 24.47 (18.2% difference). The mean GWS under NOR and HS was 1.53 and 1.10 (27.7% difference). The mean TGW under NOR and HS treatments were 47.8 g and 39.5 g (18.4 % difference). The mean value for GNP in NOR and HS treatments was 7723.6 and 6562.2 (16.1% difference). The mean value for GY in NOR was 0.75 m^{-2} and in HS was 0.46 kg m^{-2}, respectively (39.4% difference). The mean BY under NOR and HS was 1.50 and 0.89 kg (40% difference). The biggest reduction due to late sowing in GY in HS vs. NOR environment was 42% and 41 at SND, followed by 40% and 39% at BWP and ISD, due to the late sowing

respectively. The lowest reduction in GY of (31%) was observed at ISD14HS. This was associated with a significant reduction of 6-12 days in duration of grain filling in the HS environments as compared to NOR.

4.2.3 Correlations and trait heritabilities

Correlation (r) among the studied morphological and yield related parameters across 2 years under normal sowing (NOR) and late sowing (HS) were calculated and are presented (Table 2.3). Under NOR treatment, DH and DM were positively correlated with each other with the value of (0.56). DM has positive significant relation with GY (r=0.33). Significantly positive correlation was found among the PH and BY with the value of (0.24). TP were found positively correlated with GY (0.33) and BY (0.45). SL had positive significant correlation with GWS (0.18) and BY (0.30). SSP were found positively correlated with GNS (0.30), GNP (0.38), GY (0.34) and BY (0.30). Significantly positive correlation was found among the GNS, SW (0.40), GWS (0.50), GNP (r0.89), GY (0.41) and BY (0.70). SW was found positively correlated with GWS (0.74), GNP (0.42), TKW (0.43), GY (0.31) and BY (0.50). There were also found significantly positive correlation among the traits GWS, GNP(r=0.59), TGW (0.43), GY(r=0.49) and BY (0.62). GNP was found positively correlated with GY (0.43) and BY (0.78). Significantly positive correlation was found among the TGW and GY (0.31) also GY was found positively correlated with BY (0.60). Under HS treatment, DH and DM was found significantly positively correlated with each other with the r value of (0.67), while both were found negatively correlated with PH (-0.38) and GY (-0.28). PH was found positively correlated with SL (0.35), GNS (0.26), GWS (0.24), GNP (0.25) and BY (0.29). While SSP were found significantly correlated with GNS (0.25), SW (0.18), GNP (0.28), GY (0.30) and BY (0.20). Significantly positive correlation was found among the traits GNS, SW (0.39), GWS (0.54) GNP (0.85), GY (0.49) and BY (0.55). SW was found significantly correlated with GWS (0.77), GNP (0.33), TGW (0.37), GY (0.33) and BY (0.39). While, significantly positive correlation was found among the traits GWS, GNP (0.46), TGW (0.36), GY (0.40) and BY (0.39). GNP was found significantly correlated with GY (0.55) and BY (0.60). While, TGW was found positive correlated with GY (0.40) and BY (0.31). HSI_{GY} was negatively correlated with GY (-0.3). Broad sense heritability ranged between 0.52 and 0.79, with an average of 0.73 (Table 2.4). DH heritability was high (0.77) in HS 2015, moderate (0.65) in NOR 2014 and low (0.62) in NOR

Table 2.1. Descriptive statistics for phenological traits under Normal and late sowing conditions during 2014 and 2015.

	Mean	SE Mean	C.V.	Min.	Max.
Days to heading					
Normal sowing	117.38	0.6814	16.642	95.000	163.00
Late sowing	88.375	0.5389	17.484	65.000	144.000
Days to maturity					
Normal sowing	154.05	0.6092	11.338	95.000	198.00
Late sowing	114.21	0.6095	15.3	88.000	151.00
Plant height (cm)					
Normal sowing	118.12	0.5468	13.273	60.000	153.00
Late sowing	80.007	0.3552	12.728	55.000	112.00
Tillers per plant					
Normal sowing	12.523	0.0885	20.272	5.0000	22.000
Late sowing	9.3869	0.0662	20.231	4.0000	16.00
Spike length (cm)					
Normal sowing	13.689	0.1173	24.559	8.0000	30.000
Late sowing	10.145	0.0551	12.728	5.0000	18.000
Spikelets per spike					
Normal sowing	18.608	0.1901	34.916	0.0000	26.000
Late sowing	15.108	0.0994	17.699	9.0000	50.000
Spike weight (g)					
Normal sowing	2.6532	0.0215	23.24	1.0000	5.0000
Late sowing	1.9247	0.0198	29.485	0.6600	4.0800
Grains per spike					
Normal sowing	29.547	0.3428	33.262	22.000	74.000
Late sowing	24.471	0.3165	37.081	10.000	56.000
Grains weight per spike (g)					
Normal sowing	1.5392	0.0195	36.325	1.5000	3.8700
Late sowing	1.1032	0.0182	47.277	0.7826	3.8700
Thousand kernel weight (g)					
Normal sowing	47.809	0.2486	14.908	28.000	66.000
Late sowing	39.493	0.2228	16.177	14.000	58.000
Grains number plot					
Normal sowing	7723.6	72.963	27.084	3040.0	14400
Late sowing	6562.2	68.338	29.857	2160.0	13440
Grain yield (kg m^{-2})					
Normal Sowing	0.7455	0.0109	42.078	0.1600	2.340
Late sowing	0.4576	0.067	46.31	0.0800	1.250
Biological yield (kg m^{-2})					
Normal sowing	1.5093	0.0192	36.934	0.3600	4.0900
Late sowing	0.8935	0.0125	42.679	0.1600	2.3600

SE= Standard eror, CV= Coefficient of variation, Min= Minimum, Max= Maximum

Table 2.2. Analysis of variance (ANOVA) for developmental traits under normal and heat stress conditions during two years in synthetic hexaploid wheat. P-values for the mean squares are given.

Source of variations [a]	df[b]	Traits[c]												
		DH	DM	PH	TP	SL	SSP	SWS	GNS	GWS	GNP	TGW	GY	BY
Genotypes	136	141.688**	112.269**	773.575**	12.3455**	21.2219**	24.2292**	0.94881**	323.76**	0.6627*	1.68707*	186.617*	0.4013**	0.713*
Locations	2	226250**	201759**	843.316**	1021.73*	17.3324*	2.333.62*	8.142**	9062.08**	39.4542**	3.38508**	24440.18*	11.3147*	0.744*
Treatments	1	344508*	627473***	602943***	2541.32**	1212.28***	1914.28**	216.866**	8553.83***	96.7357**	5.54408**	39535.2**	61.9062**	184.39**
Years	1	3.6480*	1.78726*	2.48527*	6.6633*	3.78387**	2.78328*	0.09504**	0.5423*	0.0476*	1.56908*	6.75029*	0.3052*	49.577*
Genotype x Location	272	40.411***	32.9231**	183.733***	6.07061*	2.4523**	15.2163*	0.61097*	239.59*	0.4204*	1.14707**	144.41*	0.1817*	0.502*
Genotype x Treatment	136	55.1054***	69.5623***	222.010***	1.05399***	1.4523***	4.74549***	0.1493*	4.831**	0.0939*	176321**	27.3271**	0.0261*	0.062*
Genotype x Year	136	9.70632*	5.81632**	3.34831*	5.47033**	22.4561*	1.55532*	0.02422	0.2412*	0.0197*	791946**	1.19731*	0.044*	0.088*
Location x Treatment	2	4410.38**	105.131***	15796.5**	11.1411*	4714.45**	11.9732**	0.7329**	58.191**	0.6756*	418837***	1213.15**	0.1177**	0.026*
Location x Year	2	4.48129*	3.69728**	2.01431*	1.54031*	578.145**	1.66031**	5.1249**	0.124*	4.4484**	2.89407*	3.2373*	0.0271*	1.084**
Treatment x Year	1	7.64429***	2.94228***	4.00528***	5.08331**	12.245*	2.8673*	2.43305*	0.1745*	0.0182	1980121**	1.2633*	0.1358**	0.977*
Genotype x Location x Treatment	272	35.6050***	33.5407***	65.5085**	0.58475***	451.231***	4.43157	0.08605**	4.731*	0.0559*	157335*	34.6141**	0.0093**	0.051*
Genotype x Location x Year	272	1.62132*	1.97132**	1.04431**	4.06733**	2.3569**	6.79633	0.21131*	0.0157*	0.0999*	815799**	8.74532**	0.0403*	0.083*
Genotype x Treatment x Year	136	2.78232*	3.13832**	2.89430***	5.03234***	14.234**	5.94133	0.00923*	0.214*	0.0036*	79363*	1.00632*	0.0068*	0.02*
Location x Treatment x Year	2	4.18330**	1.11232**	1.30631***	1.45333***	11.647**	1.45733*	2.06692**	0.403*	0.2323**	3990224**	1.2083*	0.0277**	0.559**

*** ** *** Significant difference at P<0.05, 0.001, 0.0001**

[b] df: degree of freedom

[c] DH: Days to heading, DM: Days to maturity, PH: Plant height (cm), TP: Tillers per plant, SL: Spike length (cm), SSP: Spikelets per spike, GNS: Grains number per spike (g), GWS: Grains weight per spike (g), SWS: Spike weight per spike (g), TGW: Thousand grain weight (g), GNP: Grain number per unit area, GY: grain yield per unit area, BY: Biological yield.

2014, and had over all mean of 0.74. DM heritability was high (0.74) in HS 2015, moderate (0.63) in NOR 2015 and low (0.57) in NOR 2014. PH heritability was high (0.75) in HS 2015, moderate (0.63) in NOR 2015 and low (0.54) in NOR 2014. TP heritability was high (0.69) in HS 2014, moderate (0.55) in NOR 2014 and low (0.46) in NOR 2015. SL heritability was high (0.77) in HS 2015, moderate (0.64) in NOR 2015 and low (0.53) in NOR 2014. SSP heritability was high (0.78) in HS 2014, moderate (0.64) in NOR 2014 and low (0.54) in NOR 2015.GNS heritability was high (0.79) in HS 2015, moderate (0.67) in NOR 2015 and low (0.56) in NOR 2014. GWS heritability was high (0.71) in HS 2015, moderate (0.55) in NOR 2015 and low (0.46) in NOR 2014. SW heritability was high (0.69) in HS 2014, moderate (0.59) in NOR 2014 and low (0.50) in NOR 2015. TGW heritability was high (0.64) in HS 2015 and low (0.47) in NOR 2014, and had an overall mean of (0.60). GNP heritability was high in HS during 2015 (0.70) and moderate (0.63) in NOR during 2014, and had an overall mean of 0.66. GY heritability was low overall (0.58) and in all the environments except in HS 2015, where it was moderate (0.62). BY heritability was high in HS during 2015 (0.70) and moderate (0.62) in NOR during 2015, and had an overall mean of 0.65.

4.2.4 Grain yield stability in different environments

SHW accessions were ranked on the basis of GY stability across environments and in individual environments. SHW accessions AUS-30284, AUS-33384, AUS-30288, AUS-30296, AUS-33409 and AUS-30629 were the most stable overall (Table 2.5). AUS-30284 was dependably stable in HS and NOR environments while AUS-33384 was dependably stable in HS environment and accession AUS-333409 was constantly stable in normal environment.

4.2.5 Principal component analyses

To sort out the best suitable combination of the environments for getting the maximum yield of agronomic attributes, PCA were conducted. The vector length in the PCA shows the amount of disparity expounded by the respective traits in the PCA (Figure. 2.1). The primary 2 axes, i.e. PC1 and PC2, described up to 82.62% of the total variability. PCA indicated noticeably that in ISB-15NOR and ISB-14NOR the performance of genotypes regarding to agronomic attributes were superior followed by BWP-15NOR, BWP-14NOR, SND-15NOR and SND-14NOR while the BWP-14HS, SND-14HS, SND-15HS and

Table 2.3. Pearson's co-efficient of correlation among the parameters averaged in all environments. Upper trio exemplifies correlations from all heat stress treatments (HS), and lower trio exhibit the correlations from all normal environments (NOR).

Variables	DH	DM	PH	TP	SL	SSP	GNS	SW	GWS	GNP	TKW	GY	BY
DH	**1**	**0.69**	**0.40**	-0.01	**0.46**	**0.77**	**0.39**	**0.25**	-0.07	**0.30**	**0.28**	**-0.25**	**0.67**
DM	**0.56**	**1**	**0.31**	**0.55**	**0.36**	**0.33**	**0.54**	**0.18**	**0.23**	-0.05	**0.35**	-0.09	**-0.38**
PH	-0.15	-0.01	**1**	**0.60**	**0.40**	**0.37**	**0.85**	0.11	**0.26**	0.06	-0.03	0.04	-0.10
TP	-0.06	0.02	0.14	**1**	**0.39**	**0.33**	0.08	**0.28**	**0.22**	-0.12	**0.26**	-0.04	-0.09
SL	0.07	0.13	0.05	**0.29**	**1**	**0.39**	**0.49**	0.01	**0.25**	-0.10	**0.17**	-0.02	0.00
SSP	**-0.23**	**0.18**	0.06	-0.00	0.02	**1**	**0.55**	**0.30**	0.03	0.02	**0.24**	**-0.23**	**-0.21**
GNS	**-0.20**	**0.21**	**-0.21**	-0.02	0.09	**0.30**	**1**	**0.20**	**0.19**	0.02	**0.25**	**-0.23**	**-0.26**
SW	**-0.17**	**0.24**	0.05	0.03	0.13	**0.17**	**0.40**	**1**	**0.20**	0.13	0.03	-0.07	**-0.31**
GWS	-0.09	**0.18**	0.15	0.02	**0.18**	0.10	**0.50**	**0.74**	**1**	**0.32**	**0.18**	-0.05	**-0.19**
GNP	-0.14	**0.21**	**0.24**	-0.01	0.16	**0.38**	**0.89**	**0.42**	**0.59**	**1**	**0.29**	**-0.22**	-0.08
TKW	-0.10	**0.17**	-0.03	-0.02	-0.07	-0.16	0.03	**0.43**	**0.43**	0.01	**1**	-0.09	**-0.28**
GY	**0.39**	**0.33**	**0.17**	**0.33**	0.16	**0.34**	**0.41**	**0.31**	**0.49**	**0.43**	**0.31**	**1**	-0.13
BY	-0.12	-0.11	**0.24**	**0.45**	**0.30**	**0.30**	**0.70**	**0.50**	**0.62**	**0.78**	0.16	**0.60**	**1**

Values in bold are significant at * $P < 0.05$.

DH: Days to heading, DM: Days to maturity, PH: Plant height (cm), TP: Tillers per plant, SL: Spike length (cm), SSP: Spikelets per spike, GNS: Grains number per spike, GWS: Grains weight per spike (g), SWS: Spike weight per spike (g), TGW: Thousand grain weight (g) GN: Grain number per unit area, GY: grain yield per unit area, Biomass: Biological Yield.

Table 2.4. Broad sense heritability estimations of all the phenotypic and yield related traits averaged across the three locations (BWP, SND and ISD).

	Year × treatment				
	14NOR	**14HS**	**15NOR**	**15HS**	**Mean**
DH	0.62	0.74	0.65	0.77	0.70
DM	0.57	0.71	0.63	0.74	0.67
PH	0.54	0.65	0.63	0.75	0.64
TP	0.55	0.69	0.46	0.68	0.62
SL	0.53	0.75	0.64	0.77	0.70
SSP	0.64	0.78	0.54	0.76	0.70
GNS	0.56	0.76	0.67	0.79	0.72
GWS	0.47	0.67	0.55	0.71	0.61
SWS	0.59	0.69	0.50	0.67	0.63
TKW	0.47	0.61	0.57	0.64	0.60
GN	0.63	0.67	0.62	0.70	0.66
GY	0.54	0.59	0.56	0.62	0.58
BY	0.60	0.67	0.62	0.70	0.65

DH: Days to heading, DM: Days to maturity, PH: Plant height (cm), TP: Tillers per plant, SL: Spike length (cm), SSP: Spikelets per spike, GNS: Grains number per spike, GWS: Grains weight per spike (g), SWS: Spike weight per spike (g), TGW: Thousand grain weight (g) GN: Grain number per unit area, GY: grain yield per unit area, BY: Biological Yield.

BWP15HS were the lowest regarding to the performance of genotypes regarding the agronomic traits. In biplot analysis, rainfall showed negative correlation with temperature and clearly depicted that ISB-14HS and ISB-15HS experienced minimum stress as compared to SND-14HS, SND-15HS and BWP-14HS, BWP-15HS. SHWs were sort out on the biplot using the HSI^{GY} and parameters were represented as vectors (Figure. 2.2). The length of vector showed the amount of variation explicated by corresponding parameters in the PCA (Figure. 2.2).

In scatter plots, DH had positive association with DM, TKW and GNs displayed positive correlation with GY, SWS GWS and GNS had positive association with each other and positive correlation with Yield while significantly negative correlation was found among the DH and PH also both were negatively correlated with GY. Using the GY the scatter plot depicted that the genotypes AUS-30284 AUS-33384, AUS-34243, AUS-30296 AUS-30288, were found tolerant while the genotypes AUS-30282, AUS-33421 AUS-33414 were found moderately tolerant and were scattered near to TKW and GNs in the biplots.

4.2.6 Ridge regression analysis

The model of Ridge regression depicted that these phenological and yield related parameters, DH, PH, GN and TGW explicated up to 32.45% of the difference in GY across all treatments, 35.45% of GY in NOR environments and 31.14% of GY in heat stress environments (Tab. 2.6). Under NOR treatment, the R^2 value ranged between 8.80% (E1-SND14N) to 45.49% (E6-ISD15N). In HS treatment, the R^2 value ranged between 8.74% (E1-SNB14L) to 35.20% (E6-ISB14L).

4.2.7 Days to heading

There were significantly different effects of treatments, genotypes, years and their interactions at P value greater than 0.05 (Table 2.2). Maximum reduction of 28% in DH was detected when average values for days to heading in both years in NOR and HS environments were averaged for all the genotypes. Minimum decrease of (14%) in DH was observed for the accession AUS34251 while the maximum decline (33%) was being observed in the accession AUS33999 in HS. Under NOR environment, the accession AUS34251 had the lowest number of DH (109. days), while the maximum number of DH were observed for AUS30284 (126 days), while, during the HS environments the range of genotypes for DH was from 77 days (AUS33415) to 98 days (AUS34427).

4.2.8 Days to maturity

There were significantly different effects of treatments, genotypes, years and their interactions at P value greater than 0.05 (Table 2.2). In DM total decrease of 28% was experienced when the averages of both years in NOR and HS were received for averaging across all the accessions. Lowest reduction of (14%) in DM was observed for the accession AUS33419, while the maximum reduction of (34%) was well noted for the accession AUS30651 in HS environments. Under NOR environments, the genotype AUS33409 had the minimum number of DM (145. days), the maximum DM being noted for AUS34431 (160), whereas, under HS stress conditions minimum (103days) DM were recorded for genotype AUS34426 and maximum DM were recorded (128 days) for genotype AUS33419.

4.2.9 Plant height

There were significantly different effects of treatments, genotypes, years and their interactions at P value greater than 0.05 (Table 2.2). In plant height total 26.6% of reduction was received when the data was averaged for both years in NOR and HS in all the accessions. Minimum reduction of (16%) in PH was observed for the accession AUS34243 while the maximum reduction (45%) was noted in the genotype AUS33394 in HS environments. Under NOR environments, minimum PH (84. cm), was observed for the genotype AUS33384, while, the maximum PH was noted for AUS34448 (145. cm), while, under HS environment minimum (62 cm) PH were recorded for genotype AUS34255 and maximum PH were recorded (102 cm) for genotype AUS33421.

4.2.10 Tillers per Plant

There were significantly different effects of treatments, genotypes, years and their interactions at P value greater than 0.05 (Table 2.2). In TP total 2676% of reduction was received when the data was averaged for both years in NOR and HS in all the accessions. Minimum reduction of (3%) in PH was observed for the accession AUS33403 while the maximum reduction (31%) was noted in for the genotype AUS33394 in HS environments. During NOR environments, the genotype AUS34235 had the minimum number of TP (9), the maximum number of TP was noted for AUS30636 (15), While, under HS stress

Table 2.5. Grain yield stability in top ranking synthetic hexaploids, in individual environments averaged in all normal (NOR) environments, averaged across heat stress environments (HS) and averaged across all environments (AE).

