CHAPTER ONE

1.0 INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important cereal crop that ranks first globally and second in Zambia, after maize. More than 4.5 billion people in the world depend on food products made from wheat kernels. Wheat has been cultivated for over 8 000 years providing the much needed carbohydrates, proteins, vitamins, minerals and fibre to humans. Its production has over the years increased and is currently grown on more than 240 million hectares of land in the world. Its world trade exceeds all other cereal crops like maize, rice and sorghum (Braun et al, 2010; Muleoki, 1997; Lumpkin, 2011).

Wheat cultivars are classified based on their need for vernalisation as spring and winter wheat. The crop is also classified based on the hardness of the grain as soft or hard grain and on colour as red and white (Reynolds et al, 2009; FAO, 2010). Zambia only grows spring wheat mainly under irrigation.

The convenience of bread and other wheat products as fast foods has increased wheat's popularity both in urban and rural communities of Zambia. This has subsequently triggered an increase in wheat production in order to satisfy the needs of exponentially growing human population coupled with their ever changing dietary preferences. In Zambia, wheat production has thus increased from 69 000 metric tonnes in 1999 to 237 000 metric tonnes in 2011 and its consumption has also increased from 133 000 metric tonnes in 2005 to 210 000 metric tonnes in 2011(MACO/CSO, 2011).

Wheat is a C_3 plant that thrives well in cool environments. According to Reynolds et al, 2001 wheat is, however, a widely grown crop from temperate, irrigated to dry and high rainfall areas and from warm, humid to dry cold environments. In Zambia, most of wheat production is done on plateau areas (high altitude) with an elevation of 900 to 1 300 metres above sea level with temperatures reaching 10°C in July. In these areas the crop is mainly grown during cool months of the year (April to September) and the cost of irrigation raises a major challenge to production. Currently wheat production is also done in valley areas of Zambezi, Luangwa and Kafue that lie between 300 and 900 metres above sea level with ambient temperatures dropping to 19°C in July. In support of this view, large companies are opening up big farms along the country's river basins for wheat production. This trend is likely to continue since hot river valleys are endowed with abundant water resource for irrigation.

However, the production of this important cereal is limited by a number of abiotic stress factors such as drought, salinity, Aluminium toxicity and high ambient temperatures. Battisti and Naylor (2010) reported that heat stress is the most important stress factor that affects between 25 and 30 million hectares of wheat annually in the world and thereby causing significant grain yield reduction. It has thus posed a severe threat to wheat production in many countries, particularly when it occurs during reproductive and grain filling phases. Unlike drought and salinity stresses, changes in ambient temperatures occur within hours. Therefore, plants need to suppress and respond to the adverse effects of heat in a very short time (Kumar et al, 2012).

The optimum growing conditions for good yields of wheat have been reported to be between 18°C and 24°C. Exposure of the crop to temperatures above this range (28-30°C) even for 5 or 6 days short period can cause 20% or more yield losses in wheat. This is because heat stress causes an array of physiological, biochemical and morphological changes in wheat which reduce tillering capacity, shortens grain filling period and accelerates crop senescence (Bahar et al, 2011; Elbashier et al, 2012).

It further reduces the plant's photosynthetic capacity through metabolic limitation and oxidative damage to chloroplasts with concomitant reduction in dry matter accumulation and yield (Farooq et al, 2011).

The effect of heat stress on wheat production is likely to worsen with the advent of climate change (Reynolds et al, 2010). This scenario of reduced wheat yields due to heat stress has also affected Zambia both on the plateau and in hot river valleys.

Wheat in Zambia is mostly grown under irrigation during the cool season. The optimum planting dates for wheat are from late April to the last week of May. Farmers sometimes rotate wheat with crops like soya beans that require drying before harvesting and this consequently delays the time of planting. Planting late, beyond May, due to such operational problems leads to declining yields. The reason for this progressive decline in grain yield is attributed to heat stress which affects the wheat's tillering capacity, anthesis and grain filling period. Thus, high ambient temperatures reduce tillering which is a yield component.

On the other hand, valley areas generally have high ambient temperatures. In Zambia, the average wheat yields per hectare have been reported to be 4 tonnes in hot river valleys and 8 tonnes on the plateau areas (Muleoki, 1997; MACO/CSO, 2011). Therefore, wheat yields in hot river valleys are usually lower than those obtained on the plateau despite optimising all crop husbandry practices. This raises a need for wheat varieties that would give high grain yield even under high ambient temperatures.

Growing wheat only under optimum ambient temperature is currently perceived as an appropriate approach but this might not be sustainable at all levels of cropping systems and sites. The most promising approach is to understand differential tolerance of genotypes to heat stress which provides an opportunity to develop heat tolerant cultivars. Such varieties would give consistently high yields even under heat affected environments. Varieties not specifically selected for heat tolerance do not give good yields when grown in heat affected areas such as the river valleys or in situations when the crop was planted late on the plateau. Therefore, the solution is to develop varieties that have heat tolerance to mitigate the effect of heat stress on wheat production.

The development of heat tolerant varieties would be an advantage to wheat farmers in Zambia as they would be able to obtain acceptable returns from their investment. Thus, these varieties would encourage wheat production in hot river valleys that have sufficient water resources for irrigation. In addition such varieties would encourage small scale farmers in hot river valleys, especially under out grower schemes, to get involved in wheat cultivation which at the moment is solely under commercial production. Furthermore, small holder farmers participating in small scale irrigation schemes set up by Government in hot river valley areas would diversify and include growing wheat as a cash crop during winter months and thereby improve their cash- flow.