Environments	AUS30284	Rank	AUS33384	Rank	AUS30288	Rank	AUS30296	Rank	AUS33409	Rank	AUS30629	Rank
AE	1.71	1	1.55	2	1.53	3	1.46	4	1.42	5	1.41	6
NOR	1.86	1	1.66	3	1.65	4	1.58	6	1.69	2	1.55	9
HS	1.57	1	1.43	2	1.41	3	1.34	4	1.16	12	1.27	6
SND14NOR	1.75	1	1.59	6	1.54	7	1.49	15	1.65	2	1.49	16
SND15NOR	1.68	1	1.57	5	1.59	3	1.57	6	1.59	2	1.51	8
BWP14NOR	1.89	2	1.79	3	1.65	7	1.78	4	1.71	6	1.39	22
BWP15NOR	1.89	1	1.58	8	1.79	2	1.74	4	1.44	22	1.44	23
ISB14NOR	2.00	2	1.75	7	1.69	10	1.42	25	1.95	3	1.79	5
ISB15NOR	1.92	1	1.69	5	1.62	12	1.49	21	1.79	3	1.65	9
SND14HS	1.49	1	1.37	6	1.30	8	1.32	7	1.05	18	1.20	9
SND15HS	1.48	1	1.40	2	1.36	4	1.39	3	1.04	19	1.24	6
BWP14HS	1.59	1	1.56	2	1.41	4	1.49	3	1.32	7	1.16	11
BWP15HS	1.59	1	1.30	4	1.45	3	1.51	2	0.99	25	1.22	9
ISB14HS	1.75	1	1.49	3	1.47	5	1.12	23	1.34	9	1.47	6
ISB15HS	1.54	1	1.45	4	1.49	3	1.20	16	1.22	14	1.35	8

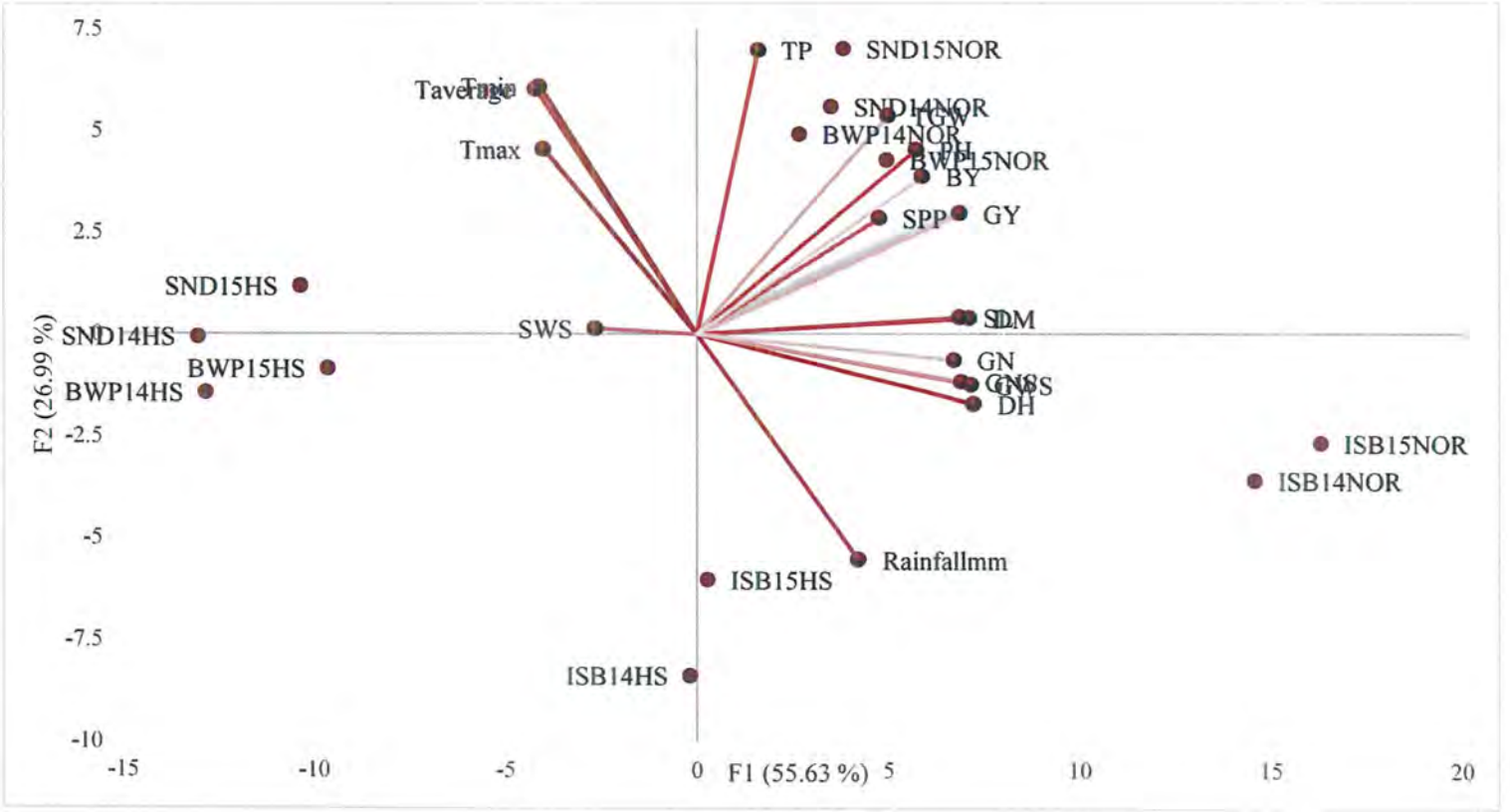


Figure 2.1. Principal component analysis showing scatter plot for environmental variables, traits and individual environments.

Table 2.6. Grain yield variation explanation model obtained by ridge regression along with estimated regression values (Est) and associated significance level in each environment and together in all normal environments (NOR), all heat stress environments (HS) and across environments (AE).

	DH		PH		GN		TGW			
Environments	Est	p	Est	p	Est	p	Est	p	R^2 (%)	p
AE	-1.23E-02	NS	1.81E-04	NS	7.19E-05	***	0.03422	***	32.14	***
NOR	-9.60E-03	NS	2.95E-04	NS	8.19E-05	***	0.03132	***	34.45	***
HS	-9.18E-03	NS	6.12E-04	NS	6.46E-05	**	0.03757	***	30.47	***
SND14NOR	9.73E-03	NS	1.43E-03	NS	4.03E-05	**	0.0106	*	8.80	*
SND15NOR	6.70E-03	NS	6.46E-04	NS	8.27E-05	***	0.01604	***	30.34	***
BWP14NOR	-7.66E-03	NS	2.57E-03	NS	5.55E-05	***	0.01654	***	18.23	**
BWP15NOR	-5.42E-03	NS	1.08E-04	NS	8.40E-05	***	0.02703	***	35.73	***
ISB14NOR	-8.94E-03	*	-1.11E-03	NS	3.55E-05	**	0.02967	***	42.46	***
ISB15NOR	-3.60E-03	NS	-1.93E-03	NS	6.45E-05	***	0.02735	***	45.49	***
SND14HS	-4.28E-03	NS	6.39E-03	NS	4.32E-05	**	0.00821	NS	8.74	*
SND15HS	-1.18E-03	NS	7.04E-03	*	7.22E-05	***	0.012	*	19.76	**
BWP14HS	-5.53E-03	NS	-4.64E-04	NS	3.27E-05	*	0.02648	***	22.22	***
BWP15HS	-3.61E-03	NS	7.15E-04	NS	7.07E-05	***	0.02163	***	23.01	***
ISB14HS	-9.69E-03	*	-3.89E-03	NS	3.15E-05	**	0.02662	***	35.20	***
ISB15HS	-1.13E-02	*	-4.19E-03	NS	6.06E-05	***	0.02054	***	32.41	***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS = non-significant.

DH: Days to heading, PH: Plant height (cm), TGW: Thousand grain weight (g), GN: Grain number per unit area,

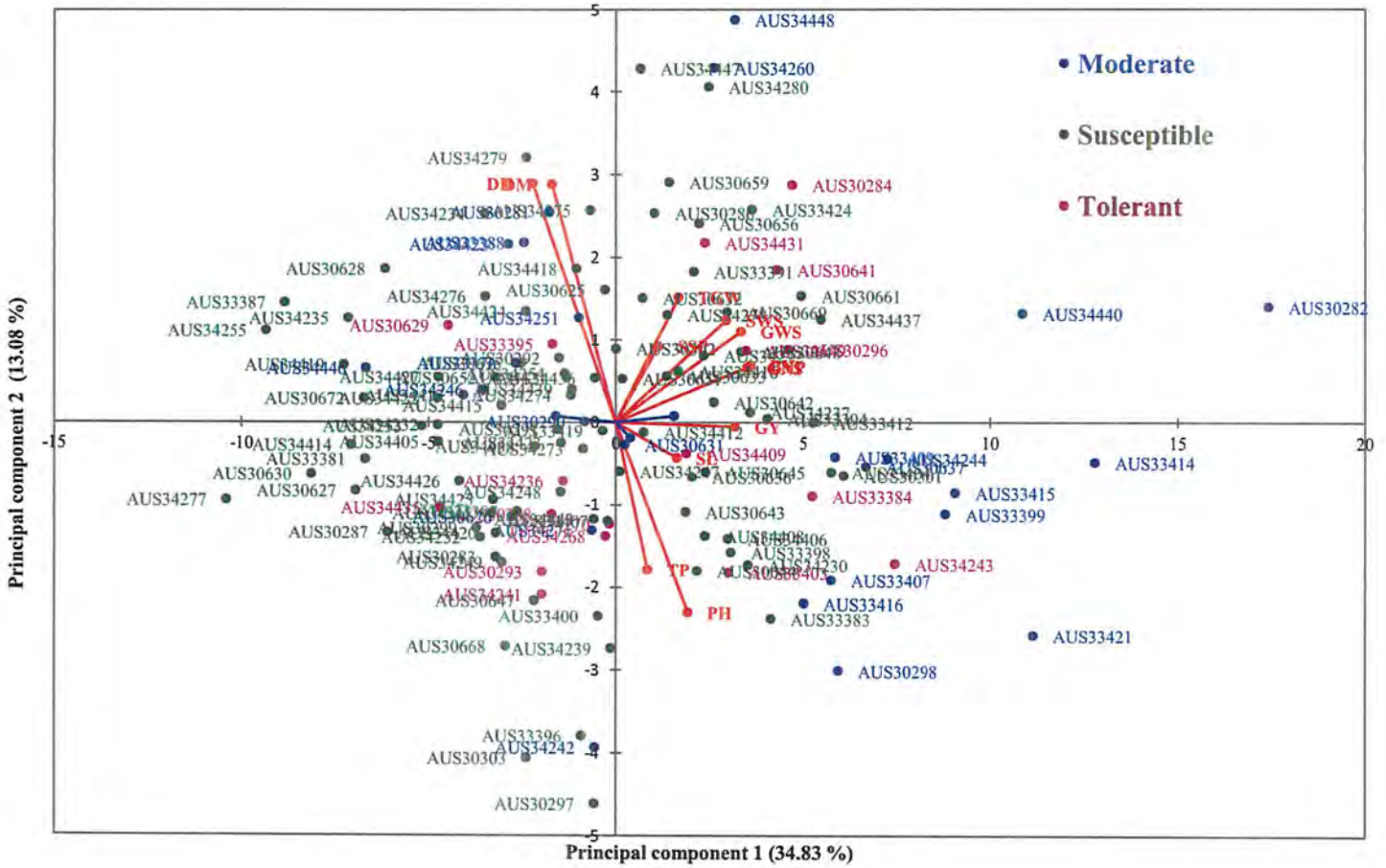
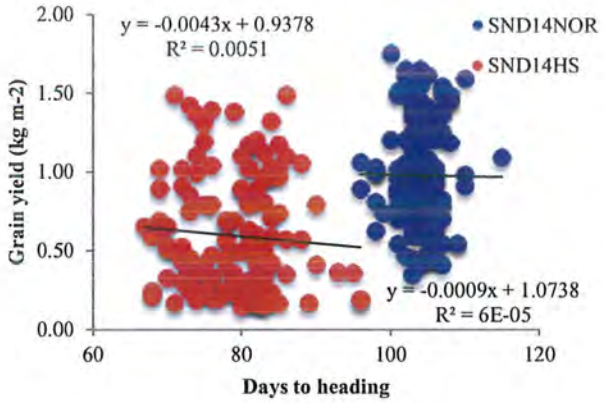
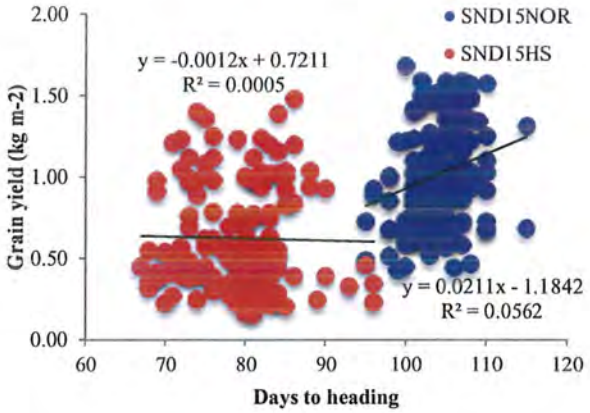


Figure 2.2. Principal component analysis based on all agronomic traits averaged over all environments. Scatterplot showing synthetic hexaploid wheats sorted into tolerant, moderate and susceptible based on heat susceptibility index, and morphological traits based on two principal components

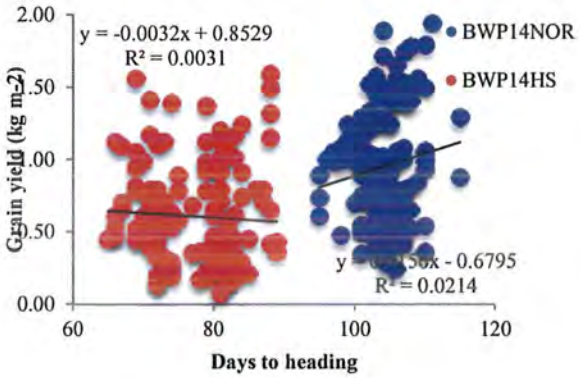
a)


y = -0.0043x + 0.9378
R² = 0.0051
SND14NOR
SND14HS
y = -0.0009x + 1.0738
R² = 6E-05
Grain yield (kg m-2)
Days to heading

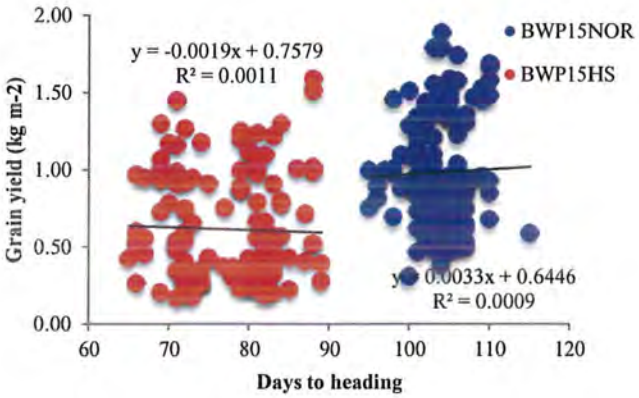
b)


y = -0.0012x + 0.7211
R² = 0.0005
SND15NOR
SND15HS
y = 0.0211x - 1.1842
R² = 0.0562
Grain yield (kg m-2)
Days to heading

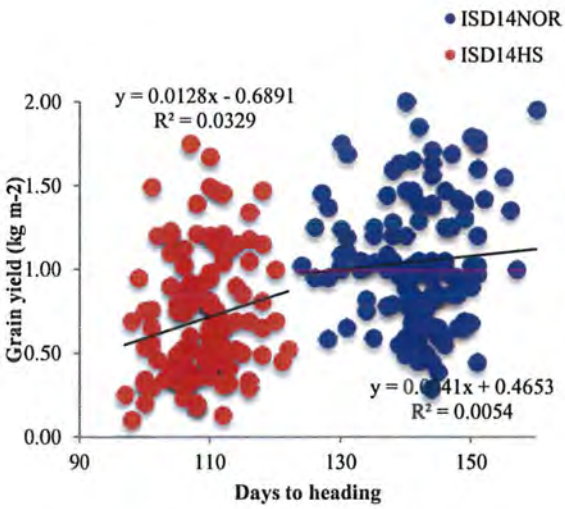
c)


y = -0.0032x + 0.8529
R² = 0.0031
BWP14NOR
BWP14HS
R² = 0.0214
Grain yield (kg m-2)
Days to heading

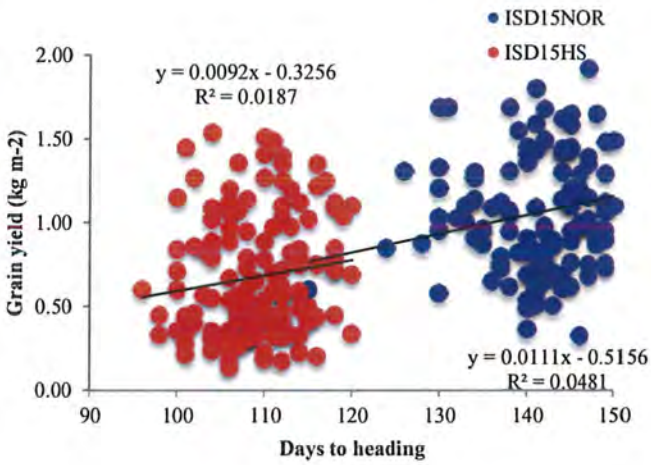
d)


y = -0.0019x + 0.7579
R² = 0.0011
BWP15NOR
BWP15HS
y = 0.0033x + 0.6446
R² = 0.0009
Grain yield (kg m-2)
Days to heading

e)


y = 0.0128x - 0.6891
R² = 0.0329
ISD14NOR
ISD14HS
R² = 0.0054
Grain yield (kg m-2)
Days to heading

f)


y = 0.0092x - 0.3256
R² = 0.0187
ISD15NOR
ISD15HS
y = 0.0111x - 0.5156
R² = 0.0481
Grain yield (kg m-2)
Days to heading

g)

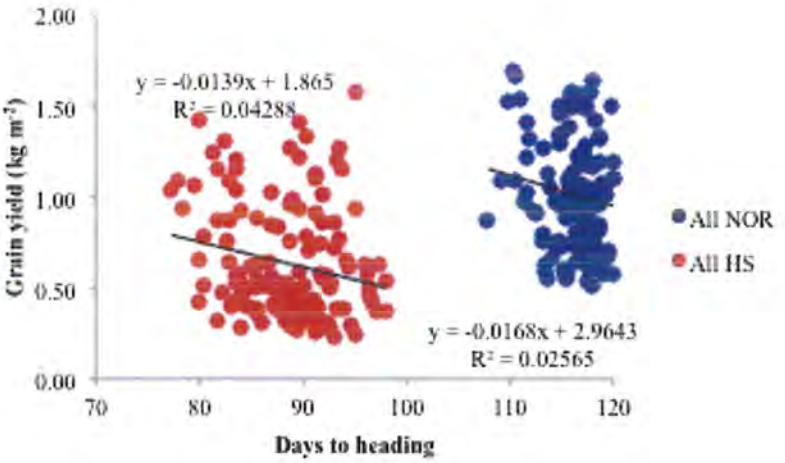
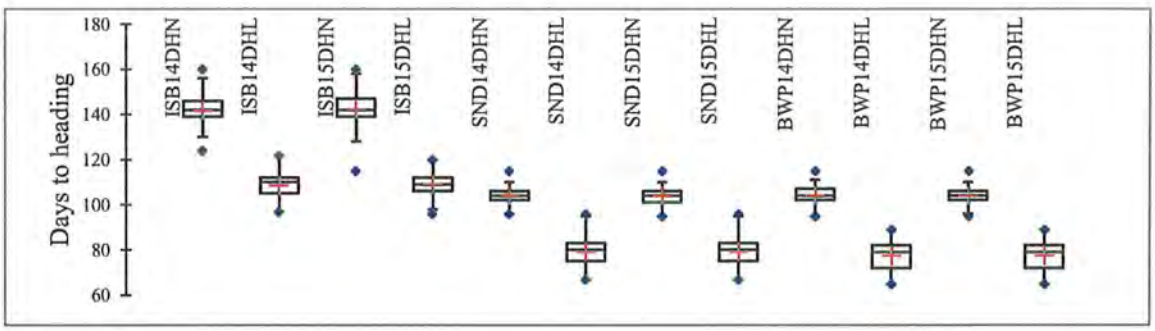
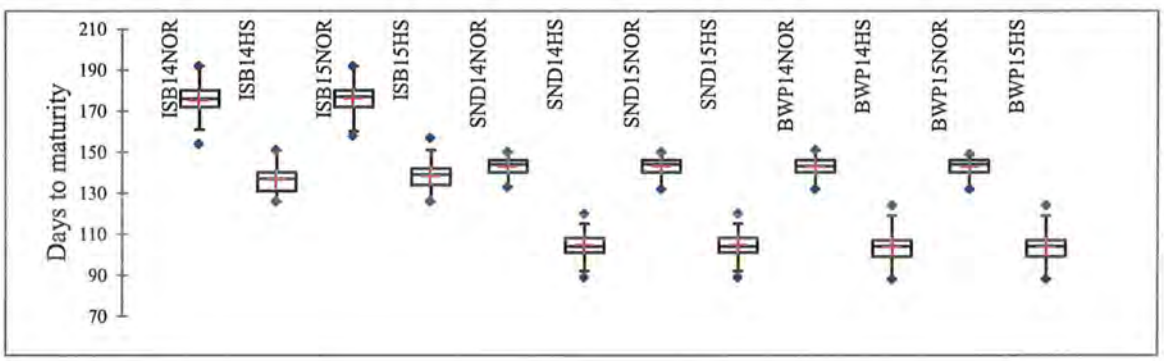


Figure 2.3. Relationship between heading days and grain yield in each environment, a) SND 2014, b) SND 2015, c) BWP 2014, d) BWP2015, e) SND 2014, f) SND 2015 and g) average across all NOR and HS environments. Each plot represents two trials (red for HS and blue for NOR) at each location during each year.