This reasonable approach requires understanding morpho-physiological responses that are associated with heat tolerance in wheat. Cool canopy and radiation use efficiency have been reported to be important for heat stress tolerance and genetic diversity for heat stress is well established (Reynolds et al, 2010; Bahar et al, 2011). Several researchers in many countries have shown that some physiological criteria provide a gain in wheat productivity under high ambient temperatures (Bahar et al, 2011). Such criteria include chlorophyll content (Yildirim et al, 2011), stomata conductance (Bahar et al, 2009), membrane thermostability (Sharifi et al, 2012), stay green (Kumar et al, 2010) and canopy temperature depression (Elbashier et al, 2012).

These studies have also revealed high genetic differences in some morphological traits such as plant height, ear length, above ground biomass, harvest index and thousand kernel weight. These characteristics were observed to be correlated to grain yield and to physiological traits like canopy temperature depression and membrane thermostability. Therefore, an understanding of morpho-physiological mechanisms among wheat genotypes would lead to identification of traits that can be used in wheat breeding programmes in Zambia. This would increase the country's wheat production and its chances of exporting wheat to other countries.

Although heat stress adaptive traits are well researched(Reynolds et al, 2010), less work has been carried out on heat tolerance in wheat in Zambia and this has rendered a limitation on the available information on heat stress in the country. It is against this background that this research was conducted.

The main objective of this study was to generate information on the mechanisms of heat stress in wheat which can be used in breeding for heat tolerant wheat.

The specific objectives of this study were:

1. To identify morpho-physiological traits associated with heat tolerance in wheat.
2. To establish the most appropriate indirect selection criteria for heat stress tolerance in wheat.

This study was carried out based on the hypothesis that there exists sufficient variation among wheat genotypes on their response to heat stress which could be used in wheat breeding programmes.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance of Wheat

Wheat(*Triticum aestivum* L.) is an important cereal crop consumed by many humans and for 8 000 years it has been the basic staple of civilised Europe, West Asia and North Africa. Today wheat is grown on more than 240 million hectares, larger than any other commercial crop and its world trade is greater than for all other crops combined (Curtis, 2002 ; Lumpkin, 2011). Wheat is a major human dietary component since it is the most important source of carbohydrates, proteins, minerals and vitamins for humans (Braun et al, 2010).

Wheat is most successful in the temperate climates of the world. Nevertheless the increasing demand of wheat products, due to a rapid increase in human population and changes in dietary preferences, has moved wheat into non-traditional areas formerly thought unacceptable for production (Reynolds, 2010; Bahar et al, 2011). Although moving wheat into non-traditional growing areas offers some promise for area expansion, production measures are more critical due to great exposure to abiotic and/or biotic stresses (Battisti and Naylor, 2009).

The optimum growing temperature for wheat has been reported to lie between 18^{o}C and 24^{o}C with minimum and maximum growth temperature of 3^{o} to 4^{o}C and 30^{o} to 32^{o}C respectively (Bahar et al, 2011).

Wheat is commonly classified into spring or winter and this generally refers to the season during which the crop is grown. Spring wheat has a mild response to vernalisation and its frost resistance is low. The winter wheat type on the other hand has a strong response to vernalisation and requires a period of cold weather to flower. In Zambia, only spring wheat is grown mainly under irrigation during the cool months of the year when disease infestation and weed pressure are low (Aquino et al, 2009; Lumpkin, 2011).

2.2 Importance of Wheat in Sub Saharan Africa

Most of the wheat produced in Sub- Saharan Africa is used for human consumption. There is a rising demand for wheat products in the region that has outstripped wheat production. To satisfy this demand some countries have resorted to importing wheat. According to Lumpkin (2011), the region has five major wheat importing countries, ‘the big 5’, that accounted for 53% of wheat imports and 64% of total wheat consumption from 2000 to 2009. These countries are Nigeria (23%), Sudan (10.7%), Ethiopia (8.2%), South Africa (6.6%) and Kenya (4.9%). The source of these wheat imports that filled the staple food deficit was mainly from USA (34%), Argentina (15%) and Australia (8%). It is based on this premise that Dixon et al (2009) have indicated that the sub Saharan Africa has a deepening wheat deficit and much of it was filled by imported wheat from other regions.

Wheat in the region is generally produced under rain fed conditions except in Zambia and Zimbabwe, where the crop is grown under irrigation (Aquino et al, 2009). Zambia is endowed with sufficient water resources and land and as such the country has great potential for increasing wheat production that can make it harness existing international markets for wheat. Zambia’s current wheat production meets the country’s requirements with a surplus of 88 000 metric tonnes being exported within the southern region (MACO/CSO, 2011).

2.2.1 Wheat Growth and Development

Wheat responds best to inputs at certain stages of plant development (Braun et al, 2010; Bauer et al, 1983). The impact of frost, heat, drought, diseases, insects and weeds can be more accurately predicted with a clear picture of the relationships between growth stage and plant response to stress (Curtis, 2002; Battisti and Naylor, 2009). Therefore, a sound understanding of plant growth and development is an essential element of efficient, economic wheat management systems (Herbek and Lee, 2009). Wheat undergoes ten major growth stages during its life cycle and these are germination, seedling, tillering, stem elongation and booting. Other stages are booting, heading, flowering or anthesis, milk, dough and ripening (Bauer et al, 1983; Farooq et al, 2011; Reynolds et al, 2007).

There are several systems that have been developed to provide numerical designations for growth and developmental stages. Among these are Feekes (Large, 1954), Haun (Haun, 1973) and Zadoks (Zadoks et al, 1974) scales that are used the most frequently. The Haun scale is most useful in defining vegetative growth stage. The Feekes scale and Zadoks' scale provide a good description for both vegetative and reproductive phases. Zadoks' scale is the most comprehensive and easiest to use as it describes all growth stages, a characteristic not considered in other scales (Herbek and Lee, 2009).