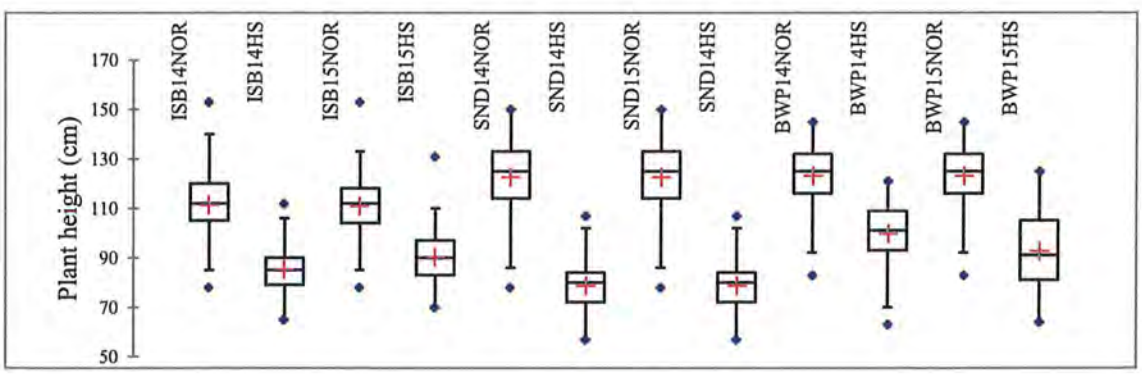
a)


Days to heading
180
160
140
120
100
80
60
ISB14DHN
ISB14DHL
ISB15DHN
ISB15DHL
SND14DHN
SND14DHL
SND15DHN
SND15DHL
BWP14DHN
BWP14DHL
BWP15DHN
BWP15DHL

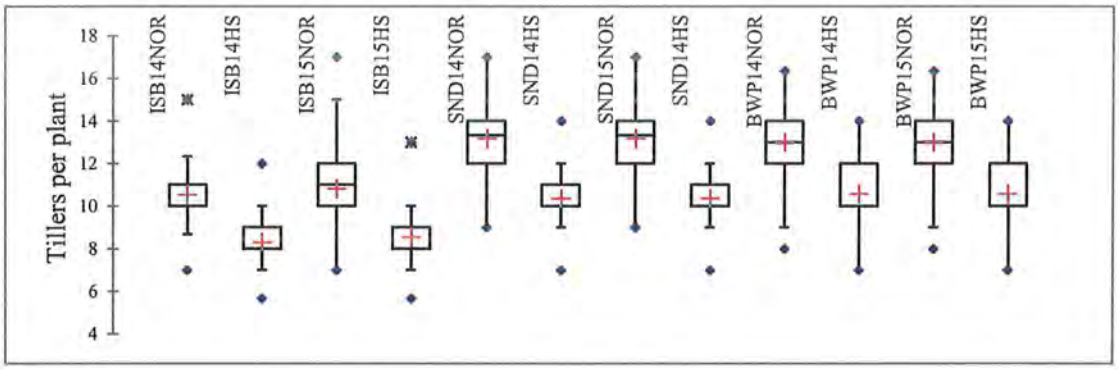
b)


Days to maturity
210
190
170
150
130
110
90
70
ISB14NOR
ISB14HS
ISB15NOR
ISB15HS
SND14NOR
SND14HS
SND15NOR
SND15HS
BWP14NOR
BWP14HS
BWP15NOR
BWP15HS

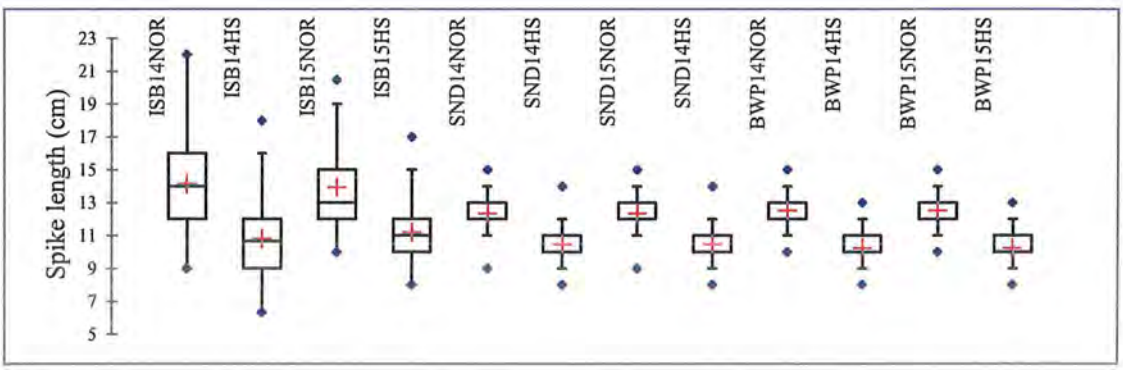
c)


Plant height (cm)
170
150
130
110
90
70
50
ISB14NOR
ISB14HS
ISB15NOR
ISB15HS
SND14NOR
SND14HS
SND15NOR
SND14HS
BWP14NOR
BWP14HS
BWP15NOR
BWP15HS

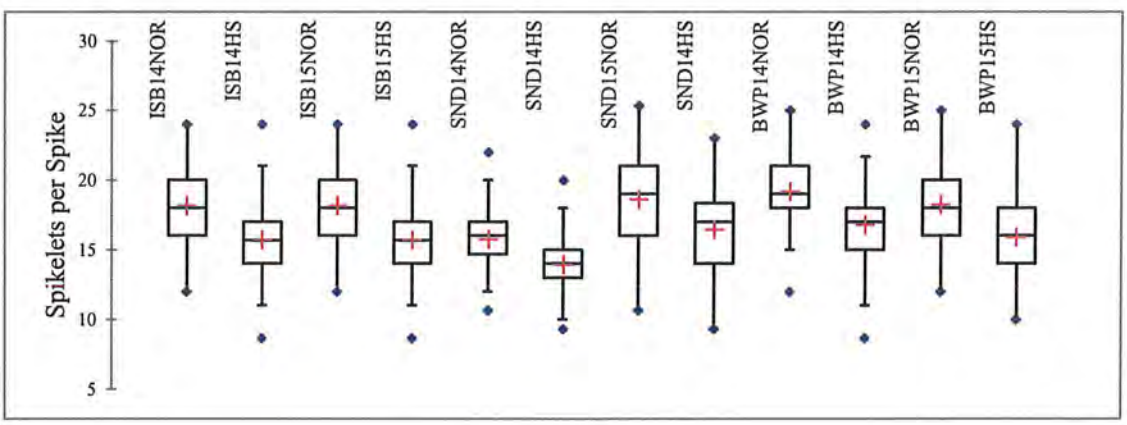
d)


Tillers per plant
18
16
14
12
10
8
6
4
ISB14NOR
ISB14HS
ISB15NOR
ISB15HS
SND14NOR
SND14HS
SND15NOR
SND14HS
BWP14NOR
BWP14HS
BWP15NOR
BWP15HS

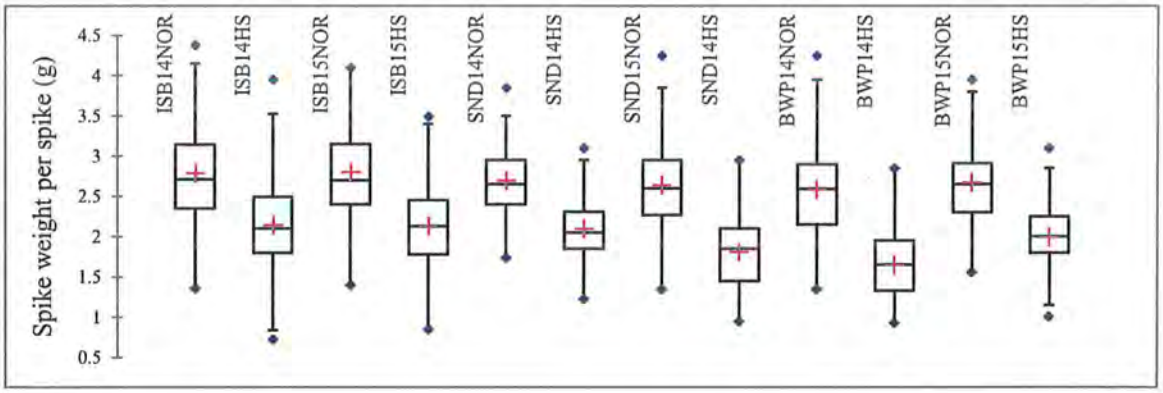
e)


Spike length (cm)
23
21
19
17
15
13
11
9
7
5
ISB14NOR
ISB14HS
ISB15NOR
ISB15HS
SND14NOR
SND14HS
SND15NOR
SND14HS
BWP14NOR
BWP14HS
BWP15NOR
BWP15HS

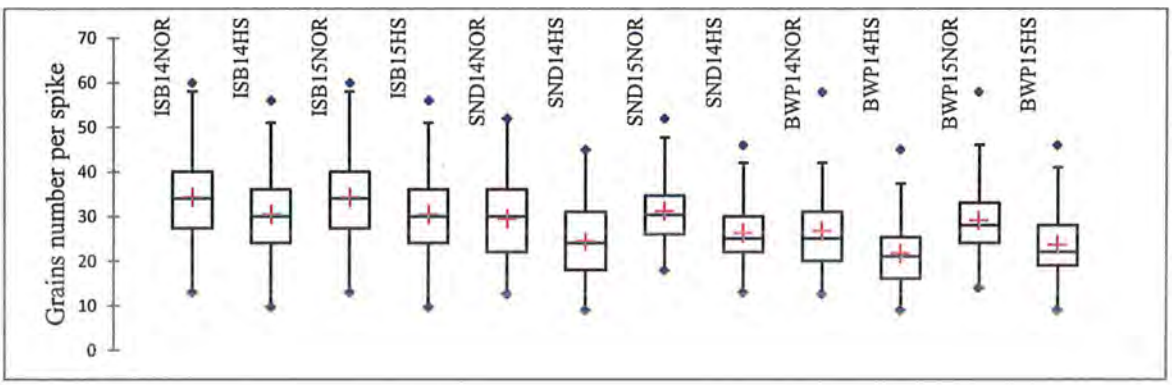
f)


Spikelets per Spike
30
25
20
15
10
5
ISB14NOR
ISB14HS
ISB15NOR
ISB15HS
SND14NOR
SND14HS
SND15NOR
SND14HS
BWP14NOR
BWP14HS
BWP15NOR
BWP15HS

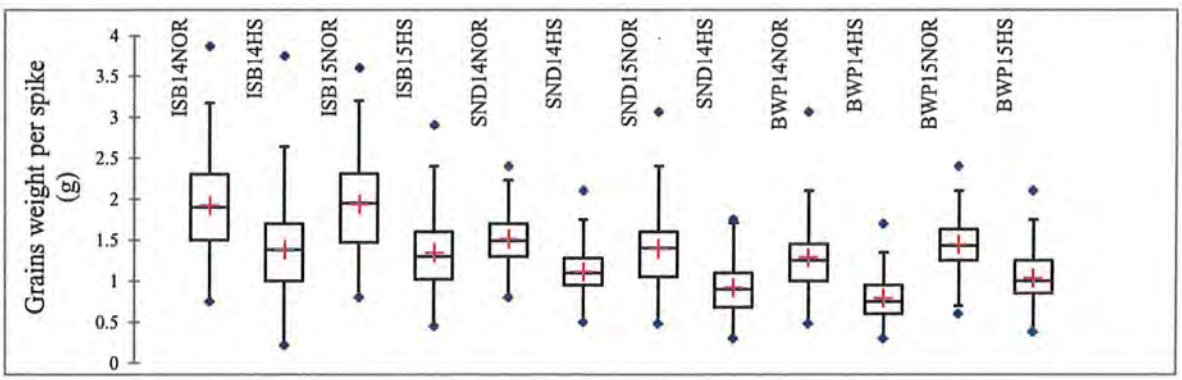
g)


Spike weight per spike (g)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
ISB14NOR
ISB14HS
ISB15NOR
ISB15HS
SND14NOR
SND14HS
SND15NOR
SND14HS
BWP14NOR
BWP14HS
BWP15NOR
BWP15HS

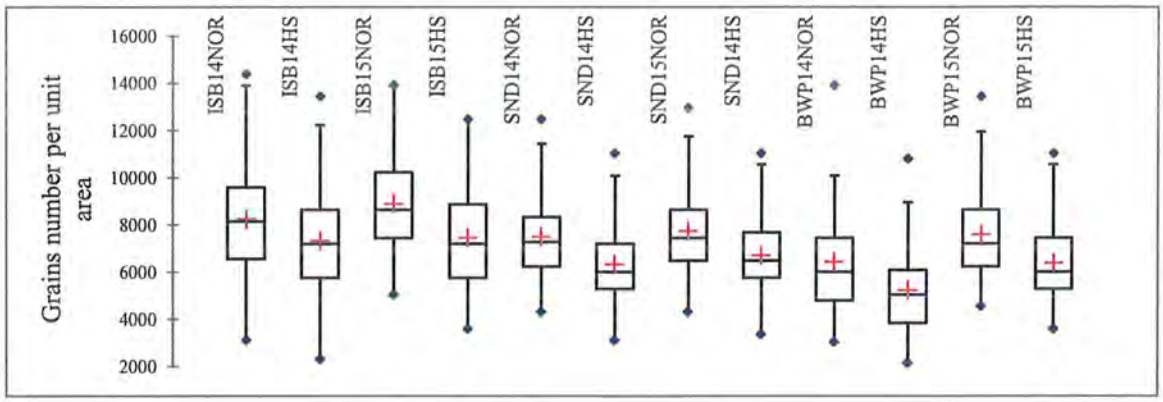
h)


Grains number per spike
70
60
50
40
30
20
10
0
ISB14NOR
ISB14HS
ISB15NOR
ISB15HS
SND14NOR
SND14HS
SND15NOR
SND14HS
BWP14NOR
BWP14HS
BWP15NOR
BWP15HS

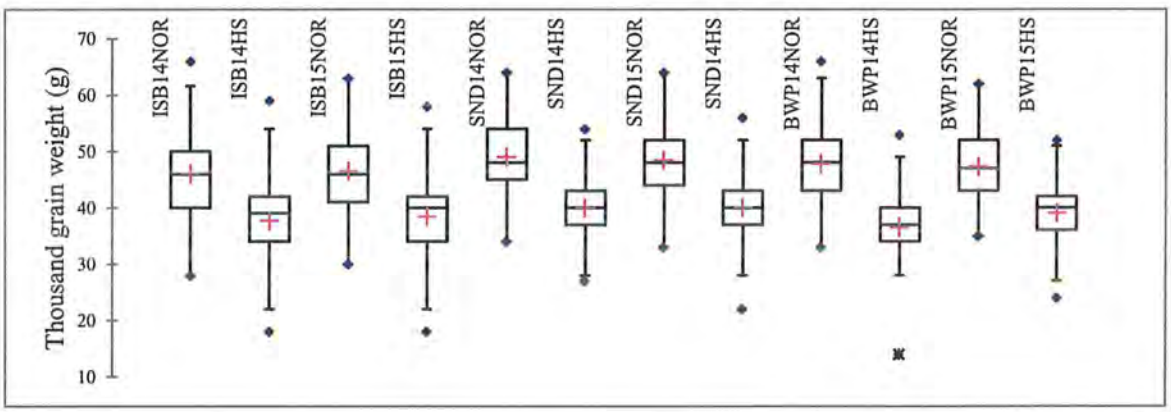
i)


Grains weight per spike (g)
4
3.5
3
2.5
2
1.5
1
0.5
0
ISB14NOR
ISB14HS
ISB15NOR
ISB15HS
SND14NOR
SND14HS
SND15NOR
SND14HS
BWP14NOR
BWP14HS
BWP15NOR
BWP15HS

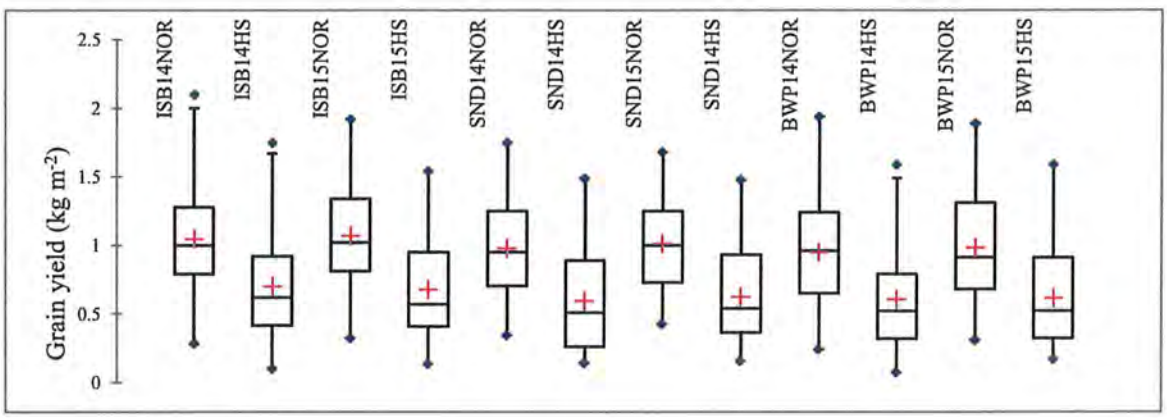
j)


Grains number per unit area
16000
14000
12000
10000
8000
6000
4000
2000
ISB14NOR
ISB14HS
ISB15NOR
ISB15HS
SND14NOR
SND14HS
SND15NOR
SND14HS
BWP14NOR
BWP14HS
BWP15NOR
BWP15HS

k)


Thousand grain weight (g)
70
60
50
40
30
20
10
ISB14NOR
ISB14HS
ISB15NOR
ISB15HS
SND14NOR
SND14HS
SND15NOR
SND14HS
BWP14NOR
BWP14HS
BWP15NOR
BWP15HS

l)


Grain yield (kg m-2)
2.5
2
1.5
1
0.5
0
ISB14NOR
ISB14HS
ISB15NOR
ISB15HS
SND14NOR
SND14HS
SND15NOR
SND14HS
BWP14NOR
BWP14HS
BWP15NOR
BWP15HS

m)

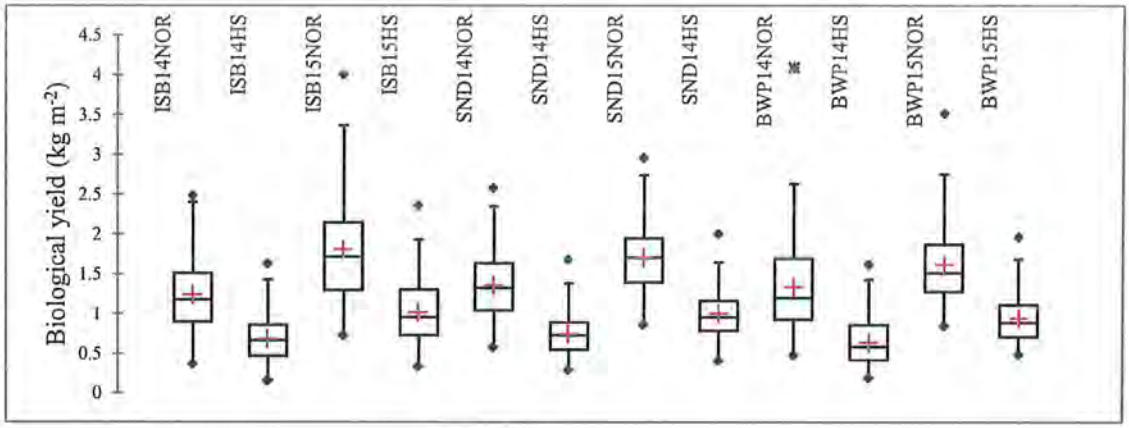
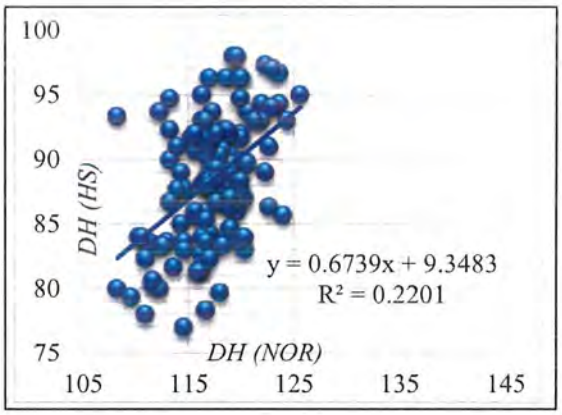
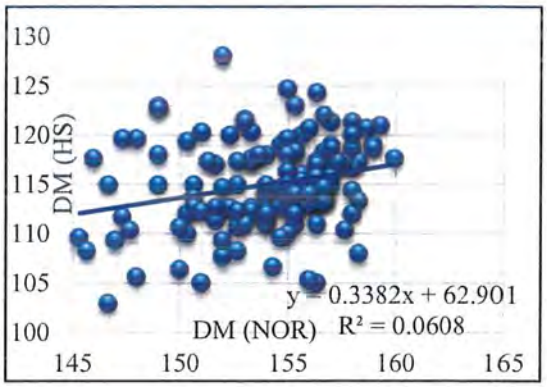


Figure 2.4. Box and whisker plots for agronomic traits across the 12 environments showing performance of 200 synthetic hexaploid wheats for a) DH: days to heading, b) DM: days to maturity, c) PH: plant height (cm), d) TP: tillers per plant, e) SL: spike length (cm), f) SPS: spikelets per spike, g) SWS: spike weight per spikes, h) GNS: grains number spike, i) GWS: grains weight per spike (g), j) GNP: grains number per unit area, k) TGW: thousand grain weight (g), l) GY: grain yield per unit area, and m) BY: biological yield. The box represent standard deviation and the vertical lines represent 90% confidence interval on lower and upper boundaries of means.