2.2.2 Optimum Growing Conditions of Wheat

Wheat can be grown successfully under a wide range of soil, rainfall and temperature conditions (Muleoki, 1997). Wheat cultivars of varying pedigree are grown under varying soil conditions and exhibit trait variations. However, for optimal production wheat requires fertile soils with adequate source of moisture during the growing season, reasonable drainage, good water holding capacity and with pH of 5.5 to 6.5 (Curtis, 2002; Dixon et al; 2009).

According to Curtis (2002) wheat can withstand the cold of the northern areas quite well; it grows successfully in hot climates if the humidity is not too high. It is not adapted to areas where warm, humid conditions prevail, largely because such conditions favour the rapid development of diseases (Muleoki, 1997). Both the amount and distribution of precipitation are of prime concern to wheat production. Wheat requires 250 to 750 mm of annual precipitation (Curtis, 2002; Blum, 1988; Bahar et al, 2011). In areas where wheat is grown with irrigation, the distribution of natural precipitation is not critical (Barnabas et al, 2008).

The optimum growing temperature for different stages of the wheat plant varies considerably (Curtis, 2002; Braun et al, 2010). For instance, the optimum mean daily temperature for germination ranges from 20 - 25°C, while the optimum temperature for good tillering is much lower (16 – 20°C) and for proper development of the wheat plant the best temperature range is 18 – 24°C (Bahar et al, 2011). At mean daily temperatures higher than 20°C in the early tillering phase, tillering is poor and heading is accelerated (Farooq et al, 2011; Ashraf and Bhatti, 1998). At temperatures higher than 25°C in the grain development phase, the plant dries prematurely (Easterling and App, 2005; Ubaidullah et al, 2006).

2.3 Heat Stress

Wheat is a cool season crop that requires optimum growing temperature of between 18°C and 24°C (Bahar et al, 2011). Temperatures above this optimum for wheat growth can be deleterious, causing injury or irreversible damage, which is called heat stress (Wahid et al, 2007). Heat stress is a function of magnitude and rate of temperature increase as well as duration of exposure to increased temperature (Wahid et al, 2007).

According to Bita and Gerats (2013), changes in ambient temperatures are sensed by plants with a complicated set of sensors positioned in various cellular compartments. The increased fluidity of the membrane leads to activation of lipid based signalling cascades and to an increased calcium ion flux and cytoskeletal reorganisation. Signalling between these routes leads to the production of osmolytes and antioxidants in response to heat stress. Saidi et al, (2009) have reported that there is a gene in plants that encodes a component of the membrane cyclic nucleotide gated calcium ion channels that act as primary thermo-sensors of land plant cells. These channels in the plasma membrane respond to increments in the ambient temperature by triggering an optimal heat shock response (Bita and Gerats, 2013; Saidi et al, 2009).

Higher plants like wheat when exposed to excess heat, at least 5°C above their optimal growing conditions exhibit a characteristic set of cellular and metabolic responses required for the plants to survive under the high temperature conditions (Guy,1999; Zhao et al, 2008; Kumar et al, 2012). These effects include changes in the organisation of cellular structures, including organelles and the cytoskeletons, and membrane function, accompanied by a decrease in synthesis of normal proteins and the accelerated transcription and translation of heat shock proteins (HSPs), the production of phytohormones such as abscisic acid (ABA) and antioxidants and other protective molecules (Maestri et al, 2002; Wahid et al, 2007; Beta and Gerats, 2013). HSPs are molecular chaperones which help the plant to tolerate extreme heat shock condition by protecting the native proteins from denaturing (Kumar et al, 2012).

Many key phytohormones including ethylene (ET), salicylic acid (SA), and ABA also increase their levels under heat stress, while others decrease, such as cytokinin (CK), auxin (AUX), and gibberellic acids (GAs); fluctuations that cause premature plant senescence (Larkindale et al, 2005). For example, the abscission of reproductive organs, an important effect of heat stress, is known to be caused by increased ABA and ET levels and reduced levels and transport of AUXs (Maestri et al, 2002).

Wheat growth can be impaired by heat stress at any developmental stage (Easterling and Apps, 2005). Many researchers have reported that heat stress during reproductive phase is more harmful than during vegetative growth due to the direct effect on grain number and grain weight (Wollenweber et al, 2003; Ubaidullah et al, 2006; Semenov, 2009; Bahar et al, 2011; Elbashier et al, 2012). They have also referred stress during reproductive growth as either 'End-of-Season or Terminal' heat stress. Stress during reproductive phase in wheat causes pollen sterility, tissue dehydration, low carbon dioxide assimilation and increased photorespiration (Munjal and Rena, 2003; Singha et al 2006).

Although elevated temperatures accelerate growth in wheat (Ubaidullah et al, 2006), they also reduce the phenology which is not compensated for by increased growth rate (Reynolds, 2010). As a result of this, many studies have found that terminal heat stress especially during grain filling period of late planted wheat as one of major environmental factors that drastically reduce wheat production (Stone and Nicolas, 1995 a; Modhej and Behdarvandi, 2006; Farooq et al, 2011).