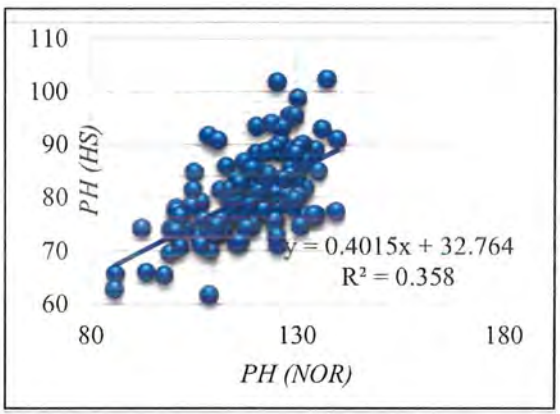
a)


100
95
90
85
80
75
DH (HS)
y = 0.6739x + 9.3483
R² = 0.2201
DH (NOR)
105
115
125
135
145

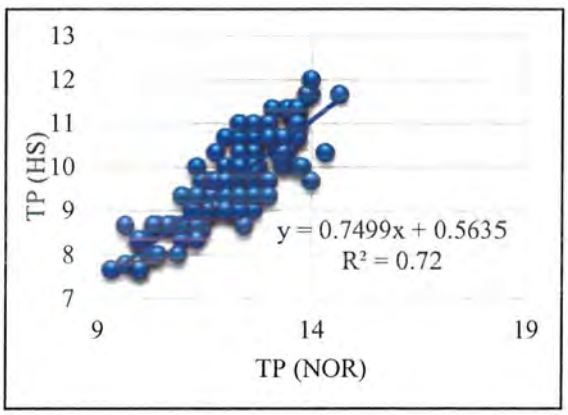
b)


130
125
120
115
110
105
100
DM (HS)
y = 0.3382x + 62.901
R² = 0.0608
DM (NOR)
145
150
155
160
165

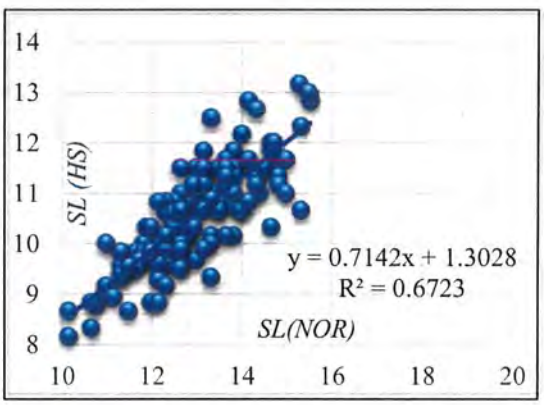
c)


110
100
90
80
70
60
PH (HS)
y = 0.4015x + 32.764
R² = 0.358
80
130
180
PH (NOR)

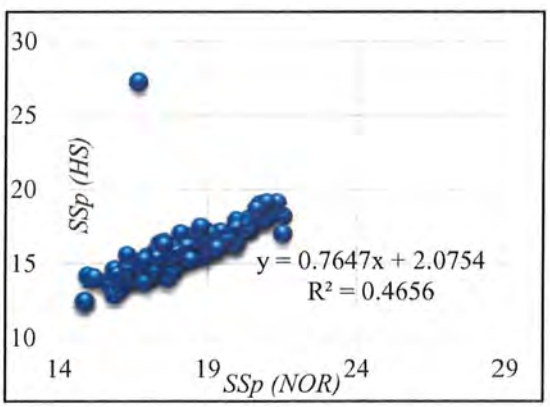
d)


13
12
11
10
9
8
7
TP (HS)
y = 0.7499x + 0.5635
R² = 0.72
9
14
19
TP (NOR)

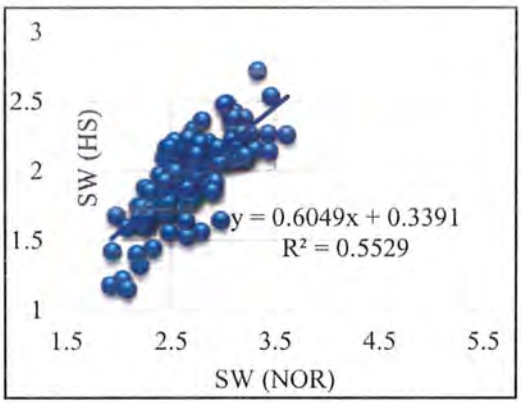
e)


14
13
12
11
10
9
8
SL (HS)
y = 0.7142x + 1.3028
R² = 0.6723
SL(NOR)
10
12
14
16
18
20

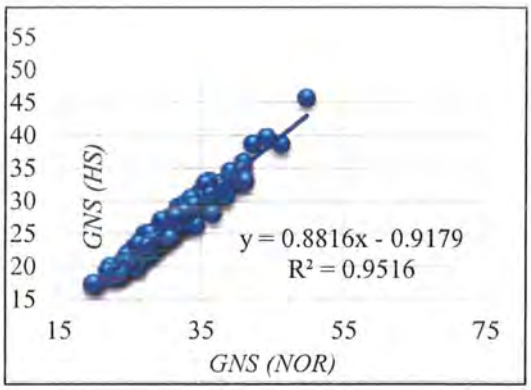
f)


30
25
20
15
10
SSp (HS)
y = 0.7647x + 2.0754
R² = 0.4656
14
19
24
29
SSp (NOR)

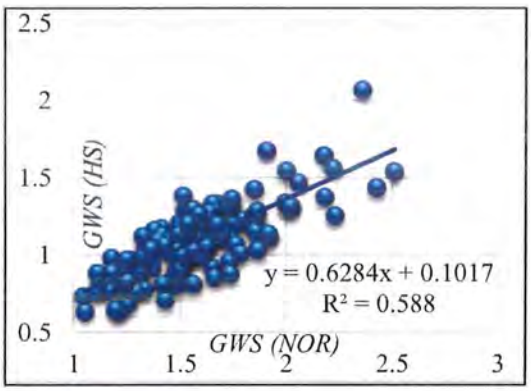
g)


SW (HS)
SW (NOR)
y = 0.6049x + 0.3391
R² = 0.5529

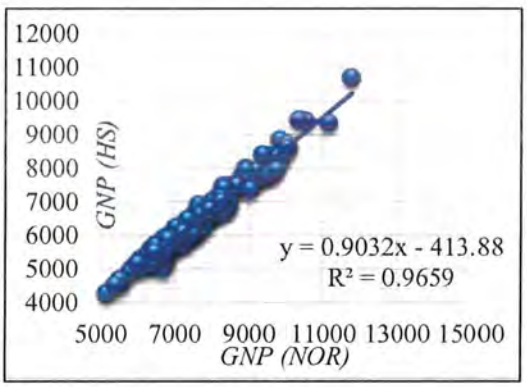
h)


GNS (HS)
GNS (NOR)
y = 0.8816x - 0.9179
R² = 0.9516

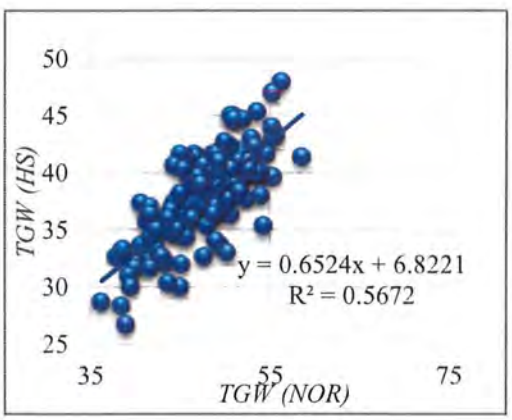
i)


GWS (HS)
GWS (NOR)
y = 0.6284x + 0.1017
R² = 0.588

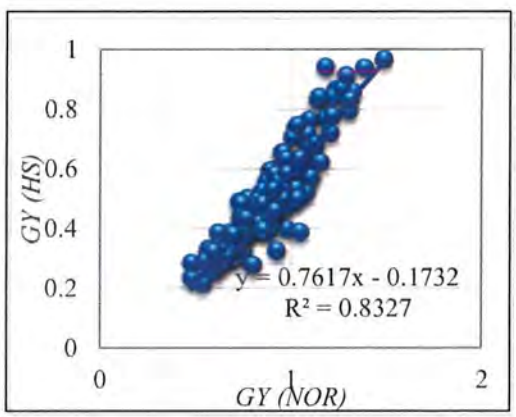
j)


GNP (HS)
GNP (NOR)
y = 0.9032x - 413.88
R² = 0.9659

k)


TGW (HS)
TGW (NOR)
y = 0.6524x + 6.8221
R² = 0.5672

l)


GY (HS)
GY (NOR)
y = 0.7617x - 0.1732
R² = 0.8327

m)

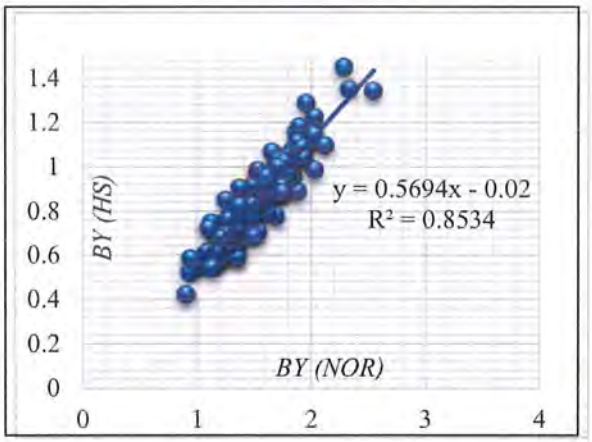


Figure 2.5. Scatterplots showing relationship between normal (NOR) and heat stress (HS) treatments averaged for across year x location for the traits including: a) DH: days to heading, b) DM: days to maturity, c) PH: plant height (cm), d) TP: tillers per plant, e) SL: spike length (cm), f) SPS: Spikeletes per spike, g) SWS: spike weight per spikes (g), h) GNS: grains number spike, i) GWS: grains weight per spike (g), j) GNP: grains number per unit area, k) TGW: thousand grain weight (g), l) GY: grain yield per unit area, and m) BY: biological yield.

conditions minimum (8) TP were recorded for genotype AUS34255 and maximum TP were recorded (12) for genotype AUS34230.

4.2.11 Spike length

There were significantly different effects of treatments, genotypes, years and their interactions at P value 0.05 (Table 2.2). In SL total 27% of reduction was received when the data was averaged for both years in NOR and HS in all the accessions. Minimum reduction of (3cm%) in SL was observed for the accession AUS33403 while the maximum reduction (35cm%) was noted for the genotype AUS33394 in HS environments. Under NOR environments, minimum SL (10cm), was noted for the genotype AUS34419, while, the the maximum SL noted for AUS34443 (16 cm), whereas, during HS environments minimum (8 cm) SL was noted for genotype AUS34255 and maximum SL were recorded (13 cm) for genotype AUS34443.

4.2.12 Spikeletes per spike

There were significantly different effects of treatments, genotypes, years and their interactions at P value <0.05 (Table 2.2). SSP were decreased by 19 % when the data of both years in NOR and HS were averaged among all the genotypes. Minimum reduction of (4%) in SSP were recorded for the accession AUS34260 while the maximum reduction of (33 %) was being noted for the genotype AUS30659 in HS environments. During NOR environments, the genotype AUS33397 had the minimum number of SSP (13), while the maximum number of SSP was noted for AUS34443 (22), while, under HS environments the minimum number of SSP (12) were noted for genotype AUS30643 and maximum number of SSP (19) were noted for genotype AUS34244.

4.2.13 Spike weight per spike

There were significantly different effects of treatments, genotypes, years and their interactions for SWS at P value <0.05 (Table 2.2). SWS were decreased by 28.6g% when the data of both years in NOR and HS were averaged among all the genotypes. Minimum reduction of (10g%) in SWS were recorded for the accession AUS34278 while the maximum reduction of (48g%) was being noted for the genotype AUS30656 in HS environments. Under NOR environments, the accession AUS30287 had the minimum SWS (1.92g), while, the maximum

SWS was noted for AUS33383 (3.62g), While, under HS environments minimum (1.13g) SWS were recorded for genotype AUS30287 and maximum SWS were recorded (2.70g) for genotype AUS30282.

4.2.14 Grains number per spike

There were significantly different effects of treatments, genotypes, years and their interactions for GNS at P value <0.05 (Table 2.2). GNS were decreased by 18.2% when the data of both years in NOR and HS were averaged among all the genotypes. Minimum reduction of (7%) in GNS were recorded for the accession AUS30841 while the maximum reduction of (28%) was being noted for the accession AUS30286 in HS environments. Under NOR environments, the accession AUS34424 had the minimum number of GNS (19), while, the maximum number of GNS being recorded for AUS30282 (47), whereas, under HS stress minimum (15) GNS were recorded for genotype AUS34424 and maximum GNS were recorded (42) for genotype AUS30282.

4.2.15 Grains weight per spike

There were significantly different effects of treatments, genotypes, years and their interactions for GWS at P value <0.05 (Table 2.2). GWS were decreased by 27.7g% when the data of both years in NOR and HS were averaged among all the genotypes. Minimum decrease of (9g%) in GWS were recorded for the accession AUS33387 while the maximum decrease of (51g%) was being noted for the accession AUS30286 in HS environments. Under NOR conditions, the genotype AUS34277 had received minimum GWS of (1.05g), the maximum GWS being recorded for AUS33421 (2.57g), whereas, under HS stress conditions minimum (0.59g) GWS were recorded for genotype AUS34277 and maximum GWS were recorded (2.07) for genotype AUS30282.

4.2.17 Grains number per unit area

There were significantly different effects of treatments, genotypes, years and their interactions for GNP at P value <0.05 (Table 2.2). GNP were decreased by 16.1% when the data of both years in NOR and HS were averaged among all the genotypes. Minimum decrease of (8%) in GNP were recorded for the accession AUS34247 while the maximum decrease of (24%) was being noted for the accession AUS34239 in HS environments. Under NOR environments,

the genotype AUS34435 had the minimum number of GNP (4776), the maximum GNP being recorded for AUS30282 (11946), whereas, under HS stress conditions minimum (4136) GNP were recorded for genotype AUS34414 and maximum GNP were recorded (10960) for genotype AUS30282.

4.2.18. Thousand grain weight

There were significantly different effects of treatments, genotypes, years and their interactions for TGW at P value <0.05 (Table 2.2). TGW was reduced by 18.4g% when the data of both years in NOR and HS were averaged among all the genotypes. Minimum reduction of (8g%) in TGW was recorded for the accession AUS34409 while the maximum reduction of (35g%) was being noted for the accession AUS34418 in HS environments. Under NOR environments, the genotype AUS30303 had the minimum TGW of (36g), the maximum TGW being recorded for AUS33423 (59g), whereas, under HS stress conditions minimum (27g) TGW were recorded for genotype AUS30297 and maximum TGW were recorded (48g) for genotype AUS30636.

4.2.19 Grain yield

There were significantly different effects of treatments, genotypes, years and their interactions for GY at P value <0.05 (Table 2.2). GY was reduced by 39.4kg% when the data of both years in NOR and HS were averaged among all the genotypes. Minimum reduction of (28kg%) in GY was recorded for the accession AUS30637 while the maximum reduction of (73kg%) was being noted for the accession AUS34417 in HS environments. Under NOR environments the genotype AUS30303 had received the minimum GY (0.36kg), the maximum GY being recorded for AUS34440 (1.55kg), whereas, under HS stress conditions minimum (0.16kg) GY were recorded for genotype AUS34246 and maximum GY were recorded (1.00kg) for genotype AUS34440.

4.2.20 Biological yield

There were significantly different effects of treatments, genotypes, years and their interactions for BY at P value <0.05 (Table 2.2). BY was reduced by 40kg% when the data of both years in NOR and HS were averaged among all the genotypes. Minimum reduction of (33kg%) in BY was recorded was noted for the accession AUS34230 while the maximum

reduction of (65kg %) was being recorded for the genotype AUS30628 under HS environments. Under NOR environments, the genotype AUS34235 had received minimum BY (0.76kg), the maximum BY being recorded for AUS34440 (2.40kg).

4.3 Physiological and biochemical analysis

4.3.1 Analysis of variance and descriptive statics for studied traits

The basic statistics including means, minimum maximum and their standard deviation are presented in (Table 3.1). ANOVA showed major variation between genotypes, treatments, and their interactions for the studied parameters except BY which remained non-significant for treatment interaction (Table 3.1) means that trait showed less response to late stage heat stress. Results were consistent with the studies of earlier reported for bread wheat in identifying significant variations in agronomic, biochemical and physiological traits and strong genotype by environment interactions (Motamedi et al., 2012; Tadesse et al., 2012; Degewione et al., 2013; Wahid et al., 2007). High temperature stress imposed after anthesis resulted variation in physio-biochemical parameters such as proline content (PRC), total soluble sugar (SS), Membrane stability index (MSI) and yield related parameters. Proneness to heat stress may differ with the phase of plant growth, but all growth and reproductive phases are affected by high temperature stress to some degree (Wahid et al., 2007).

4.3.2 Yield performance of wheat genotypes

Over all heat stress reduced the TKW and GY by the 23% and 30%, respectively as compared the control treatment (Table 3.2). While, maximum reduction for TKW (28%) and GY (40%) were found for the genotypes AUS30652, while, minimum TKW (11%) and GY (13%) were found for the genotype AUS30824. BY reduced by (15%) in heat stress as compared to the normal environment. Maximum reduction was recorded for the genotype AUS30633 (20%), While genotypes AUS34339 and AUS34448 have increased the (6.0%) and (12%).We used HSI to define tolerant genotypes as identified by other workers (Mohammadi et al. 2008; Pinto et al. 2010). On the basis of HSI^{TKW} and HSI^{GY}, accessions were ranked from highly tolerant ($HSI \leq 0.50$), to moderately tolerant ($0.50 < HSI \leq 1.00$) or intolerant ($HSI > 1.00$) to heat stress. We found the most intolerant genotype AUS30652 on the basis HSI^{TKW} and HSI^{GY} while the most tolerant genotype for $HSIT^{KW}$ was AUS34448 for GY the most tolerant genotype was

Table 3.1. Analysis of variance for genotype (G), treatment (T) and genotype × treatment (G × T) and effects and descriptive statics for studied traits in normal and heat stress environment.

Traits	G	T	G x T	Normal treatment		Heat stress	
				Range	Mean±Std.	Range	Mean±Std.
Chlorophyll a	<0.001	<0.010	<0.015	0.88-1.62	1.16±0.26	0.61-1.29	0.88±0.24
Chlorophyll b	<0.001	<0.011	<0.021	0.46-0.96	0.77±0.16	0.37-0.81	0.55±0.13
Total chlorophyll	<0.001	<0.011	<0.031	1.05-227	1.65±0.37	0.85-1.79	1.27±0.29
Proline contents	<0.001	<0.001	<0.001	1.23-5.78	3.40±1.45	2.04-7.66	4.84±1.84
Soluble sugar	<0.001	<0.001	<0.002	254.28-757.36	462.34±135.27	320.12-885.65	601.86±173.37
Membrane stability index	<0.001	<0.001	<0.004	24.34-62.36	42.90±10.64	17.23-50.23	26.74±9.85
Thousand grain weight	<0.001	<0.004	<0.002	30-66	49.21±9.47	22-59	40.61±9.11
Grain yield	<0.001	<0.001	<0.010	0.54-2.01	1.04±0.43	0.32-1.75	0.79±0.45
Biological yield	<0.001	<0.082	<0.158	0.53-1.94	1.21±0.45	0.33-1.48	0.75±0.35

Table 3.2. Percent reduction and increase in the studied yield, biochemical and physiological traits in synthetic hexaploid wheats under heat stress environment and HSITKW and HSIGY.

AUS ID	Pedigree	Chla (%)	Chlb (%)	TChl (%)	SS (%)	MSI (%)	PRC (%)	$HSIT^{KW}$	HSI^{GY}
AUS30284	AAZ_3/AE.SQUARROSA (398)	-18.28	-12.96	-14.62	+24.11	-17.56	+77.7	0.58	0.38
AUS30290	ALTAR 84/AE.SQUARROSA (502)	-14.99	-18.39	-17.73	+29.01	-19.45	+63.89	0.90	0.72
AUS30292	DOY1/AE.SQUARROSA (516)	-26.54	-31.31	-23.55	+40.73	-40.83	+33.94	0.82	1.10
AUS30302	CETA/AE.SQUARROSA (1030)	-23.46	-35.49	-21.03	+36.12	-39.47	+25.43	1.31	1.19
AUS30633	SCOOP_1/AE.SQUARROSA (358)	-31.24	-42.35	-28.31	+46.72	-44.83	+18.24	1.10	1.54
AUS30652	6973/WARD.7463//74110/3/AE.SQUARROSA (665)	-35.86	-55.49	-29.02	+36.43	-49.69	+22.94	1.52	1.61
AUS33383	GARZA/BOY//AE.SQUARROSA (374)	-38.49	-33.15	-32.90	+45.32	-43.42	+26.47	1.25	1.55
AUS33388	DOY1/AE.SQUARROSA (458)	-16.57	-13.62	-18.51	+19.02	-23.59	+44.73	0.61	0.45
AUS33396	LCK59.61/AE.SQUARROSA (536)	-19.31	-20.41	-22.81	+41.18	-35.53	+31.42	1.47	0.85
AUS34239	ALTAR 84/AE.SQUARROSA (198)	-40.69	-40.52	-30.73	+39.35	-48.09	+36.16	0.52	1.80
AUS34409	CROC_1/AE.SQUARROSA (210)	-19.32	-20.11	-18.68	+17.12	-29.21	+65.71	0.64	0.50
AUS34439	CROC_1/AE.SQUARROSA (662)	-16.67	-19.47	-19.05	+21.32	-26.71	+40.81	0.51	0.69
AUS34448	ALTAR 84/AE.SQUARROSA (205)	-13.96	-20.08	-17.46	+19.69	-28.76	+49.68	0.47	0.64
SH-2002	INQALAB-91/FINK'S'	-16.65	-20.15	-19.5	32.01	-26.13	+49.13	0.56	0.62
Wafaq-01	OPATA/RAYON//KAUZ	-55.71	-53.12	-48.08	11.09	-40.15	19.13	1.36	1.47

Values in negative are showing the (%) decrease and values in positive are showing the (%) increase under heat stress environment.

Chla, chlorophyll a (mg/g); Chlb, chlorophyll b (mg/g); TChl., total chlorophyll (mg/g); SS, soluble sugars (µg/g); MSI, membrane stability index; PRC, proline content (µmol/g); TKW, thousand kernel weight; GY, grain yield; BY, biological yield, HSITKW, heat susceptibility index thousand kernel weight; HSIGY, heat susceptibility index grain yield.

AUS30824 (Table 3.2).

4.3.3 Correlation and factor analysis of the traits

The knowledge about important correlation between different plant parameters is vital for any breeding program as it assist in identifying the genotypes devouring suitable traits (Ali et al., 2009). Correlation of the traits were presented in Table 3.3. Positive significant correlation between GY and TGW was found in both normal and heat stress treatment while there is poor correlation were found between TKW and GNP. TKW and GY have negative association with $HSIT^{KW}$ and HSI^{GY} which is in agreement to previous studies (Mason et al 2010). Physiological and biochemical traits have significant correlation with each other. PRC have significant positive correlation (r= 0.61) with MSI in controlled environment. MSI have positively correlated with Chla (r=0.63), Chlb (0.56) and TChl (0.58) in normal environment. In heat stress environment, Chla, Chlb and TChl have significant positive correlation with each other and significant positive correlation with MSI.

To find out the relationships between the traits within each treatment, PCA and pairwise association tests were performed (Figure 3.1). The vector length exhibit the level of difference explicated by each trait in the PCA. The first two axes, i.e. PC1 and PC2, explained up to 56.22% in normal environment (Figure 3.1) while under heat stress condition the first 2 axes, i.e. PC1 and PC2, described up to 58.39% of the entire variability (Figure 3.2).

4.3.4 Biochemical and physiological potential of genotypes

The tolerance to heat stress of wheat can be examined via the estimation of yield performance or assessing the agronomic and physio-biochemical parameters under

Table 3.3. Pearson's co-efficient of correlation of the studied yield, biochemical and physiological traits under normal and heat stress environment. Upper trio exhibit correlations from normal environment (NOR), and lower trio exhibit correlations from heat stress environment (HS).

Variables	Chla	Chlb	TChl	SS	MSI	PRC	TKW	GY	HSITKW	HSIGY
Chla	**1**	**0.665**	**0.856**	0.206	**0.633**	0.374	-0.016	0.331	0.058	0.326
Chlb	**0.634**	**1**	**0.818**	0.445	**0.557**	0.175	-0.385	0.085	0.073	0.249
TChl	**0.849**	**0.710**	**1**	0.304	**0.584**	0.302	-0.327	0.198	0.008	0.026
SS	0.109	0.298	0.304	**1**	0.283	0.283	0.009	-0.225	0.011	0.476
MSI	**0.596**	**0.647**	0.372	0.118	**1**	**0.608**	-0.252	-0.005	0.125	0.483
PRC	0.514	0.360	0.431	0.293	0.501	**1**	-0.264	-0.238	0.111	0.236
TKW	0.067	-0.256	0.214	-0.208	0.048	-0.290	**1**	0.440	**-0.583**	0.348
GY	0.383	0.266	0.124	-0.347	0.420	0.105	**0.673**	**1**	**-0.558**	**-0.836**
HSITKW	0.058	0.073	0.008	0.011	0.125	0.111	**-0.583**	**-0.558**	**1**	0.492
HSIGY	0.326	0.249	0.026	0.476	0.483	0.236	-0.348	**-0.836**	0.492	**1**

Values in bold are different from 0 with a significance level alpha=0.05

Chla, chlorophyll a (mg/g); Chlb, chlorophyll b (mg/g); TChl., total chlorophyll (mg/g); SS, soluble sugars (µg/g); MSI, membrane stability index; PRC, proline content (µmol/g); TKW, thousand kernel weight; GY, grain yield; HSITKW, heat susceptibility index thousand kernel weight; HSIGY, heat susceptibility index grain yiled.

stress conditions (Shipler and Blum 1991; Zhao et al. 2007; Reynolds et al. 1994, 2001; Al-Khatib and Paulsen 1984). Different physio-biochemical parameters such as PRC, MSI, soluble carbohydrates, and chlorophyll contents were studied to evaluate the tolerance of genotypes under late heat stress (Reynolds et al. 1994; Khan et al., 2015).

4.3.5 Chlorophyll contents

Heat stress had a substantial effect on the content of chlorophyll a and b, and on the TChl (Table 3.1). Content of Chla reduced because of heat stress and reduction of Chla, was minimum (13.96%), in the genotype AUS34448 and AUS30284 (14.99%) respectively (Table 3.2). While, maximum decrease was recorded for the genotype AUS34239 (40.69) and genotypes AUS33383 (38.49). Chlb maximum decrease were recorded for AUS30652 (55.49%) and AUS30633 (42.35%) respectively. Minimum decrease was found for the genotypes AUS30284 (12.96%) and AUS33388 (13.65%). TChl were decreased in all the studied genotypes but the minimum decrease was recorded for the genotypes AUS30284 (14.62%) and AUS34448 (17.46%) respectively. While, maximum decrease was recorded for the genotypes AUS33383 (32.90%) and AUS34239 (30.73%).

4.3.6 Soluble sugars

Heat stress significantly represented the changes of flag leaf soluble carbohydrates amount under the studied genotypes (Table 3.1). The minimum SS accumulated under the heat stress as compared to normal by the genotypes AUS33388 (19.02%) and genotype AUS34448 (19.69%). While, maximum accumulation of SS under heat stress were recorded for the genotype AUS30633 (46.72%) and genotype AUS33383 (45.32%).

4.3.7 Proline contents

Heat stress after grain filling stage significantly increased the proline contents flag leaves of all the wheat genotypes observed (Table 3.1) in the synthetic hexaploid wheat maximum increase was found for the genotypes AUS30284 (77%) and (63%) in AUS30290 and lowest increase of (18%) and (22%) was found for the genotypes AUS30633 and AUS30652.

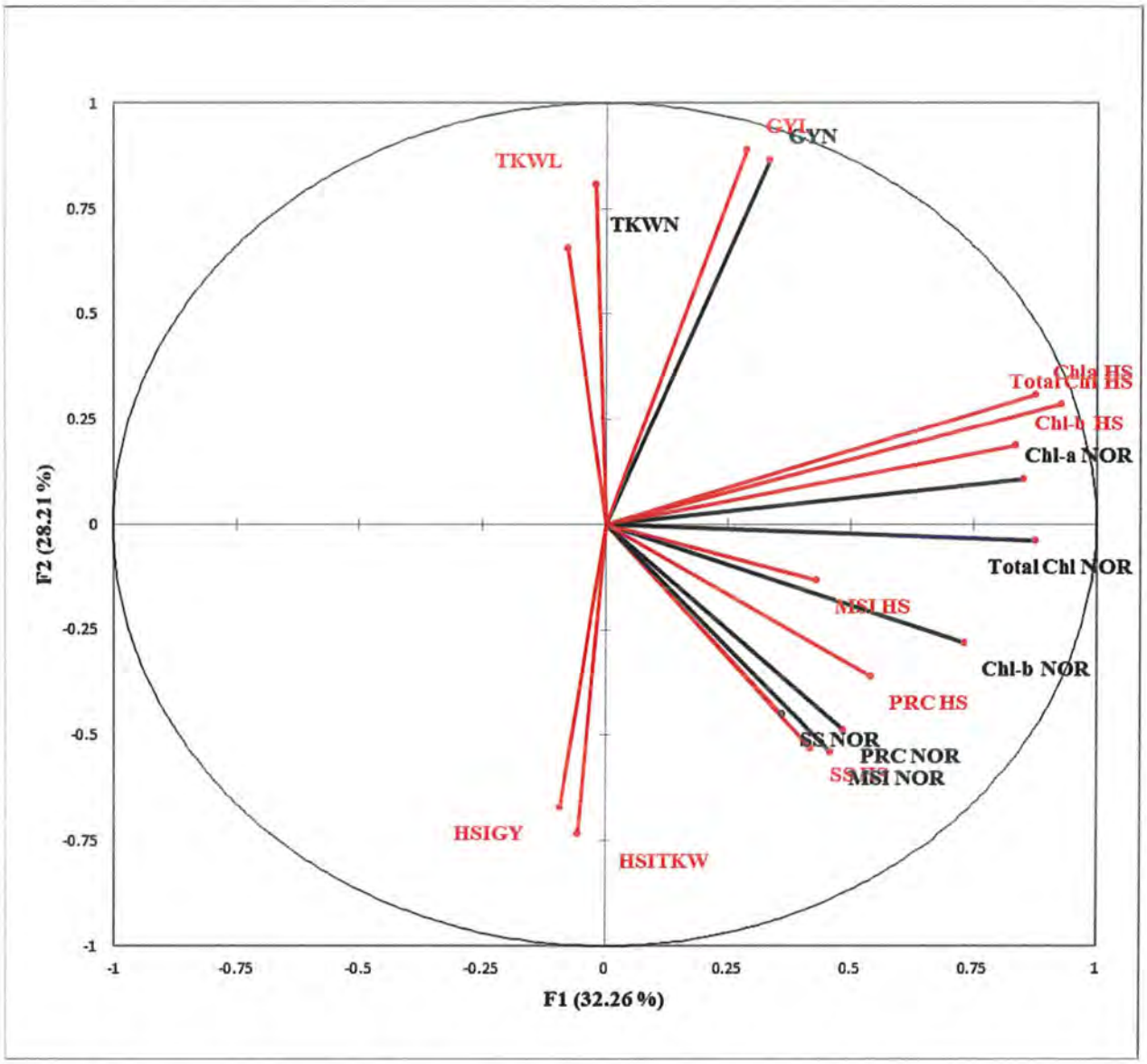
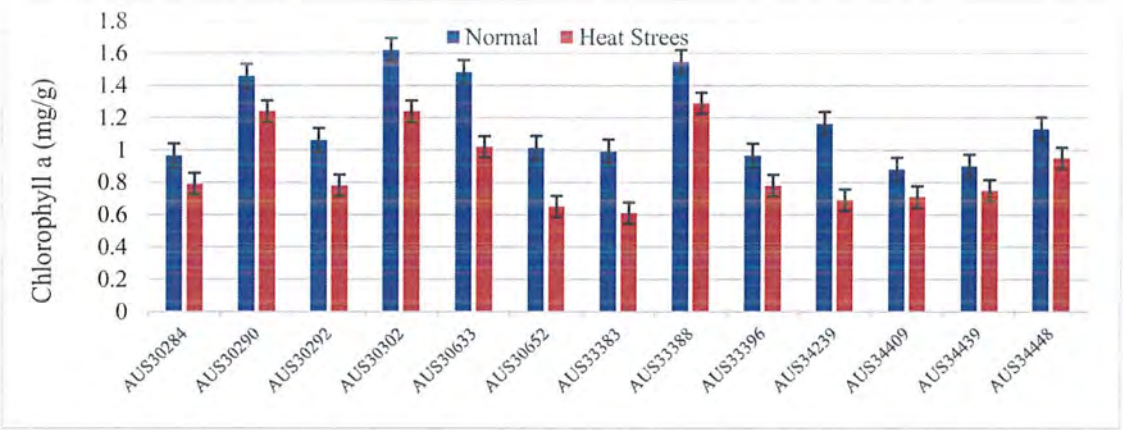
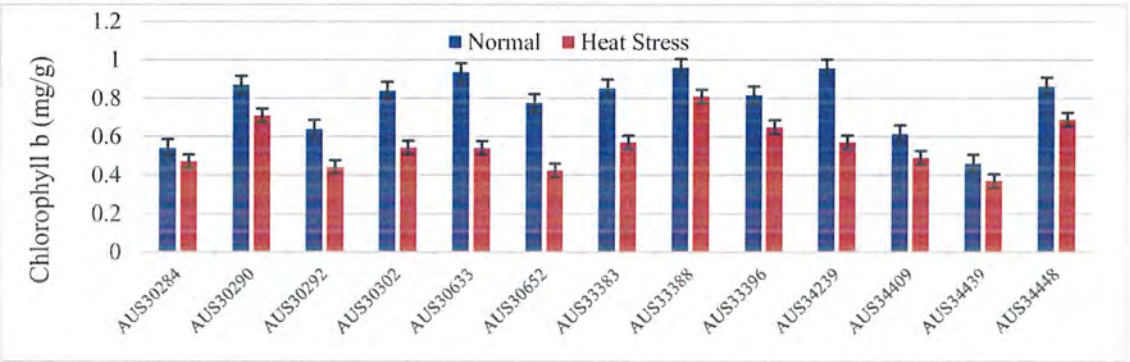


Figure 3.1. Principal component analysis (PCA), showing traits in normal and heat stress environment respectively. Chla, chlorophyll a (mg/g); Chlb, chlorophyll b (mg/g); TChl., total chlorophyll (mg/g); SS, soluble sugars (µg/g); MSI, membrane stability index; PRC, proline content (µmol/g); TKW, thousand kernel weight; GY, grain yield.

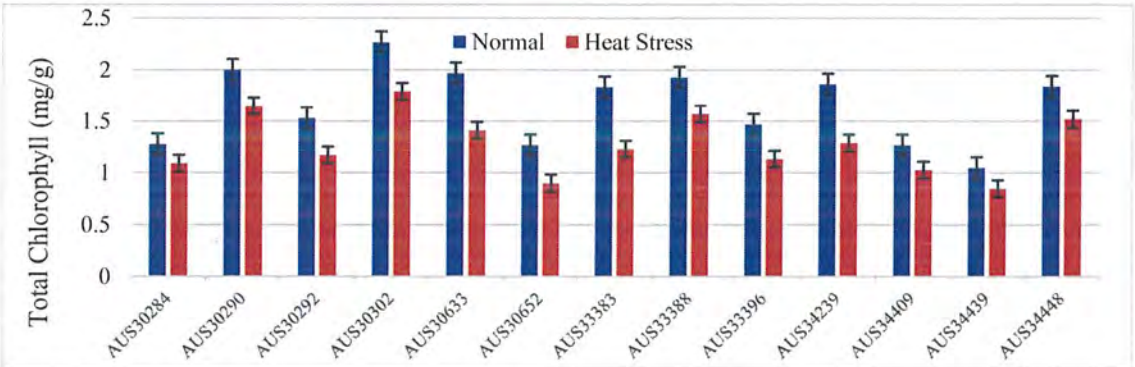
a)


Chlorophyll a (mg/g)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Normal
Heat Strees
AUS30284
AUS30290
AUS30292
AUS30302
AUS30633
AUS30652
AUS33383
AUS33388
AUS33396
AUS34239
AUS34409
AUS34439
AUS34448

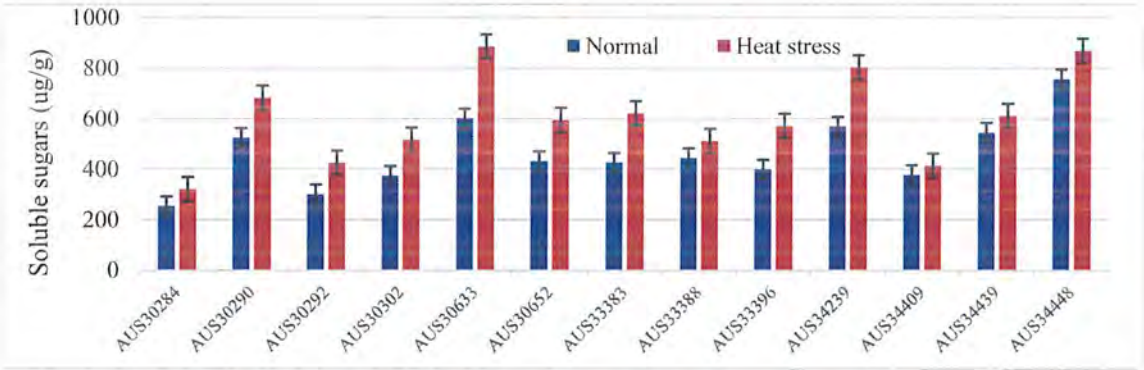
b)


Chlorophyll b (mg/g)
1.2
1
0.8
0.6
0.4
0.2
0
Normal
Heat Stress
AUS30284
AUS30290
AUS30292
AUS30302
AUS30633
AUS30652
AUS33383
AUS33388
AUS33396
AUS34239
AUS34409
AUS34439
AUS34448

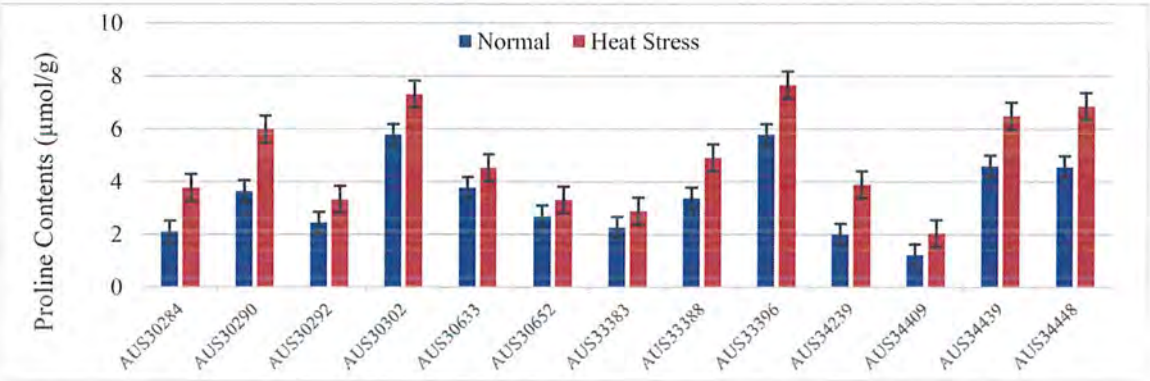
C)


Total Chlorophyll (mg/g)
2.5
2
1.5
1
0.5
0
Normal
Heat Stress
AUS30284
AUS30290
AUS30292
AUS30302
AUS30633
AUS30652
AUS33383
AUS33388
AUS33396
AUS34239
AUS34409
AUS34439
AUS34448

d)



e)



f)

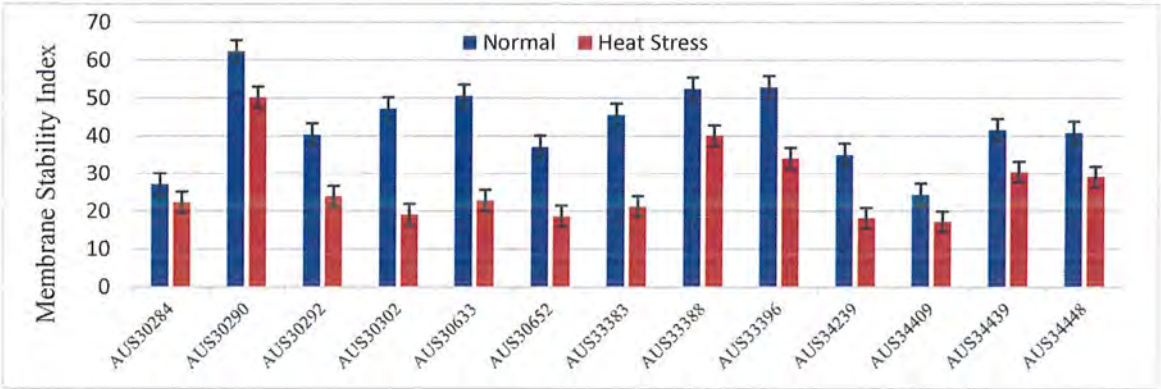


Figure 3.2. a) Chla, chlorophyll a (mg/g); **b)** Chlb, chlorophyll b (mg/g); **c)** TChl., total chlorophyll (mg/g); **d)** SS, soluble sugars (µg/g); **e)** MSI, membrane stability index; **f)** PRC, proline content (µmol/g); showing the in control and heat-treated plants. The vertical bars indicate the LSD values ($\alpha = 0.05$) for mean comparisons between genotypes within control (blue bar) or heat (red bar) stress.

4.3.8 Membrane stability index

High temperature stress at late grain filling stage decreased the MSI in all the tested synthetic hexaploid genotypes (Table 3.1). The decrease in MSI of all the tested wheat genotypes ranged from 17.56 to 49.69 %). However, the lowest decrease in MSI was recorded in AUS30284 (17.56 %) followed by AUS30290 (19.644%), respectively. While maximum decrease in MSI were found for AUS30265 (49.65%) followed by AUS34239 (48.09) respectively.

4.4.1 Genome-wide association study in synthetic hexaploid wheats for heat stress

Using the bi-allelic DArT markers the 231 synthetics were genotyped. On the basis of more than 100 mapping population the consensus genetic map of DArT markers was generated to assign the chromosomal position (Detering et al., 2010). For final genetic association total, 834 polymorphic DArT markers were used. 40 markers per chromosome was the final density in this population. Average density of 1 marker per 3.11 cM with total genetic distance covered by 2,608 cM DArT markers integrated into the framework genetic map. The total number of markers per chromosome ranged between 9 (chromosomes 5D and 5A) and 103 (chromosome 3B). Though, the marker coverage for D-genome chromosomes was very low (20.29 per chromosome) as linked to A and B genomes. Polymorphic information content (PIC) value ranged from 0.07 to 0.499 with an average of 0.38.

4.4.2 Population structure and kinship analysis

Structure software was used to perform the population structure analysis by dispensation genotypic data of DArT markers, scattered across the whole wheat genome (Pritchard et al., 2000). The admixture model was applied (Falush et al., 2003) using correlated allelic frequencies with burn in phase of 1 lac iterations and MCMS (Markov chain Monte Carlo, representing number of replications) 1 lac to test for genetic structure (K= number of subpopulations) values set from 1 to 20 and performed 10 runs for K values. The likely number of subpopulations present was determined according to Evanno et al. (2005) by plotting LnP(D) against K and further confirming by plotting ΔK against the subclasses K. Population structure matrix (Q) was recorded by running structure at K=3 and 5 (Rasheed et al., 2014).

4.4.3 Linkage disequilibrium

Linkage disequilibrium was assessed via r^2 at $P \leq 0.001$ from total pairs of the DArT markers. Nearly 59.1 % of total pairs of loci were in major LD on a genome-wide level (Table 3.20). Genome-wide LD r^2 average was 0.08. Allocated DArT markers to position of their map were further tested for the assessment of intra- and inter-chromosomal LD. Inter-chromosomal pairs of loci were in important LD for about 28 %, with r^2 average of 0.09, whereas intra-chromosomal pairs of loci were insignificant LD of about 42 % with *r2* average of 0.3. The amount of LD and its distribution is displayed graphically by plotting a graph between the genetic distance in centiMorgan (cM) at $P \leq 0.001$ against intra-chromosomal r^2 values in significant LD for loci and a second-degree locally weighted polynomial regression-based (LOESS) curve was fitted (Figure 3.24). The critically important value for the significance of r^2 estimation was at 0.2 explained by Breseghello and Sorrells (2006), therefore the $r^2 > 0.3$ were likely to be due to genetic linkage for all values. The connection of baseline with the LOESS curve at 8 cM was measured as the estimation of the range of LD in the population of SHWs, whereas in a few circumstances higher levels of LD were seen over longer distances ($r^2 = 1$ at a genetic distance of 167 cM). An average *r2* LD decays to 0.069 from 0.246 as the genetic distance increases to >10 cM and the markers in complete LD also reduced to 1 from 238 As a result, 6 cM map coverage was estimated suitable to perform association analysis on the population of SHWs.

4.4.4 Marker-trait association

Marker-traits associations (MTAs) for agronomic traits were recognized in 201 SHWs by genome wide association studies analysis using mixed linear model (MLM) approach. MTAs for seven important agronomic traits are presented (Table 4.1). In total, 31 MTAs with -log P >3 were detected using the MLM model, while 14 were detected in NOR environments and 17 were detected in HS environments.

For GNS 14 markers were find significantly associated on chromosome 1A, 3D, 5B and 7B. Markers (wPt9757, and wPt6280) were find associated under two HS environments on chromosome 1A, while marker wPt2527 were find associated in NOR environment (Table 4.1). The phenotypic variation varied for markers and maximum variation with the r^2 value of 9.3% explained by wPt2527 in BWP15HS followed by the marker wPt6280 in BWP14HS with the r^2

value of 7.3%. On chromosome 3D marker rPt1806 were found associated in both NOR and HS environments and explained maximum phenotypic variation with the r^2 value of 9.5% in NOR environment. On chromosome 5B only one marker wPt6880 was find associated in NOR environment and explained the phenotypic variation of 4.1%. On chromosome 7B two markers (wPt4298) were find associated in both NOR and HS environments. While marker tPt7362 find associated only in NOR environments and explained the maximum phenotypic variation of 5.7%.

For GNP total three markers were find associated in both NOR and HS environments on chromosome 3B (wPt7984), 5B (wPt6880) and 7B (wPt4298) (Table 4.1). The phenotypic variation varied for markers and maximum variation explained by the marker wPt6880 with the r^2 value of 8.1% in BWP15HS, followed by wPt4298 with the r^2 value of 0.75%.

For PH two markers wpt3342 and wpt5138 were find associated on chromosome 3B and 7B under HS environments. Maximum phenotypic variation explained by wpt3342 with r^2 value of 0.74, while marker wpt5138 explained the phenotypic variation with the r^2 value of 0.9% (Table 4.1).

For SL two markers wPt5432 (3B) and wPt2923 (3D) find associated in 4 NOR environments, while wPt5432 explained the phenotypic variation of 3.5% and maker wPt2923 showed maximum association of 4.4% (Table 4.1).