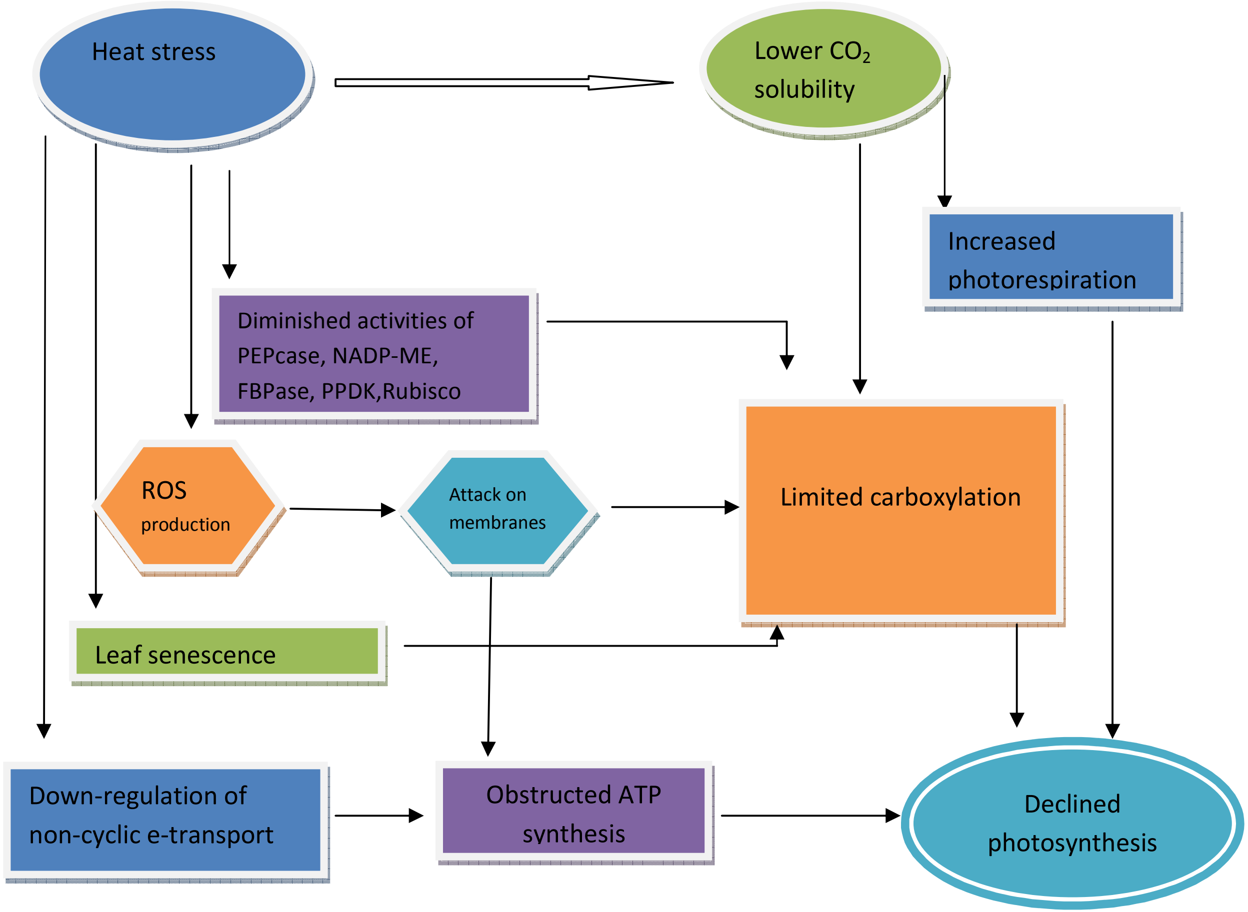
Over one half of the total wheat area worldwide is already prone to periods of heat stress (Braun et al, 2010), and climate models suggest further increases in average temperatures as well as extreme temperature anomalies, which are already detectable (Hansen et al , 2012). Thus, occasional or prolonged exposure of wheat to high temperatures causes many biochemical and morpho-physiological responses in wheat (Tahir et al, 2006; Elbashier et al, 2012).

2.4 Morpho- physiological Consequence of Heat Stress

Heat stress causes different morpho-physiological and biochemical changes in wheat (Reynolds et al, 2008). These changes may lead to severe cellular injury and even cell death in a very short time. Moderately high temperatures can cause injury in wheat only after the crop is exposed to such temperatures for a long time. According to Farooq et al (2011), injuries that are directly induced by high temperatures include protein denaturing and aggregation, and increased fluidity of membranes. Heat stress also inactivates enzymes in the chloroplasts and mitochondria, inhibits protein synthesis and induces membranes to lose integrity.

Kumar et al (2012) have reported that heat stress also induces oxidative stress in plants caused by generation and accumulation of super oxides (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-), which are commonly known as reactive oxygen species (ROS). Oxidative stress may induce lipid peroxidation leading to protein degradation, membrane rapture and enzyme inactivation (Zhao et al, 2008). According to Hays et al (2007) and Kumar et al (2012) high temperatures accelerates plant development and thereby reducing the vegetative growth and seed setting in wheat, and initiates grain abortions, early transition to dry seed stage and low grain yield. Heat stress accelerates the rate of grain filling whereas grain filling duration is shortened (Dias and Lidon, 2009). For instance, 5^oC increases in temperature above 20^oC increased the rate of grain filling and reduced the duration by 12 days in wheat (Yin et al, 2009).