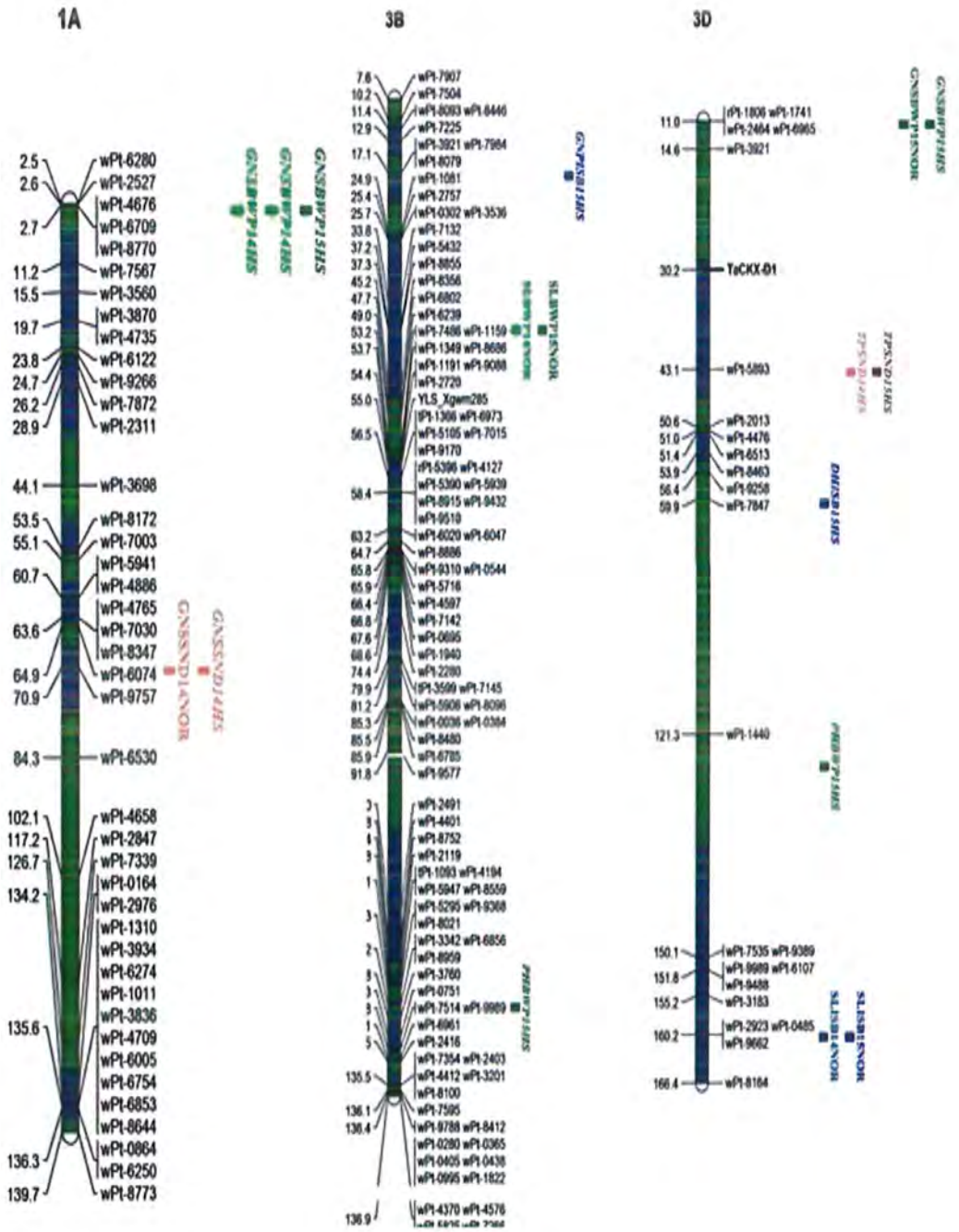
Two markers wPt1440 and wPt5893 were find associated for TP on chromosome 3D in three HS environments, while marker wPt5893 explained .86% phenotypic variation in and marker wPt1440 explained 2.4% phenotypic variation.

For TGW only one marker wPt8153 (6B) find associated in BWP15HS and explained 1.2% of phenotypic variation.

One marker wPt7847 on chromosome 3D for DH was associated in ISB15HS and explained the phenotypic variation with the r^2 value of 3.9%. Only two markers wPt6880 on chromosome 5B and wPt4298 on chromosome 7B were multi-trait MTAs because they were find associated with two traits GNS and GNP combined in NOR and HS environments, while remaining markers were associated with single trait.

Table 4.1. Marker trait associations (MTAs) observed in MLM for developmental traits under normal and heat stress conditions during two years in synthetic hexaploid wheat.

Traits	Env.	Marker	Chr.	Pos.(cM)	p-value (-log10)	Marker r^2 (%)
DH	ISB15HS	wPt7847	3D	60	3.16	3.9
GNS	SND14NOR	wPt9757	1A	71	3.01	3.8
	SND14HS	wPt9757	1A	71	3.48	9.4
	BWPI4HS	wPt6280	1A	3	3.02	7.3
		wPt2527	1A	3	3.02	2.4
	BWP15HS	wPt2527	1A	3	3.1	9.3
	BWP15NOR	rPt1806	3D	11	3.38	2.7
		rPt1806	3D	11	3.2	9.5
	BWPI4NOR	wPt6880	5B	129	3.06	4.1
	ISB14NOR	wPt4298	7B	196	3.3	1.4
		tPt7362	7B	196	3.02	5.7
	ISB14HS	wPt4298	7B	196	3.14	3.9
	ISB15NOR	wPt4298	7B	196	3.3	1.4
		tPt7362	7B	196	3.02	5.7
	ISB15HS	wPt4298	7B	196	3.14	3.9
GNP	ISB15HS	wPt7984	3B	17	3.01	3.0
	BWP14NOR	wPt6880	5B	129	3.06	4.0
	BWP15NOR	wPt6880	5B	129	3.45	7.5
	BWP15HS	wPt6880	5B	129	3.42	8.1
	ISB14NOR	wPt4298	7B	196	3.25	8.6
	ISB14HS	wPt4298	7B	196	3.15	3.8
PH	BWP15HS	wPt3342	3B	125	3.22	7.4
	ISB15HS	wPt5138	7B	215	3.39	0.9
SL	BWP14NOR	wPt5432	3B	37	3.38	3.5
	BWP15NOR	wPt5432	3B	37	3.38	3.5
	ISB14NOR	wPt2923	3D	160	3.33	4.4
	ISB15NOR	wPt2923	3D	160	3.33	4.4
TP	ISB14HS	wPt1440	3D	121	3.69	2.4
	SND14HS	wPt5893	3D	43	3.16	8.6
	SND15HS	wPt5893	3D	43	3.16	8.6
TGW	BWP15HS	wPt8153	6B	36	3.03	1.2


1A
2.5 wPt-6280
2.6 wPt-2527
wPt-4676
2.7 wPt-6709
wPt-8770
11.2 wPt-7567
15.5 wPt-3560
wPt-3870
19.7 wPt-4735
23.8 wPt-6122
24.7 wPt-9266
26.2 wPt-7872
28.9 wPt-2311
44.1 wPt-3698
53.5 wPt-8172
55.1 wPt-7003
wPt-5941
60.7 wPt-4886
wPt-4765
63.6 wPt-7030
wPt-8347
64.9 wPt-6074
70.9 wPt-9757
84.3 wPt-6530
102.1 wPt-4658
117.2 wPt-2847
126.7 wPt-7339
134.2 wPt-0164
wPt-2976
wPt-1310
wPt-3934
wPt-6274
wPt-1011
135.6 wPt-3836
wPt-4709
wPt-6005
wPt-6754
wPt-6853
wPt-8644
136.3 wPt-0864
wPt-6250
139.7 wPt-8773
3B
7.6 wPt-7907
10.2 wPt-7504
11.4 wPt-8093 wPt-6446
12.9 wPt-7225
wPt-3921 wPt-7984
17.1 wPt-8079
24.9 wPt-1081
25.4 wPt-2757
25.7 wPt-0302 wPt-3536
33.8 wPt-7132
37.2 wPt-5432
37.3 wPt-8855
45.2 wPt-8356
47.7 wPt-6802
49.0 wPt-6239
53.2 wPt-7486 wPt-1159
53.7 wPt-1349 wPt-8686
54.4 wPt-1191 wPt-9088
wPt-2729
55.0 YLS_Xgwm285
wPt-1366 wPt-6973
56.5 wPt-5105 wPt-7015
wPt-9170
wPt-5396 wPt-4127
58.4 wPt-5390 wPt-5939
wPt-8915 wPt-9432
wPt-9519
63.2 wPt-6020 wPt-6047
64.7 wPt-8886
65.8 wPt-9310 wPt-0544
65.9 wPt-5716
66.4 wPt-4597
66.8 wPt-7142
67.6 wPt-0695
68.6 wPt-1940
74.4 wPt-2280
79.9 wPt-3599 wPt-7145
81.2 wPt-5908 wPt-8096
85.3 wPt-0036 wPt-0384
85.5 wPt-8480
85.9 wPt-6785
91.8 wPt-9577
wPt-2491
wPt-4401
wPt-8752
wPt-2119
wPt-1093 wPt-4194
wPt-5947 wPt-8559
wPt-5295 wPt-9368
wPt-8021
wPt-3342 wPt-6856
wPt-8959
wPt-3760
wPt-0751
wPt-7514 wPt-9989
wPt-4961
wPt-2416
wPt-7354 wPt-2403
135.5 wPt-4412 wPt-3201
wPt-8100
136.1 wPt-7595
136.4 wPt-9788 wPt-8412
wPt-0280 wPt-0365
wPt-0405 wPt-0438
wPt-0995 wPt-1822
136.9 wPt-4370 wPt-4576
3D
11.0 wPt-1806 wPt-1741
wPt-2464 wPt-6965
14.6 wPt-3921
30.2 TaCKX-D1
43.1 wPt-5893
50.6 wPt-2013
51.0 wPt-4476
51.4 wPt-6513
53.9 wPt-8463
56.4 wPt-9258
59.9 wPt-7847
121.3 wPt-1440
150.1 wPt-7535 wPt-9389
151.8 wPt-9989 wPt-6107
wPt-9488
155.2 wPt-3183
160.2 wPt-2923 wPt-0485
wPt-9662
166.4 wPt-8184

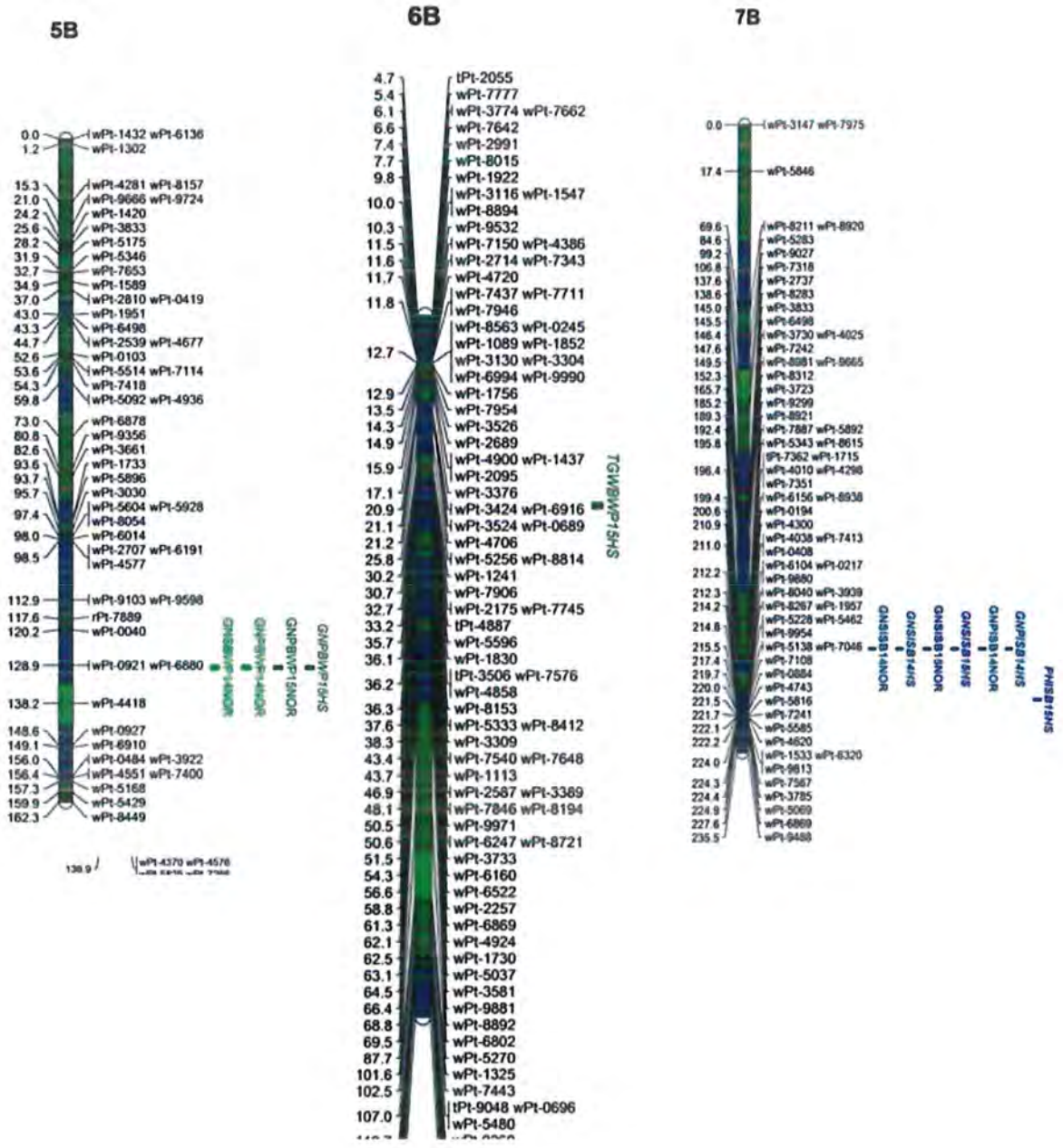


Figure 4.1. Consensus linkage map of DArT markers showing significant marker-traits associations (MTAs) for various heat stress and control traits along wheat chromosom

Chapter 5

5. DISCUSSION

5.1 Grain filling experiment under controlled conditions

In this experiment, genotypes showed a varied response to SGW and chlorophyll content losing from 47 to 57 % in response to a small episode of heat stress, respectively. Results advocated a marked result of high temperature on the performance of wheat genotypes and also suggested a considerable yield improvement under high temperature conditions, these findings are in agreement with prior studies (Stone and Nicolas, 1994; Wardlaw et al. 1989b). At various phenological phases, heat stress effects the wheat to various changing levels i.e vegetative phase and reproductive stage. But heat stress for reproductive stage is more injurious as compared to vegetative stage due to the direct result on dry weight and number of grains (Wollenweber et al., 2003). During grain filling stage, high temperature affects both the rate and length depending upon genotype and stress intensity, which results in a net decrease in final SGW (Hunt et al., 1991; Zahedi and Jenner, 2003). As a result of high temperature, genetic variation has been observed for both for grain filling and duration rate in various synthetic hexaploid genotypes. Numerous studies reported that under high temperature, differences in rate of grain filling is more as compared to duration of grain filling duration, also reported genotypic differences between GFD and GFR by (Hunt et al. 1991; Wardlaw and Moncur, 1995; Zahedi and Jenner, 2003). Stone and Nicolas (1995a) for both rate of grain filling and duration of grain filling under severe heat stress (40 °C) for 5 days at different stages of grain filling found major difference between two genotypes that were differing in heat tolerance. They also perceived a strong association of rate of grain filling and duration of grain filling in response to high temperature stress.

Earlier, Talukder et al. (2013) pragmatic a significant difference among bread wheat genotypes for grain filling rate response to one day of severe heat stress (35 °C, at 7-10 DAA) in both field and control environments. The time from anthesis to grain filling duration trait were reduced by the heat treatment by an average of 13%, but this response did not show any important G x T interaction or a significant association with SGW or GWS responses (Table 1.2). This exhibit that modifications in tolerance (SGW response) were driven mainly by changes in the response of grainfill rate, rather than duration.

Although, it may also have showed the trouble of measuring the grain fill duration more precisely by this subjective method. For the selection of tolerant and intolerant genotypes the factor which is more significant in determining the tolerance variation will require destructive sampling of grains over time for dry weight determination. Senescence is a genetically determined phenomenon which interacts with biotic and abiotic stresses and results in remobilization of reserves to young reproductive parts of the plant chlorophyll loss and reduced photosynthesis (Vijayalakshmi et al., 2010). Mobilized stem reserves and assimilates derived from current photosynthesis contribute to grain growth, that depends on the environment (Hossain et al., 1990).

Under non stressed conditions stem reserves contribute less to the grain growth, whereas in stressed condition it shows major contribution depending on wheat genotype (Yang et al., 2002a). Delayed senescence can reduce yield, by delaying remobilization of stored reserves to the reproductive parts of the plant. One of the major cause for delayed senescence that causes yield reduction due to reduced mobilization to grain is heavy application of nitrogen fertilizer (Yang et al., 2002; Yang and Zhang 2006). The prolonged consumption of glucose for continued nitrogen absorption and protein synthesis by green leaves and grains, which deprive the assimilation for grain filling is described for adverse effect of stay green starch synthesis (Hirel et al., 2007; Kipp et al., 2014). In the present study, FLSe and AUSC of control plants showed significant negative relationships with GWS and SGW (Table 1.2), indicating there may have been a yield penalty for stay green genotypes. The control plants were well watered and fertilized and not subjected to heat stress – conditions that may have resulted in slower than optimal rate of senescence for grain yield in some of the genotypes.

Accelerated senescence caused by biotic or abiotic stress can have both positive and negative consequences for crop yield. It can help yield by increasing remobilization of stem reserves to the grain during late grain filling, but it can also reduce the capacity for late generation of assimilates that normally contribute significantly to grain yield particularly in wheat (Lopes and Reynolds 2012; Yang and Zhang 2006). Chlorophyll content by SPAD is used to describe the declining photosynthetic capacity under terminal stress because SPAD readings and PS II efficiency showed strong association and maximum photosynthetic rate under high temperature (Ristic et al., 2007; Ristic et al., 2008).

In the present study, stay-green by SPAD based traits was positively associated with grain yield GWS and SGW under heat conditions. Moreover, chlorophyll content responses to heat during grain filling were significantly positively associated with the GWS and SGW responses (Table 1.2) suggesting that under these conditions, genotypes that responded with less chlorophyll loss under heat were also better able to maintain grain weight. Those genotypes were likely to have been able to better maintain photosynthetic competence under heat, which could have contributed positively to grain filling.

Earlier studies (Lopes and Reynolds 2012; Reynolds et al., 2000; Rosyara et al., 2009; Rosyara et al., 2010b) showed the advantage of stay green in wheat under biotic and abiotic stress condition. It is also found, that as an alternative, grain filling and stay green may have been affected by heat stress independently, rather than related by any indirect effect. In spite of that, this information recommends that a SPAD meter may be responsible for an easy and economical tool to indirectly select stress tolerant varieties and stay-green trait suggesting their well performance under heat stress conditions. Some genotypes did not conform well to general relationship between stay-green and heat tolerance for grain weight, signifying that there may have been other factors at play. For example, AUS33391, AUS33394 and AUS33377 showed tolerance for heat in case of chlorophyll retention but heat susceptible for SGW (Kumar et al., 2010; Thomas and Howarth, 2000). In the developing wheat grain heat stress can also limit the conversion of sucrose into starch by affecting several enzyme activities of the starch synthesis pathway (Hawker and Jenner 1993), particularly soluble starch synthase (Hawker and Jenner, 1993; Jenner and Hawker, 1993). When the temperatures are returned to normal (Jenner 1991b) even the inauspicious effect of high temperature on soluble starch synthase can continue for some time. Thus, AUS33391, AUS33394 and AUS33377 may have had forms of soluble starch synthase that were particularly heat-sensitive, less abundant, or less able to recover, preventing these genotypes from being able to convert carbohydrates afforded by the stay-green trait from being efficiently converted to grain mass starch synthesis AUS3080, AUS3081 and AUS33379 were very meager for stay-green but exhibited good performance for SGW maintenance during heat. Probably these genotypes had predominantly high levels of stem reserves and proficient mechanisms of carbon remobilization, which protected them against the difficulty afforded by their low stay-green. Authenticating the stay-green-independent mechanisms of heat tolerance in these 'outlying' genotypes also require genetic mapping of stay-green traits. Genotypes with

higher SGW and GWS revealed resilient responses of these traits to heat treatment, i.e. less tolerance. This is equivalent with Yang et al. (2002a) who testified a positive link between yield potential and heat susceptibility index which specified a stronger response for genotypes with higher yield potential. Days to anthesis negatively correlated with SGW and GWS under stress and positively correlated with GWS and SGW under non stressed conditions. In contrast, genotypes with higher senescence rate potential is more likely to experience higher acceleration of senescence in response to heat. This helps to suggest that heat predominantly affected the senescence by accelerating senescence processes that occurred under non-stress conditions, in spite of that by causing impairment that was heat-specific in nature. SGW was negatively correlated with GFD under control conditions which suggests that shorter GFD was linked with higher grain filling rate. On the contrary, under stress enviornments, SGW response was positively associated with GFD response and GFD potential. Genotypes with a shorter GFD and higher grain filling rate under control conditions therefore showed more respond to heat. Lower SGW response was also negatively associated with potential plant size (CL and ShW under control conditions; $p < 0.05$). *Rht-B1* and *Rht-D1* are the main loci affecting plant height and size in wheat and the wild-type (tall) alleles act via gibberellic acid (GA) signaling (Blum and Sullivan, 1997). This may propose that intolerance was favored by a tall stature or more vegetative mass, rather than by GA-insensitive *Rht* alleles per se. Limited semi-dwarf genotypes such as AUS30291, and AUS33394 were also susceptible. There are contradictory reports linking GA-insensitive dwarfing alleles to reduced abiotic stress tolerance. A marked sensitivity to heat and/or drought stress at booting stage in genotypes carrying GA-insensitive alleles (*Rht-B1b, Rht-D1b* and *Rht-B1c*) were also found by Law et al. (1980) and Law and Worland (1985). Smaller plants (carrying dwarfing allele *Rht-B1b, Rht-D1b* or *Rht-B1c*) appeared to be more tolerant to top-root drying in terms of reduction in plant height, tillering, shoot and root biomass (Blum and Sullivan 1997). Plants carrying dwarfing gene/s and lower growth rate potentials revealed higher tolerance to both drought and heat temperature and lower response to ABA, as observed in the plant growth rate response in seedlings (Blum et al., 1997). Under stress conditions Butler et al. (2005) testified a grain yield and grain weight advantage of tall lines, as compared with semi-dwarf lines. Various factors including environmental factors, growth habit (spring vs. winter), genetic background affected the yield benefits associated with dwarfing genes (Alghabari et al., 2014) which may at least partially explicate the conflict between the

results of the aforementioned studies, and between some of these and the current study. Overall, the association of tolerance (lower SGW response) with lower rates of senescence and grain filling and smaller plant size (shorter CL and lower ShW) suggests a penalty for genotypes with higher trait potentials and bigger plant size. This may indicate a hindrance for breeding varieties that will be high yielding in both non-stressed and heat-stressed environments. The observed associations between traits such as plant size, flowering time, grain yield spike-1, SGW, flag leaf senescence, and tolerance (lower SGW response) might be due to control of these traits by common gene(s). On the other hand, some of these associations could have simply reflected chance association of genes within the small sample of genotypes. Distinguishing these possibilities will require GWAS mapping of the traits and responses.

5.2 Filed experiment for terminal heat stress at three different locations

5.2.1 Variation for agronomic traits in synthetic hexaploid wheats

Previously, very few SHWs and their derivatives have been evaluated for heat tolerance and were characterized mainly to describe their superiority over the conventional bread wheat genotypes (Sharma et al., 2014; Sehgal et al. 2011; Cossani and Reynolds, 2015). We reported here the enactment of a relatively large array of 200 SHWs with timely and late sowing to find out the difference for important phenotypic and yield related traits and their relationships during terminal heat stress environment. ANOVA depicted substantial variation due to genotypic, treatment, location, year and their interactions among them, and the results were in accordance with those earlier reported for bread wheat in detecting significant variations in agronomic traits and strong genotype by environment interactions (Motamedi et al., 2012; Tadesse et al., 2012; Degewione et al., 2013). SHWs have shown large variation for all the traits that can be utilized in breeding for high temperature tolerance (Cossani and Reynolds, 2105). The variation in GY is significantly explained by temperature and rainfall patterns (90%) and other traits (57%). The high broad sense heritability for DH showed that this parameter has great response in selecting the genotypes and large variations in SHWs under HS conditions could be reliable in indirect high temperature tolerance breeding program.in wheat germplasm to avoid heat stress. Early DH and early DM is desirable for achieving high yield in temperate environments. Though, the GY of the lines was decreased during HS environments. On the other hand, low yield was associated with late heading and late maturity suggesting that the high yield is attributed to their adaptation

and ability to escape late heat stress. Early heading and maturity enabled the lines to fill their grains normally and escape the late heat stress occurring at the end of the season. PH exhibited great to temperate heritability estimates and might be a valuable parameter in heat tolerance breeding. Although PH had non-significant correlation with HSI but the correlation was three times higher in HS treatment (r = - 0.11) as compared to NOR treatment (r = -0.04). This showed that heighted plants tend to be highly tolerant to high temperature probably because of maximum amassing of stem carbohydrates that are soluble in water. Cossani and Reynolds (2015) also concluded that in synthetic derived bread wheats there is high GY because of increased water-soluble carbohydrates due to taller stems that significantly increased TGW. GY is used for wheat improvement and is considered as main benchmark in breeding program. The present study showed that in future it will be not easy to use GY as direct selection method exhibited low heritability estimation. Hence, to increase the GY under heat stress with the help of yield traits are considered as main criteria in order to screen for tolerance contrasting to drought and heat stress.

5.2.2 Effects of heat stress in synthetic hexaploid wheats

The effects of temperature treatments (NOR and HS) on all the characters were greater than effects of genotype and genotype by environment interaction. Furthermore, significant genotype by treatment interactions was also observed, showing that genotypes responded differently to the treatments across three locations. Sharma et al. (2012) explained that GY is highly influenced by environment. Because of genotype by environment interactions, and genotype effect helps to identify only six SHWs with good GY stability across environments (Table 2.5). Major decrease in various growth and yield characters were find due to HS treatments. High temperature decreased the PH (Singh et al., 2007), time to flowering (Rahman et al 2009), time to maturity (Joshi et al. 2007), grains number per spike and grains weight per spike (Cao et al., 2015), TGW (Wardlaw and Wrigley, 1994; Reynolds et al., 1994), (Tewolde et al., 2006). Heat stress also cause reduction in GY which was high in 2015 than 2014 (39% vs. 36%) due to higher temperatures. So the 2°C temperature increase in 2015 reduced 5% GY as compared to 2014 in our experiment. Previously, it was reported that under controlled conditions wheat production reduced to 3.5 to 4.5% for every rise of 1°C above 15°C, and number of grains decreased by 13% with rise in temperature from 25/20°C to 35/20°C (Wardlaw and

Wrigley1994). Similarly, global simulations have indicated that wheat production will decline on average by 6% for each additional 1oC temperature increase, which correspond to 42 million tons yield reduction (Asseng et al., 2015).

5.2.3 Relationship among agronomic traits and multivariate analysis

We used HSI_{GY} to define tolerant genotypes as described by many other researchers (Mohammadi et al., 2008; Pinto et al., 2010). In the scatter plot (Figure 2.2), the genotypes were categorized into tolerant, moderately tolerant and intolerant on the basis of HSI_{GY}. The positive correlation of TKW and GN with GY in the PCA proposed that the TKW and GN are the vital contributing traits for improving the GY in both HS and NOR environments. HSI_{GY} is proposed to be a better parameter to identify heat-tolerant plants and has been used in earlier studies for estimating the high temperature tolerance in wheat (Mohammadi et al., 2008; Mason et al. 2010, 2011). Further, in the scatter plot, DH had negative correlation with GY during HS environments signifying that earlier flowering time allowed to escape of terminal heat stress. Early DH permitted SHWs to normally fill out their grains and spurt the late high temperature happening at the time of maturity to fill the grains. While DH has positive correlation with GY under NOR conditions proposing the lengthier the maturation of the plants particularly if the duration of grain filling is extended, the more GY increase will be achieved. The genotypes that going to mature early during the normal environments would be more appropriate for high temperature environments due to stress escaping mechanisms (Kazan and Lyons, 2015). Similarly, there was slightly lower and negative correlation in NOR conditions, and was opposing to Lopes et al. (2012) and del Pozo et al. (2016) reporting non-significant association among DH and GY in drought stress and high temperature stress environments. This is primarily attributed to the higher variation for time to flowering in SHWs owing to the usage of wild *Ae. tauschii* accessions (Jones et al. 2013) as compared to improved germplasm displaying short range to time of flowering (Lopes et al., 2012). In the current study, there was no correlation between GN and TGW in the NOR or HS treatments, however both have significant correlation with GY under NOR and HS treatments. Genetic progress for GY might be attained by improving TGW while sustaining or, if probable, improving the grains number per unit area. But it is usually identified that TKW and GNP are in deprived or negatively associated with each other (Slafer and Rawson, 1994). This might be due to the decreased availability of assimilates in single grain

due to the increased number of grains per unit area, especially in the course of stage of grain filling when resource precincts happen. In current study both GN and TGW are contributing independently in grain yield and regression models projected the difference in GY under HS environment was due to variation in TGW more than GN. The SHWs with desirable HSI_{GY} were capable to sustain the TKW had greater yields during terminal heat stress. SHWs showed major differences for the weight of grains likened to bread wheat and TGW of up to 68 g and related results have been described in Mexico (Calderini and Reynold, 2011).

5.3. Morphological, physiological and biochemical evaluation

The basic statistics including means, minimum maximum and their standard deviation are presented in (Table 3.1). ANOVA explained substantial variations between genotypes, treatments, and their interactions for the studied traits except BY which remained non-significant for treatment interaction (Table 3.1) means that trait showed less response to late stage heat stress. Results were consistent with those earlier stated for bread wheat in identifying significant variations in agronomic, biochemical and physiological traits and strong genotype by environment interactions (Motamedi et al., 2012; Tadesse et al., 2012; Degewione et al., 2013; Wahid et al., 2007). Heat stress given after anthesis stage showed significant variations in physio-biochemical traits such as total proline content, total soluble sugar, membrane stability index and yield parameters. Plant developmental stages may have affected to high temperatures at various stages, but most importantly morphological and developmental stages are affected by high temperature to some degree (Wahid et al., 2007). SHWs have shown greater variation for all the attributes that can be utilized in breeding programs for tolerance to high temperature (Cossani and Reynolds 2015).

Over all heat stress reduced the TKW and GY by the 23% and 30%, respectively as compared the control treatment, the significant decrease in grain yield and other yield related traits in HS environment in agreement with several reports of high temperature responses of wheat. High temperature decreased PH (Singh et al., 2007), flowering time (Rahman et al., 2009), TKW (Wardlaw and Wrigley, 1994; Reynolds et al., 1994), and GY (Tewolde et al., 2006).

HSI_{GY} was used in earlier reports to measuring the high temperature tolerance in wheat and measured as best parameter in selecting high stress tolerant plants (Mohammadi et al., 2008; Mason et al., 2010, 2011).

It is essential to know about significant correlation between different plants attributes as it helps in selecting the genotypes with suitable characters for a breeding program (Ali et al., 2009). Correlation of the traits were presented (Figure 3.1). Positive significant correlation between GY and TGW with the value of was found in both normal and heat stress treatment while there is poor correlation were found between TKW and GNP. The findings suggested that under high temperature TGW along with GY might be used as direct selection criteria for high temperature tolerance in wheat. Genetic improvement for higher grain yield might be attained by increasing TKW or, if likely, improving the grains number per unit area. Mostly, TKW and number of grains unit area negatively correlated with each other (Slafer and Rawson, 1994). TKW and GY have negative association with HSI^{TKW} and HSI^{GY} which is in agreement to previous studies (Mason et al 2010). Physiological and biochemical traits have significant correlation with each other. PRC have significant positive correlation with MSI in controlled environment. MSI have positive significant correlation with Chla, Chlb and TChl in normal environment. In heat stress environment, Chla, Chlb and TChl have significant positive correlation with each other and significant positive correlation with MSI. This suggested that heat stress increased the activity of and antioxidants (SOD and POD) and osmoprotectants thus resulted in a little decrease in chlorophyll contents. Under abiotic stresses such insignificant decreases or little reformed chlorophyll in other species have also been described (Kyparissis et al., 1995).

The tolerance of wheat to high temperature can be determined through estimation of GY presentation or determining agronomic and physio-biochemical parameters under abiotic stress environments (Shipler and Blum, 1990; Zhao et al., 2007; Reynolds et al., 1994, 2001; Al-Khatib and Paulsen 1984). Different physio-biochemical traits such as proline contents, MSI, soluble carbohydrates, and chlorophyll contents were studied to evaluate the tolerance of genotypes under heat stress environments (Reynolds et al., 1994; Khan et al., 2015).

High temperature had important influence on the content of chl a, b, and TChl (Table 3.2). Chla and Chlb and TChl contents decreased because of heat stress. Findings of such a

decline in the contents of chl in many crops among abiotic stress environments have also described by (Kyparissis et al., 1995). Under drought stress condition reduction in total chlorophyll indicate lowered capability to harvest light (Herbinger et al., 2002).

Heat stress significantly represented the changes of flag leaf soluble carbohydrates amount under the studied genotypes (Table 3.2). Comparing the mean value of flag leaf soluble sugar quantity in synthetic hexaploid wheat genotypes exemplified here as a result of high temperature stress increased the accumulation of SS and reduced assimilates utilization that relates to inhibit the activity of invertase or sucrose synthase and also declines the source to sink translocation. Osmotic balance further suggests that balance in wheats, mainly in leaves as sink, SS are amongst the key contributing plant organic solutes. Soluble sugars have a role in ROS scavenging mechanisms through NADPH reported by Coue et al. (2006). They have further indicated that of its association in directive and expression of few ROS linked genes like SOD, signifying that soluble sugars have capacity to act upon as indications, important for the crops in identifying and governing the balance of cellular redox in an intricate method.

Heat stress after grain filling stage showed high proline contents in fresh leaves of all the synthetic wheat genotypes under study (Table 3.2). Increase in compatible organic solutes, for example proline is one of the utmost common responses of many crops exposed to abiotic stresses. It has been suggested that proline shows a protective role in plants between cytoplasmic vacuole, and it has the potential to detoxifying the reactive oxygen species (ROS) and also stabilizing antioxidant enzymes and thus protects the integrity of the membrane (Ashraf and Foolad 2007). During the high temperature stress, accumulation of proline contents in plants resulted whichever due to suppressed activity of proline degradation or augmented expression of proline synthetic enzymes (Hong et al., 2000). In current study under high temperature increased the proline contents was found high in AUS30284 and lowest in AUS30633. Ahmed and Hassan (2011) in their study reported the same genotypic differences in proline accumulation under high temperature stress. Leaf proline increase under high temperature stress and act as an effective index to screen wheat genotypes in relating to differences in heat tolerance.

Terminal heat stress at late stage of grain filling reduced the MSI in all the tested synthetic hexaploid genotypes (Table 3.2). Increase in solute seepage is an indication of decrease in the thermal stability of cell membrane, and also been used as an unintended extent of high

temperature stress tolerance amongst the diverse plant species, as well as wheat (Blum et al., 2001, Wahid et al., 2007). In current study, high temperature decreased the MSI at vegetative and reproductive stages in all the studied synthetic hexaploid wheat genotypes. Our findings showed harmony with the Khan et al. (2015) in their study concluded that MSI was a beneficial selection criterion for high temperature tolerance in wheat, showing modifications in MSI at various growth stages among different wheat cultivars. Khan et al. (2015) observed major association between MSI of flag leaf and yield of wheat genotypes, and proposed that MSI can be used in the high temperature tolerance studies of wheat genotypes under heat stress conditions.

5.4.1 Genetic diversity and population structure in D-genome SHWs

Many marker systems have been used so far to study the SHWs and genetic diversity within *Ae. tauschii* (Ogbonnaya et al., 2013). These are formed by *Ae. Tauschii sub species and tetraploid wheat crosses which have genes for breeding* showing proportion of genetic variation also. The mechanism of linkage is affected by genome variability and population structure. Kinship matrix and variation in genetic relation decreases the number of false positives. (Yu et al., 2006). In our study findings supported the fact that five sub-structures were suitable in outlining synthetic hexaploid wheats structure. Lately, Mulki et al. (2012) calculated an extensive range of SHWs and designated occurrence of seven sub-structures were fitting in defining the population structure. Slight alteration to the outcomes may be attributed to the higher number of *Ae. tauschii* used as likened to current study. *Ae. tauschii* existence of accessions among the Synthetics hexaploid was divergent from single to a great of five however the durum best lines extended from 1 to 46, a sign of intricacy of the crossing. STRUCTURE algorithm has been recommended that does not do congregate to an optimum K once complex genetic assemblies occur, such as resilient kinship inside some crops (Camus-Kulandaivelu et al., 2007). LD is inclined by degree of recombination, occurrence of alleles and selection of population structure (Flint-Garcia et al., 2003). Many readings propose that linkage disequilibrium is not constant among the complete genome, or alongside a particular chromosome. LD can arise above great spaces but might also be educe for close loci (Neumann et al., 2011). In current work, genetic distance increase but LD commonly reduced with the rise of genetic distance with very robust LD among duos of loci detected at genetic distance of up to

8 cM, indicative of LD sustained by linkage. Our fallouts were constant with previously reported studies in wheat. In a comparable work utilizing a sub-set of 92 synthetics, Emebiri et al. (2010) described that the overall trend was great among LD up to 16 cM, and a drop afterward. LD was probable to extended to around 11 cM between 43 USA elite bread wheat cultivars and breeding population (Chao et al., 2007) WAMI association mapping panel (Sukumuran et al., 2015 and Edae et al., 2104). In a worldwide assemblage of barley cultivars, Malysheva-Otto et al. (2006) recognized genetic regions where LD stretched up to 51 cM, whereas Crossa et al. (2007) indicated that some LD lumps strained up to 88 cM in a set of 170 bread wheat lines. Breseghello and Sorrells (2006) recommended that LD might differ amongst populations and might require to be assessed for each population on a case-by-case basis. A sum of LD centered mapping and diversity studies in wheat have been piloted at the genome or chromosome stages (Breseghello and Sorrells 2006; Chao et al., 2007; Somers et al., 2007; Horvath et al., 2009; Chao et al., 2010; Hao et al., 2011; Maccaferri et al., 2010). Yet, it is vibrant to represent germplasm for considering the amount of LD to study the genomic diversity.

Our study revealed extensive genetic difference for heat tolerance that can be exploited for wheat improvement and enhance the GY. Continuous variation in all parameters under heat stress is a channel towards applying GWAS studies. Our study epitomized total of 31 significant MTAs over the whole-wheat genome using the mixed linear model among them, 14 MTAs were identified under NOR and 14 MTAs were found under HS conditions. Previously, Sukumuran et al., (2015) in their GWAS study Marker trait association findings have been discussed by genotyping a core collection wheat association mapping initiative (WAMI) population of 287 elite, spring wheat lines grown under temperate irrigated of wheat lines with DArT and SNP markers. DArT is a high-throughput cost-effective genotyping platform covering whole genome to screen out large number of polymorphic loci and has the potential for linkage mapping and genome wide diversity analysis. New diversity has repercussions in improvement of Heat tolerance germplasm after genetic dissection allowing irreplaceable *Ae. Tauschii* accessional diversity authentication to mark synthetic wheats as donors for breeding purposes. Our results are like those of Jaccoud et al. (2001) who recognized DArT for rice and also used efficiently to genotype huge genomes and new species e.g. *Triticeae* crops for genetic map construction and analysis for diversity (Wenzl et al., 2006; Neumann et al., 2011; Roy et al., 2011; Varshney et al., 2012).

Developmental phases of bead wheat such as emergence ear (days to heading), days to anthesis and days to maturity are controlled by group of three, vernalization (*Vrn*), photoperiod (*Ppd*), and the earliness per se genes (Kosner and Pankova, 1998) and plays a key role in adaptation of bread wheat by their expression in diverse locations (Gororo et al., 2001). For DH we have identified one significant MTA under HS condition on chromosome 3D. Bennett et al. (2012b) in their study reported some QTLs for days to heading on chromosomes 2B, 2D and 7B. Furthermore, it has been reported that homoeologous groups of chromosomes 2, 3, 5, 6 and 7 of wheat encompass important genes for tolerance to a-biotic stresses (Dubcovsky et al., 1995, Somers et al., 2004; Golabadi et al., 2011). For number of grains per unit area and number of grains per spike we identified the markers associated in both NOR and HS environments on the chromosomes 1A, 3B, 5B and 7B. Not any of these MTAs are in agreement with earlier identified QTL in bi-parental populations with the exception of MTA noted for kernel number per unit area on chromosomes 3B by (Pinto et al., 2016). In plant breeding piling the QTL from different chromosome regions that govern traits of significance into single background is a challenging and also time consuming task. For this, increasing the QTL pyramiding efficacy by expending multi-trait markers in marker-assisted breeding. We identified the pleiotropic markers for the GNS and GNP on the chromosome 5B and 7B.

PH is another significant parameter to use specially in abiotic environments. Green Revolution in the Indian sub-continent major contributors to the success were semi-dwarf cultivars. Environment specific MTAs on chromosome 3B and 7B were identified in current study. While none of both MTAs were in agreement to other QTL mapping studies in abiotic stresses. MTA for PH on chromosome 2B was within confidence intervals of previously identified QTL (Börner et al., 2002; Groos et al., 2003).

It is important to know about significant correlation between different plants attributes as it helps in selecting the genotypes with suitable characters for a breeding program (Ali et al., 2009). Positive significant correlation between GY and yield contributing traits with was found in both normal and heat stress treatment while there is poor correlation were found between TKW and GNP. The findings suggested that under high temperature TGW along with yield may be utilized as direct selection benchmarks for tolerance to heat in wheat. It has been found that genetic advancement for high grain yield might be attained by increasing TKW or, if likely,

enhancing the grains number per unit area. Due to positive significant correlation of SL and TP with each other we identified the 7 environment specific MTAs on chromosome 3B and 3D. Earlier, stable MTAs were identified on chromosomes 3B, 5A, and 5B for yield and related traits (Pinto et al., 2010; Edae et al., 2014; Lopes et al., 2014; Sukumuran et al., 2015).

For TGW we identified one MTA on chromosome 6B under HS condition. In previous studies none of the MTAs found associated on chromosome 6B except, Pinto et al. (2016) who identified a MTA for TKW on 6B chromosome under HS condition. It has been found that genetic advancement for high grain yield might be attained by increasing TKW or, if likely, enhancing the grains number per unit area. Generally, TKW and grains per unit area show negative association (Slafer and Rawson 1994). Therefore, enhancing the TKW without significant reduction in number of grains to identify the important genomic regions is challenging for the breeders (Griffiths et al., 2015). In current study, the MTAs linked with these 2 significant traits were self-governing of each other and the amount of trade-offs among number of grains and TKW might be decreased. Though genetic gain for yield has been attained by enhancing number of grains, however it is probable to produce cultivars with high yield and large grains.

Utilization of DArT markers for the coverage of genome is a significant prerequisite for experiment of any bi-parental and genome wide association studies (GWAS). Recently, DArT markers have been used in a several number of wheat genome wide association studies (Lu et al., 2013; Skinnes et al., 2010; Edae et al., 2014; Sukumuran et al., 2015; Tadesse et al., 2015; Huang et al., 2012; Crossa et al., 2007). Few multi trait markers (more than one trait) were identified on chromosomal regions signifying either linkage or pleiotropic effects in association studies on this SHWs population. For the improvement of genetic dissection of wheat there is a need of more markers to map more QTLs, extensive coverage of genome could be helpful in finding QTLs with major effects for heat tolerance in SHWs.

Conclusion

We evaluate the potential of SHWs under field and controlled conditions. This study provided evidence for the suitability of SHWs for exploitation in breeding for heat stress adaptation. The results here suggested that SHWs possessing good tolerance to high temperature stress along with greater genetic variations for important yield related traits under field conditions. On the basis GY stability we recognized six SHWs viz. AUS-30284, AUS-33384, AUS-30288, AUS-30296, AUS-33409, and AUS-30629 as best stable genotypes under heat stress treatment. Under controlled condition experiment at late grain filling stage results showed that a brief episode of severe high temperatures can considerably affect growth and senescence related traits. However, more work is required to find procedures that will result in greater reproducibility. Further work is also required to identify the physiological/molecular processes and genetic loci controlling the variation in heat responses at the vegetative stage. Overall, the significant association of heat responses grain weight and chlorophyll at the grain filling stage may suggest a possible physiological/genetic link between heat responses at different developmental stages, with implications for developing more efficient heat tolerance screening methods. Such methods may assist breeding for heat tolerance and identification of heat tolerance genes.