High temperatures further affects physiological processes associated with carbon assimilation like transpiration, respiration and photosynthesis (Streck, 2005). Wahid et al (2007) reported that photosynthesis is the most sensitive process to elevated temperatures. Thus, heat stress reduces photosynthesis through disruptions in the structure and function of chloroplasts, and reductions in chlorophyll content (Xu et al, 2004). Zhao et al (2008) observed that heat stress affects a key enzyme, Rubilose-1, 5-biphosphate carboxylase/oxygenase (Rubisco), that regulates carboxylation during photosynthesis. This scenario lowers the enzyme’s affinity for carbon dioxide and acts as oxygenase and this consequently increases the rate of photorespiration and lowers photosynthesis (Xu et al, 2004). A specific effect of high temperatures on photosynthetic membranes includes the swelling of grana stacks and aberrant stacking. Such structural changes are accompanied by ion-leakage from leaf cells exposed to heat and changes in energy allocation to the photo systems (Wahid and Shabbir, 2005). Figure 1 illustrates the possible mechanism in which photosynthesis is reduced under heat stress.



Source: M. Farooq et al (2011)

Figure 1: Photosynthetic Mechanism under Heat Stress

2.5 Physiological Parameters associated with Heat Adaptation

According to Kumar et al, 2012, changes in ambient temperature occur within hours, unlike drought and salinity stresses. Therefore plants need to suppress and respond to the adverse effects to heat in a very short time. Plants tend to reduce the impact of heat-induced damage through a number of mechanisms such as leaf rolling, leaf shedding, reducing leaf size, thickening of leaves, reducing growth duration, transpirational cooling and other adjustments in morphology and ontogeny (Wahid et al, 2007). Plant responses to heat stress are mediated by an intrinsic capacity to endure basal thermotolerance and, after acclimation, the ability to gain thermotolerance. The capacity of crop plants to survive and produce good grain yield under heat stress is generally regarded as heat tolerance (Wahid et al, 2007).

Producing an economically significant yield under heat stress conditions depends on several plant physiological parameters and mechanisms that contribute to heat tolerance in the field, such as amendments to essential processes like photosynthesis, and concomitant increases of transcripts coding for proteins involved in protection (Nagarajan et al, 2010). The accumulation of osmo- protectants is also an important adaptive mechanism in plants subjected to extreme temperatures, as primary metabolites participate directly in the osmotic adjustment (Bita and Gerats, 2013). For instance, accumulation of proline, glycine betaine, and soluble sugars is necessary to regulate osmotic activities and protect cellular structures from increased temperatures by maintaining cellular water balance, membrane stability, and by buffering the cellular redox potential (Farooq et al, 2011).

Despite advances in understanding genes of major effect conferring disease resistance in wheat (Krattinger et al, 2009), the genetic basis of heat adaptation is poorly understood. Currently no ‘heat tolerance’ genes have been cloned. For the time being, physiological traits associated with heat adaptation constitute the best available ‘handle’ for genetic improvement of crops, since they represent de facto favourable combination of alleles. Such alleles are still quite elusive using quantitative trait locus (QTL) approach, because they show interaction with both environment and genetic background, which typically includes genes of major effect (Reynolds and Tuberosa, 2008; Pinto et al, 2010).

In recent years researchers have shown that physiological traits such as canopy temperature depression (Karimizadeh and Mohammadi, 2011), photosynthetic rate (Koc et al, 2003), stomata conductance (Bahar et al, 2009), membrane thermostability (Kumar et al, 2012), chlorophyll content (Yildirim et al, 2011) and stay green (Harris et al, 2007) provide a gain on wheat. Therefore, understanding the physiological basis of yield can complement traditional breeding in three main ways: (i) by identifying traits that serve as an indirect selection criteria for yield; (ii) by developing selection methodologies that increase the efficiency of parental and progeny selection; and (iii) by providing insights into physiological and genetic basis for raising yield potential (Reynolds et al, 2010).

(a) Canopy Temperature

Physiological and biochemical processes in plants are affected by temperature extremes and high temperatures induce heat stress in plants. Researchers have reported that plants under heat stress exhibit higher canopy temperature (CT) than non- stressed plants both at vegetative and reproductive stages (Siddique et al, 2000; Reynolds et al, 2010; Bahar et al, 2011). These studies have also shown genetic variability in canopy temperatures among wheat genotypes when exposed to high temperatures and that genotypes with cooler canopy temperatures have been associated with high grain yield (Cossani and Reynolds, 2012). Therefore cool canopy in heat stressed areas is regarded as one of the physiologically efficient way of attaining high grain yields in wheat (Elbashier et al 2012). Thus, the interest when selecting for heat tolerance in wheat is to find genotypes that have lower canopy temperature as compared with other genotypes under similar field conditions.

Contrary to the above assertion, Singh and Kinemasu (1983) announced that pearl millet (*Pennisetum glaucum* L.) genotypes showed significantly higher yields with warmer canopy temperature under irrigated conditions than with cooler canopy temperature under non-irrigated conditions.

Canopy temperature is measured remotely by the infrared thermometer (IRT). Canopies emit long wave infrared radiation and the thermometer senses this radiation and converts it to an electrical signal, which is displayed as temperature. Measurements are taken in the afternoon on cloudless and windless day when ambient temperatures are high (Reynolds et al, 2001).

(b) Canopy Temperature Depression

Canopy Temperature Depression (CTD) is the deviation of plant canopies in comparison to ambient temperatures (Canopy temperature Depression= Air temperature – Canopy temperature). Kumar et al, 2012 reported that vapour pressure deficit has a large effect on CTD, while net radiation, air temperature and wind speed have slight effects. CTD, effected by biological and environmental factors like water status of soil, wind evapotranspiration, cloudiness, conductance systems, plant metabolism, air temperature, relative humidity and continuous radiation (Reynolds et al, 2001), have been preferably measured in high air temperature and low relative humidity because of high pressure deficit conditions (Amani et al, 1996 ; Bahar et al, 2008).

Wheat genotypes have exhibited great genetic variability in CTD and cultivars with high CTD values have been generally associated with high grain yield. This has made CTD a suitable selection criterion for grain yield under heat stress (Bahar et al, 2008). However, Elbashier et al (2012) observed that some wheat cultivars despite having high CTD had low grain yield and did not take advantage of cool plant canopies.

The positive correlation of CTD with grain yield and other related traits under dry, hot and irrigated conditions has drawn major attention to this trait as an avenue for increasing grain yield under these environments (Elbashier et al, 2012). High CTD has been used as selection criteria for tolerance to drought and high temperature stress in wheat breeding and the breeding method used is generally mass selection in early generations like F_3. According to this method bulks which show high CTD value (have cool canopy) are selected in F_3 generation. Later single plants which show high stomata conductance with cool canopies are selected. CTD measurements have been used as an effective tool at International Maize and wheat improvement centre (CIMMYT) since they are associated with yield increase among wheat cultivars on different irrigated experiments (Reynolds et al, 2001; Bahar et al, 2011 and Elbashier et al, 2012).

The physiological basis of the association of CTD and yield is unknown. However, since CTD is a direct function of evapotranspiration rate which is determined by a number of physiological and metabolic processes CTD becomes a suitable selection criterion under heat stress environment (Cossani and Reynolds, 2012). As a result of this, CTD has been widely used by researchers in yield improvement experiments under heat stress. In support of this view, Bahar et al (2008) compared performance of durum wheat genotypes and bread wheat genotypes under heat stress in Mediterranean region using CTD and grain yield measurements. They showed that durum wheat genotypes stayed cooler than bread wheat under heat stress and also found a positive and significant correlation between CTD and grain yield ($r=0.45^*$; $P\leq0.05$) at half heading stage; but non- significant positive correlation ($r=0.39$) at anthesis. They recommended that CTD can be used to identify plants with cooler canopies with the aim of increasing yield under non-stressed conditions. Ginkel et al (2004) reported that the overall CTD showed relatively high correlation with yield under optimum conditions ($r=0.74$; $P\leq0.01$).

Besides grain yield, Canopy Temperature Depression measurements have also been observed to have a positive and significant correlation with other traits like above ground biomass, stay green duration, chlorophyll content and some yield components (Kumar et al, 2010; Reynolds et al, 2010; Sareen, et al, 2012).

(c) Membrane Thermostability

Membrane Thermostability (MTS) is a measure of electrolyte diffusion resulting from heat induced cell membrane leakages (Fokar et al, 1998). It is an important physiological mechanism of heat tolerance in spring wheat and is responsible for adaptation of plants to high temperatures (Blum et al, 1988; Islam et al, 2011). Cell membrane is one of the first targets of plant stresses and the ability of plants to maintain membrane integrity and function under heat stress is what determines tolerance towards heat stress (Abdullah et al, 2011). Wheat genotypes exhibit genetic variation in membrane thermostability and cultivars with high solute leakages are susceptible to heat stress.

Heat stress on plasma membrane destroys membrane integrity causing solute leakage from the cells and the extent of which can be estimated by conductometric measurement of electrolyte leakage of solute from leaf tissue after a heat shock. The electrolyte leakages are measured by an electrical conductivity meter (Islam et al, 2011).

Membrane thermostability is a fair index of genetic variation for heat tolerance and has a reasonable relationship to plant performance under heat stressed environment. It is therefore considered as a possible selection criterion for yield under heat stress (Blum et al, 2001).

Researchers have observed that membrane thermostability could be used as an early generation selection criteria to identify high yielding genotypes among segregating generations under irrigated high temperature environments. Shanahan et al (1990) observed a significant yield increase of spring wheat in hot locations by selection of membrane stable lines, as determined by measurements on flag leaves at anthesis. Also Islam et al, 2011 observed significant correlation between MTS and biomass, grain filling rate and grain yield in most of the crosses at genotypic and phenotypic levels.

The physiological basis for association of MTS with heat tolerance is unknown (Fokar et al, 1998; Reynolds et al, 2010), but results from previous studies suggest that MTS has a potential use as an indirect selection criteria for grain yield under heat stress environment (Blum et al, 2001).

(d) Chlorophyll Stability Index

Chlorophyll (Chl) is a green pigment in plants that absorbs light during the process of photosynthesis. It is therefore an essential factor in the process of photosynthesis. Chlorophyll a and b are two main forms of chlorophyll which contribute to green coloured matter in plants. Chlorophyll a is yellowish- green whereas chlorophyll b is bluish- green. Chlorophyll a donates energy directly to the photosynthetic reaction and all other pigments transfer their absorbed energy to it (Rad et al, 2012).

Chlorophyll content in plants can be determined by using destructive method or non-destructive method. The destructive method involves the removal of the leaf from the plant and putting it into chlorophyll extracting organic solvents. Polar solvents such as acetone, methanol, ethyl acetate, pyrimidine and dimethylsulfoxide (DMSO) are most effective chlorophyll extractors (Nikulopoulos et al, 2008).

Stein and Braga (2010) proposed dimethylsulfoxide as a more superior solvent than acetone to extract chlorophyll a and b from plants. They discovered that the acetone method is slow since it requires grinding and centrifugation, and this limits its application under field conditions. On the other hand DMSO requires only leaf immersion in determinate volume of this solvent. Irrespective of the method used, chlorophyll content is calculated based on the absorbance readings obtained through a spectrophotometer using prescribed formulae (Sharifi et al, 2012).