The number of QTLs have been identified under heat stress was slightly more than under normal conditions (17 vs. 14), a phenomenon which has been noted also in other mapping populations. GWAS recognized chromosomal regions linked with yield and yield related parameters under heat stress and normal environment in primary D-genome SHWs population that can be utilized to pinpoint desirable QTLs to be used in heat tolerant cultivar development. These MTAs for DH, GNS and TGW on chromosome 3D, 6B and 7B can be vital goals for MAS, marker-based breeding and fine mapping of functional genes after further validation. Thus, a breeding strategy based on marker assisted selection would need to incorporate novel genomic selection methods.

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Table S1: Pedigree of 137 synthetic hexaploid wheats in this study

Sr. No	GENOTYPE ID	PEDIGREE
1	AUS30280	CROC_1/AE.SQUARROSA (275)
2	AUS30281	STY-US/CELTA//PALS/3/SRN_5/4/AE.SQUARROSA (277)
3	AUS30283	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (334)
4	AUS30284	AAZ_3/AE.SQUARROSA (398)
5	AUS30286	DOY1/AE.SQUARROSA (418)
6	AUS30287	STY-US/CELTA//PALS/3/SRN_5/4/AE.SQUARROSA (418)
7	AUS30288	CROC_1/AE.SQUARROSA (466)
8	AUS30290	ALTAR 84/AE.SQUARROSA (502)
9	AUS30291	CROC_1/AE.SQUARROSA (516)
10	AUS30292	DOY1/AE.SQUARROSA (516)
11	AUS30293	CETA/AE.SQUARROSA (525)
12	AUS30296	DOY1/AE.SQUARROSA (1016)
13	AUS30297	DOY1/AE.SQUARROSA (1024)
14	AUS30298	CETA/AE.SQUARROSA (1025)
15	AUS30299	DVERD_2/AE.SQUARROSA (1027)
16	AUS30301	CETA/AE.SQUARROSA (1027)
17	AUS30302	CETA/AE.SQUARROSA (1030)
18	AUS30303	CETA/AE.SQUARROSA (166)
19	AUS30625	GAN/AE.SQUARROSA (180)
20	AUS30626	GAN/AE.SQUARROSA (257)
21	AUS30627	D67.2/P66.270//AE.SQUARROSA (257)
22	AUS30628	LCK59.61/AE.SQUARROSA (308)
23	AUS30629	ARLIN/AE.SQUARROSA (308)
24	AUS30630	LCK59.61/AE.SQUARROSA (313)
25	AUS30631	LCK59.61/AE.SQUARROSA (324)
26	AUS30632	SRN/AE.SQUARROSA (358)
27	AUS30633	SCOOP_1/AE.SQUARROSA (358)
28	AUS30635	GAN/AE.SQUARROSA (408)
29	AUS30636	STY-US/CELTA//PALS/3/SRN_5/4/AE.SQUARROSA (431)
30	AUS30637	YAV_2/TEZ//AE.SQUARROSA (437)
31	AUS30641	YAR/AE.SQUARROSA (513)
32	AUS30642	SCA/AE.SQUARROSA (518)
33	AUS30643	TK SN1081/AE.SQUARROSA (519)
34	AUS30644	SCA/AE.SQUARROSA (523)
35	AUS30645	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (528)
36	AUS30647	CIT71/CPT//AE.SQUARROSA (629)
37	AUS30648	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (629)
38	AUS30649	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (633)
39	AUS30651	SCOOP_1/AE.SQUARROSA (662)
40	AUS30652	6973/WARD.7463//74110/3/AE.SQUARROSA (665)
41	AUS30656	LCK59.61/AE.SQUARROSA (783)
42	AUS30659	GARZA/BOY//AE.SQUARROSA (171)
43	AUS30661	GARZA/BOY//AE.SQUARROSA (270)
44	AUS30668	DVERD_2/AE.SQUARROSA (295)

45 AUS30669 GARZA/BOY//AE.SQUARROSA (300)
46 AUS30672 KAPUDE/AE.SQUARROSA (314)
47 AUS33376 ALTAR 84/AE.SQUARROSA (333)
48 AUS33381 RASCON/AE.SQUARROSA (367)
49 AUS33382 GARZA/BOY//AE.SQUARROSA (374)
50 AUS33384 GARZA/BOY//AE.SQUARROSA (427)
51 AUS33387 GARZA/BOY//AE.SQUARROSA (439)
52 AUS33388 DOY1/AE.SQUARROSA (458)
53 AUS33391 ALTAR 84/AE.SQUARROSA (507)
54 AUS33394 GARZA/BOY//AE.SQUARROSA (520)
55 AUS33395 DOY1/AE.SQUARROSA (532)
56 AUS33396 LCK59.61/AE.SQUARROSA (536)
57 AUS33397 CROC_1/AE.SQUARROSA (170)
58 AUS33398 CETA/AE.SQUARROSA (170)
59 AUS33399 CETA/AE.SQUARROSA (174)
60 AUS33400 CROC_1/AE.SQUARROSA (177)
61 AUS33403 DOY1/AE.SQUARROSA (256)
62 AUS33404 GAN/AE.SQUARROSA (259)
63 AUS33407 DOY1/AE.SQUARROSA (318)
64 AUS33409 CROC_1/AE.SQUARROSA (362)
65 AUS33412 SCA/AE.SQUARROSA (409)
66 AUS33414 GAN/AE.SQUARROSA (413)
67 AUS33415 CROC_1/AE.SQUARROSA (444)
68 AUS33416 GAN/AE.SQUARROSA (459)
69 AUS33419 CETA/AE.SQUARROSA (540)
70 AUS33421 DVERD_2/AE.SQUARROSA (1022)
71 AUS33423 DVERD_2/AE.SQUARROSA (1027)
72 AUS33424 CETA/AE.SQUARROSA (1027)
73 AUS34230 68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (164)
74 AUS34232 DOY1/AE.SQUARROSA (188)
75 AUS34234 ALTAR 84/AE.SQUARROSA (191)
76 AUS34235 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (191)
77 AUS34236 ALTAR 84/AE.SQUARROSA (193)
78 AUS34237 CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (193)
79 AUS34239 ALTAR 84/AE.SQUARROSA (198)
80 AUS34241 CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (205)
81 AUS34242 CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (208)
82 AUS34243 SORA/AE.SQUARROSA (208)
83 AUS34244 CROC_1/AE.SQUARROSA (210)
84 AUS34245 D67.2/P66.270//AE.SQUARROSA (211)
85 AUS34246 SORA/AE.SQUARROSA (211)
86 AUS34247 CROC_1/AE.SQUARROSA (213)
87 AUS34248 DVERD_2/AE.SQUARROSA (214)
88 AUS34249 ROK/KML//AE.SQUARROSA (214)
89 AUS34251 YUK/AE.SQUARROSA (217)
90 AUS34252 ALTAR 84/AE.SQUARROSA (219)

91	AUS34253	D67.2/P66.270//AE.SQUARROSA (220)
92	AUS34254	DVERD_2/AE.SQUARROSA (221)
93	AUS34255	D67.2/P66.270//AE.SQUARROSA (221)
94	AUS34260	D67.2/P66.270//AE.SQUARROSA (223)
95	AUS34268	SCA/AE.SQUARROSA (279)
96	AUS34270	GARZA/BOY//AE.SQUARROSA (286)
97	AUS34271	ACO89/AE.SQUARROSA (290)
98	AUS34272	GARZA/BOY//AE.SQUARROSA (307)
99	AUS34273	LARU/AE.SQUARROSA (309)
100	AUS34274	68.111/RGB-U//WARD/3/AE.SQUARROSA (321)
101	AUS34275	68.111/RGB-U//WARD/3/AE.SQUARROSA (322)
102	AUS34276	SORA/AE.SQUARROSA (323)
103	AUS34277	68.111/RGB-U//WARD/3/AE.SQUARROSA (325)
104	AUS34278	68.111/RGB-U//WARD/3/AE.SQUARROSA (328)
105	AUS34279	ALTAR 84/AE.SQUARROSA (328)
106	AUS34280	68.111/RGB-U//WARD/3/AE.SQUARROSA (329)
107	AUS34405	ALTAR 84/AE.SQUARROSA (193)
108	AUS34406	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (193)
109	AUS34407	ALTAR 84/AE.SQUARROSA (198)
110	AUS34408	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (205)
111	AUS34409	CROC_1/AE.SQUARROSA (210)
112	AUS34412	CROC_1/AE.SQUARROSA (213)
113	AUS34414	ROK/KML//AE.SQUARROSA (214)
114	AUS34415	D67.2/P66.270//AE.SQUARROSA (217)
115	AUS34416	ALTAR 84/AE.SQUARROSA (219)
116	AUS34417	D67.2/P66.270//AE.SQUARROSA (220)
117	AUS34418	DVERD_2/AE.SQUARROSA (221)
118	AUS34419	D67.2/P66.270//AE.SQUARROSA (222)
119	AUS34420	TK SN1081/AE.SQUARROSA (222)
120	AUS34421	D67.2/P66.270//AE.SQUARROSA (223)
121	AUS34422	CROC_1/AE.SQUARROSA (224)
122	AUS34423	CROC_1/AE.SQUARROSA (224)
123	AUS34424	ALTAR 84/AE.SQUARROSA (224)
124	AUS34425	GAN/AE.SQUARROSA (236)
125	AUS34426	YAV_2/TEZ//AE.SQUARROSA (243)
126	AUS34427	SCA/AE.SQUARROSA (279)
127	AUS34431	68.111/RGB-U//WARD/3/AE.SQUARROSA (329)
128	AUS34435	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (460)
129	AUS34436	DOY1/AE.SQUARROSA (511)
130	AUS34437	6973/WARD.7463//74110/3/AE.SQUARROSA (35A)
131	AUS34438	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (633)
132	AUS34439	CROC_1/AE.SQUARROSA (662)
133	AUS34440	CROC_1/AE.SQUARROSA (725)
134	AUS34443	CROC_1/AE.SQUARROSA (784)
135	AUS34446	FGO/USA2111//AE.SQUARROSA (658)
136	AUS34447	D67.2/P66.270//AE.SQUARROSA (221)
137	AUS34448	ALTAR 84/AE.SQUARROSA (205)

138	AUS33382	GARZA/BOY//AE.SQUARROSA (374)
139	AUS33384	GARZA/BOY//AE.SQUARROSA (427)
140	AUS33386	GARZA/BOY//AE.SQUARROSA (433)
141	AUS33387	GARZA/BOY//AE.SQUARROSA (439)
142	AUS33390	GARZA/BOY//AE.SQUARROSA (503)
143	AUS33394	GARZA/BOY//AE.SQUARROSA (520)
144	AUS33389	GREEN/AE.SQUARROSA (458)
145	AUS30672	KAPUDE/AE.SQUARROSA (314)
146	AUS34273	LARU/AE.SQUARROSA (309)
147	AUS30628	LCK59.61/AE.SQUARROSA (308)
148	AUS30630	LCK59.61/AE.SQUARROSA (313)
149	AUS30631	LCK59.61/AE.SQUARROSA (324)
150	AUS33396	LCK59.61/AE.SQUARROSA (536)
151	AUS30655	LCK59.61/AE.SQUARROSA (689)
152	AUS30656	LCK59.61/AE.SQUARROSA (783)
153	AUS34233	RABI//GS/CRA/3/AE.SQUARROSA (190)
154	AUS30671	RASCON/AE.SQUARROSA (314)
155	AUS33381	RASCON/AE.SQUARROSA (367)
156	AUS34249	ROK/KML//AE.SQUARROSA (214)
157	AUS34414	ROK/KML//AE.SQUARROSA (214)
158	AUS34451	ROK/KML//AE.SQUARROSA (214)
159	AUS34268	SCA/AE.SQUARROSA (279)
160	AUS34427	SCA/AE.SQUARROSA (279)
161	AUS33412	SCA/AE.SQUARROSA (409)
162	AUS30642	SCA/AE.SQUARROSA (518)
163	AUS30644	SCA/AE.SQUARROSA (523)
164	AUS30633	SCOOP_1/AE.SQUARROSA (358)
165	AUS30634	SCOOP_1/AE.SQUARROSA (407)
166	AUS30650	SCOOP_1/AE.SQUARROSA (659)
167	AUS30651	SCOOP_1/AE.SQUARROSA (662)
168	AUS30660	SCOT/MEXI_1//AE.SQUARROSA (186)
169	AUS30673	SCOT/MEXI_1//AE.SQUARROSA (314)
170	AUS33406	SKARV_2/AE.SQUARROSA (304)
171	AUS34432	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (412)
172	AUS30645	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (528)
173	AUS30648	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (629)
174	AUS30649	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (633)
175	AUS34243	SORA/AE.SQUARROSA (208)
176	AUS34246	SORA/AE.SQUARROSA (211)
177	AUS34411	SORA/AE.SQUARROSA (211)
178	AUS34276	SORA/AE.SQUARROSA (323)
179	AUS30632	SRN/AE.SQUARROSA (358)
180	AUS34449	STY-US/CELTA//PALS/3/SRN_5
181	AUS30281	STY-US/CELTA//PALS/3/SRN_5/4/AE.SQUARROSA (277)
182	AUS30287	STY-US/CELTA//PALS/3/SRN_5/4/AE.SQUARROSA (418)
183	AUS30636	STY-US/CELTA//PALS/3/SRN_5/4/AE.SQUARROSA (431)
184	AUS33417	STY-US/CELTA//PALS/3/SRN_5/4/AE.SQUARROSA (502)

185	AUS34256	TK SN1081/AE.SQUARROSA (222)
186	AUS34258	TK SN1081/AE.SQUARROSA (222)
187	AUS34420	TK SN1081/AE.SQUARROSA (222)
188	AUS30643	TK SN1081/AE.SQUARROSA (519)
189	AUS30641	YAR/AE.SQUARROSA (513)
190	AUS34265	YAV_2/TEZ//AE.SQUARROSA (243)
191	AUS34426	YAV_2/TEZ//AE.SQUARROSA (243)
192	AUS30637	YAV_2/TEZ//AE.SQUARROSA (437)
193	AUS30638	YAV_2/TEZ//AE.SQUARROSA (457)
194	AUS30657	YAV_2/TEZ//AE.SQUARROSA (882)
195	AUS34457	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA (498)
196	AUS30639	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (457)
197	AUS34435	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (460)
198	AUS30640	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (490)
199	AUS34251	YUK/AE.SQUARROSA (217)
200	AUS34459	YUK/AE.SQUARROSA (217)
201	AUS34249	ROK/KML//AE.SQUARROSA (214)
202	AUS34414	ROK/KML//AE.SQUARROSA (214)
203	AUS34268	SCA/AE.SQUARROSA (279)
204	AUS34253	D67.2/P66.270//AE.SQUARROSA (220)
205	AUS34250	D67.2/P66.270//AE.SQUARROSA (217)

Table S2: Meteorological information of the Bahawalpur for Normal planting and late planting during the two cropping season 2013/14 and 2014/15.

Month	Max. Temp (°C)	Min. Temp (°C)	Avg. Max Temp (°C)	Avg. Min. Temp (°C)	Max. RH (%)	Min. RH (%)	Avg.RH (%)
Nov-13	31	11.1	28.9	12.8	74	46.1	60.6
Dec-13	28.5	1.7	22.5	8.5	97.5	48.5	71.7
Jan-14	27.2	0.6	20.7	5.6	96	44.3	60.9
Feb-14	26.2	5.8	22.3	8.5	80.3	45.3	61.4
Mar-14	33.6	9	27.8	13.7	73.7	38.3	57.4
Apr-14	43	16.7	35.9	20	58.3	24	42.6
May-14	45.5	19.5	39	25	66.3	26.3	41.8
Nov-14	31.5	9.7	29.4	12.7	72	40	50.8
Dec-14	28.5	1.7	20.7	7.3	93.3	49.7	69.2
Jan-15	24.3	1.9	18.3	6.3	95.3	49	75.7
Feb-15	27.5	6.9	23.8	10.7	82	42.3	65.4
Mar-15	38.2	8.1	27	14.1	92	47	64.4
Apr-15	44.5	15.6	36.6	21.8	71.1	24.7	44.2
May-15	44.5	21.8	40.4	25.4	63.3	21	36.5

Max. Temp: Maximum temperature, Min. Temp: minimum temperature, Avg. Min Temp: average minimum temperature, Avg. Max. Temp: average maximum temperature, Max. RH: maximum relative humidity, Min. RH: minimum relative humidity, Av. RH: average relative humidity,

Table S3: Meteorological information of the Sind for Normal planting and late planting during the two cropping season 2013/14 and 2014/15.

Month	Max. Temp (°C)	Min. Temp (°C)	Avg. Max Temp (°C)	Avg. Min. Temp (°C)	Max. RH (%)	Min. RH (%)	Avg.RH (%)
Nov-13	35.5	12	28.9	12.8	80	46.1	59.3
Dec-13	32	3	25.9	10.3	68.3	45.1	62.8
Jan-14	29.5	2	23.4	7	68.3	39.3	52
Feb-14	29	5	26.5	10.1	67.7	32.3	50.2
Mar-14	37	11.5	32	15.6	72.5	25.7	49
Apr-14	44.5	15.5	39.2	21.1	60	29.7	42.9
May-14	45.5	23.5	41.6	28	56	24.3	31.6
Nov-14	38	12	25.5	8.8	58	40.3	50.8
Dec-14	32.5	4	25.5	8.8	72.7	37.3	53.8
Jan-15	30	4.5	24.2	8.3	78.7	47.2	62.7
Feb-15	35	8	28.5	12.7	68.7	35.3	57.6
Mar-15	42	6.5	32.7	14.8	72.3	32	46.9
Apr-15	44	20	39.7	22.1	65	26.7	43.2
May-15	47	21	41.9	25.3	63	32.3	49.3

Max. Temp: Maximum temperature, Min. Temp: minimum temperature, Avg. Min Temp: average minimum temperature, Avg. Max. Temp: average maximum temperature, Max. RH: maximum relative humidity, Min. RH: minimum relative humidity, Av. RH: average relative humidity,

Table S4: Meteorological information of the Islamabad for Normal planting and late planting during the two cropping season 2013/14 and 2014/15.

Month	Max. Temp (°C)	Min. Temp (°C)	Avg. Max Temp (°C)	Avg. Min. Temp (°C)	Max. RH (%)	Min. RH (%)	Avg.RH (%)
Nov-13	26.4	4.5	28.9	12.8	92	57	67.5
Dec-13	24.5	-1	19.9	3.9	80	44	68.4
Jan-14	24	-2	18.9	2.3	80.3	38.3	57.5
Feb-14	24	2.5	19.3	5.5	94	43.7	61.6
Mar-14	28.2	3	22.4	9	96.3	43	61.9
Apr-14	39	9.5	29.6	14.4	81.7	35.7	51.2
May-14	41	14	33.5	19.2	95.5	31.3	46.5
Nov-14	27.5	3.2	25	6.5	78	44.3	55.5
Dec-14	26.5	0	20.5	2.9	71.7	46	59.5
Jan-15	22.5	2	18.7	4.4	90	43	62.9
Feb-15	25	4.5	20.8	8.4	92.7	41.7	62.5
Mar-15	32	6.5	23	11.3	96	40	64.4
Apr-15	35	13.5	29.1	17.2	95	40.7	58
May-15	39.5	16.5	35.6	21.1	80.3	29.7	41.2

Max. Temp: Maximum temperature, Min. Temp: minimum temperature, Avg. Min Temp: average minimum temperature, Avg. Max. Temp: average maximum temperature, Max. RH: maximum relative humidity, Min. RH: minimum relative humidity, Av. RH: average relative humidity,

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RESEARCH

Genotypic Variation and Genotype × Environment Interaction for Yield-Related Traits in Synthetic Hexaploid Wheats under a Range of Optimal and Heat-Stressed Environments

Abdul Aziz, Tariq Mahmood, Zahid Mahmood, Kanwal Shazadi, Abdul Mujeeb-Kazi, and Awais Rasheed*

ABSTRACT

Adaptation of wheat (*Triticum aestivum* L.) to high temperature could be improved by introgressions from wild relatives. The response of 137 D genome synthetic hexaploid wheats (SHWs) to high temperature was evaluated to determine their potential for wheat improvement. Field experiments were conducted in two temperature scenarios (normal sowing time [NOR] and late sowing time to expose the plants to heat stress [HS]) for 2 yr at three locations to assess the effect of terminal high temperature on yield-related traits. High temperature stress overall led to a 46.9% reduction in grain yield and significant reductions of 25.2% in days to heading, 26.6% in plant height, 16.1% in grain number per square meter, and 18.3% in thousand grain weight. In ridge regression analysis, agronomic traits explained 8.74 to 35.2% of the variation in grain yield in the HS treatments with an average of 30.47%. In NOR treatments, agronomic traits explained 8.85 to 45.5% of the variation in grain yield, with an average of 34.5%. Days to heading was negatively correlated with grain yield in the heat-stressed environments but did not explain significant variation in grain yield in optimal environments. Thousand-grain weight explained the highest variation in grain yield in all environments, followed by grain number per square meter. The top ten highest grain-yielding SHWs in the HS treatment were also tolerant to heat stress, with a heat susceptibility index ranging from 0.33 to 0.40. These SHWs could be a promising source to introduce yield-related traits to develop high-yielding wheat cultivars for heat-stressed environments.

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Abbreviations: BWP, Bahawalpur in Punjab province; DH, days to heading; GN, grain number per square meter; GY, grain yield; HS, late sowing time to expose the plants to heat stress; HSI_{GY}, heat susceptibility index for grain yield; ISB, Islamabad; NOR, normal sowing time; PCA, principal component analysis; PH, plant height; SHW, synthetic hexaploids wheat; SND, Tando Jam in Sindh province; TGW, thousand-grain weight.

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide. It is cultivated on >20% of the world's arable land and forms a part of the daily diet for 21% of the global population (Ortiz et al., 2008). Wheat production faces a serious challenge from climate change, and continuously rising global temperatures could have a significant negative impact (Stone and Nicolas, 1995). World production is projected to decline by an average of 6% (42 Tg) for each degree Celsius increase in temperature (Asseng et al., 2015). High temperatures in tropical and subtropical areas affect crop development at all growth stages, imposing morphological and physiological changes that result in considerable losses in grain yield (GY) (Tewolde et al., 2006). Currently, wheat crops in India, Pakistan, the United States, Australia, and Mexico are exposed to high temperatures during the grain-filling period, adversely affecting yield and grain quality. High temperature shortens the duration of grain filling and decreases the time to senescence and harvest maturity (Altenbach

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