High chlorophyll content is a desirable characteristic under heat stress because it indicates a low degree of photo inhibition of photosynthetic apparatus, therefore reducing carbohydrate losses for grain growth (Ananthi, et al, 2013). Thus, the stability of chlorophyll under a stress condition is very important and it is expressed as chlorophyll stability index (CSI). CSI gives an indication of the available chlorophyll in the plant. High chlorophyll stability index helps the plants to withstand stress through availability of chlorophyll that leads to increased photosynthetic rate and more dry matter production (Madhan Mohan et al, 2000). CSI is determined using several methods (Arnon, 1949; Chapple et al, 1992; Sairam et al, 1997) and according to Sharifi et al (2012) the index is calculated as follows:

CSI= (Total Chl under stress/ Total Chl under control) x 100

There is considerable genetic variability among wheat genotypes with respect to chlorophyll content and CSI under heat stress that has been reported (Elbashier et al, 2012; Reynolds et al, 2010; Mohammadi et al, 2008), and genotypes with high CSI have been found to be associated with high grain yield in wheat. This has conferred chlorophyll content and CSI as possible indirect selection criteria for yield under heat stressed conditions (Mohammadi et al, 2008).

(e) Stay Green Duration

Leaf senescence is initially characterised by structural changes in the chloroplast, followed by a controlled vacuole collapse, and a final loss of integrity of plasma membrane and disruption of cellular homeostasis (Lim et al, 2007). Delay in the expression of senescence related genes permits some genotypes to maintain photosynthesis (Yildirim et al, 2009).

Therefore, the maintenance of leaf chlorophyll and photosynthetic capacity is called ‘Stay Green’ (SG) and the period it lasts from planting is called ‘Green Leaf Duration or Stay Green Duration’. Stay green is considered as an indicator of heat tolerance (Fokar et al, 1998; Bahar et al, 2011). Because the loss of chlorophyll is associated with less assimilation of carbon into grains, stay green genotypes should be better able to maintain grain filling under elevated temperatures (Farooq et al, 2011).

Kumar et al (2010) have reported that stay green or delayed senescence plays a crucial role in grain development in wheat when assimilates are limiting, and stay green cultivars are well adapted to drought and heat stress. Stay green has been evaluated in several crops (Harris et al, 2007; Kumari et al, 2007), but breeding for this trait has been limited in wheat. Under heat stress Stay Green is determined by the number of days from planting to a stage when 95% of wheat leaves turn yellow. However, a challenge associated with the measurement of stay-green is often lack of control of phenology where both early and late genotypes are evaluated or where information on phenology is simply not considered. Also, very often spikes are ignored and these are known to contribute to grain yield under resource limitations (Maydup et al, 2010).

Kumar et al (2006) screened Indian and CIMMYT germplasm for stay green on a visible scoring (0 to 9 scale) and found significant differences between SG and non-SG genotypes for CTD. They further reported that SG together with CTD could be used as an effective selection criterion for heat stress tolerance.

While SG is recognised as an adaptive physiological trait for stress conditions, the optimal pattern of senescence/pigment loss in terms of improving grain yield under heat stress has not been identified. This is partly because chlorosis is an integral part of programmed senescence, where there are unavoidable tradeoffs between maintaining photosynthetic area and remobilisation of nitrogen to maturing grain (Vijayalakshmi et al, 2010).

2.6 Yield, Yield Components and Other Traits

(a) Grain Yield and Above Ground Biomass

Yield is a complex trait that is determined by several characters. Stress factors especially high ambient temperatures affect plant growth and development and cause a sharp decrease of plant productivity (Reynolds et al, 2010; Ubaidullah et al, 2006). However, there are differences in grain yield obtained due to genetic variation. Evaluating grain yield under heat stress has long been practiced by breeders to identify genotypes better adapted to hot conditions. Hansan et al (2007) reported that the growing conditions and wheat cultivars interact significantly to govern the grain yield. They observed that post anthesis heat stress condition decreased grain yield significantly in all wheat genotypes but the amount of reduction was not equal in different cultivars.

The biological yield also called above ground biomass is a combined contribution of yield components (Karamanos et al, 2012). The above ground biomass and grain yield in wheat have been reported to be significantly associated with some physiological traits like CTD (Bahar et al, 2008), membrane thermal stability (Kumar et al, 2012; Islam et al, 2011), stay green (Fokar et al, 1998) and chlorophyll content (Elbashier et al, 2012).

(b) Spike Length and Spikelets per Spike

The ear length and spikes per ear vary among wheat cultivars and are greatly influenced by the interaction of growing conditions and the cultivars (Hansan et al, 2007). Both the spike length and spikelets per spike are sensitive to elevated temperature and the effect of temperature on each of these components of yield depends on developmental phase at which heat stress occurs (Farooq et al, 2011). Heat stress speeds up the development of spikes and thereby reducing spike length and number of spikelets per spike (Porter and Gawith, 1999). For instance, temperatures above 20°C between spike initiation and anthesis may reduce grain yield substantially. Significant reduction in spike length and spikelets per spike has been observed in late sown wheat that was subjected to heat stress (Hansan et al, 2007).

(c) Thousand Kernel Weight

Individual kernel weight directly influences the grain yield. It differs significantly due to combined effect of growing conditions and wheat genotypes. Elevated temperatures reduce the duration between anthesis and physiological maturity, which is associated with reduction in grain weight (Streck, 2005). The weight of kernels is determined by the number of filled spikelets and by the size of grains. Wheat single grain weight decreases as temperature rises above 20°C because the rate of grain filling does not increase enough to compensate for decreased duration of grain filling (Mohammadi et al, 2004). Kernel weight was reported as a good trait to use for indirect selection for yield. Kumari et al (2012) observed a decline in grain yield, biomass, grain filling duration and 1000 kernel weight (TKW) under late sowing conditions owing to terminal heat stress at anthesis and later stages.

(d) Harvest Index (HI)

Harvest index (HI) is the ratio of grain yield to the total above ground biomass and is a key parameter for crop yield predictions. It determines the physiological efficiency of the crop to mobilise photosynthates and transports it to organs of economic value (Mushtaq et al, 2011). Sighn (2009) reported that modern semi dwarf wheat varieties have high harvest index and grain yield due to greater remobilisation of carbohydrates deposited to the economic part. It has been reported that various agronomic and environmental conditions have great influence on harvest index (Hansan et al, 2007). Data from hot wheat growing environments show that grain number is often reduced more than might be expected from reduction in biomass, leading to relatively low harvest index under heat stress (Reynolds et al, 2007). Any or combinations of morpho-physiological that can give high HI and high grain yield should be considered in wheat breeding programmes.

(e) Plant Height

Plant height differs among wheat cultivars and it reveals the overall vegetative growth of the crop in response to environmental conditions (Ubaidullah et al, 2006). Mishra et al (2000) observed significantly negative effects of delayed planting on plant height and these results were similar to earlier findings of Ibrahim et al (1986) who found a reduction of 20% in plant height due to late sowing. The decrease in plant height occurred due to shortening of growth and photosynthetic period as a result of terminal heat stress. Tall plants in response to genetic potential are generally associated with high biological and economic yield in wheat (Reynolds et al, 2010).

(f) Number of Tillers/m²

The number of viable seeds planted and the number of tillers produced per plant sets the upper limit on the number of heads that can be produced by the wheat crop. The capacity to produce tillers is a function of genotype and growing conditions (Hansan et al, 2007). Tillers produced must survive to maturity to contribute to gain yield. Tillering capacity is strongly influenced by heat stress and it is favoured by temperature range of between 16 and 20°C. The higher the number of productive tillers per unit area the higher the grain yield. Thus the number of tillers per unit area has been used as an indirect selection for grain yield under high ambient temperatures (Farooq et al, 2011; Reynolds et al, 2007).

(g) Days to 50 % Flowering (Heading)

This is the number days from planting to the time when 50% of plants are in flower. Days to 50% flowering has been found to be correlated to grain yield and researchers have reported that plants that flower early obtained a higher grain yield that those that flower late Mohammadi et al, 2008; Sareen et al, 2012). Thus, Tewolde et al (2006) reported that early heading varieties performed better than later heading varieties because they; (i) produced fewer leaves per tiller and retained more green leaves, (ii) had longer grain filling periods, and (iii) completed grain filling earlier in the season when air temperatures were lower.

(h) Flag Leaf Area

The flag leaf is responsible for about 75% of photosynthesis during grain filling period in wheat and is an important source of carbohydrates storage for wheat kernels (Reynolds et al, 2010). The area of the leaf is a function of the genotype and the environment. A greater leaf area under normal sowing has been reported in wheat in comparison to late sowing that was subjected to heat stress (Ubaidullah et al, 2006). Similarly, reduced leaf area in response to heat stress has been reported by Ashraf and Bhatti (1998). Flag leaf area is highly correlated with biomass and canopy photosynthesis (Shankarrao et al, 2010).

2.7 Breeding for Heat Tolerance

Heat stress is a major limitation to wheat productivity in environments of the world that are prone to occasional or prolonged high ambient temperatures. Consequently, the development of heat tolerant cultivars is of major concern in wheat breeding programmes (Mohammadi et al, 2007). Heat tolerant cultivars have the ability to consistently produce high grain yields even under high temperatures.

Heat tolerant cultivars are developed through selection and breeding. Physiological traits like canopy temperature depression, membrane thermostability, stay green duration; photosynthetic rate and leaf chlorophyll content provide potential selection criteria for yield improvement under heat stress (Cossani and Reynolds, 2012). The use of these traits as indirect selection criteria for yield in a breeding programme will depend on their genetic correlation with grain yield under heat stress, ease and cost of measurement, extent of genetic variation, heritability, genotype x environment interactions and whether they are associated with adverse pleiotropic effects or genetic drag (Richards, 2002; Mohammadi et al, 2007).

Landraces have been reported to be a source of genetic material for heat stress mitigation. Landraces are varieties adapted to their native environments and significant variability for heat tolerance has been observed in such cultivars. For instance heat tolerant landraces tend to have high chlorophyll content and high stomata conductance (Hede et al, 1998). These materials may be used in breeding programmes aimed to induce tolerance in wheat.

Maintaining grain weight under heat stress is a measure of heat tolerance (Tyagi et al, 2003; Singha et al, 2006). In this regard, Dias and Lindon (2009) proposed that high potential grain weight can be a useful selection criterion for improving heat tolerance.

Genetic engineering is a potential means of improving heat tolerance in wheat. It would involve the introduction of individual genes of interest into the candidate genotype in order to help in improving heat tolerance against heat stress (Barnabas et al, 2008). However, wheat's complex genome has hampered research on genetic modification as compared with other plant species.

Another promising approach in developing heat tolerant cultivars in wheat is the use of quantitative trait loci (QTLs). Recently, several quantitative trait loci have been identified in wheat for heat tolerance during reproductive phase. For instance, Kumar et al (2010) identified three QTLs for the stay green character. Likewise, Pinto et al (2010) identified QTL on chromosome 4A-a for canopy temperature depression under heat.

Various stress indices have been proposed and used by various researchers to differentiate genotypes based on heat tolerance (Fischer and Maurere, 1977; Fernandez, 1992; Nouri et al, 2010; Mohammadi et al, 2011). These indices include heat tolerant index (HTI) and heat susceptibility index (HSI). The mean production (MP), geometric mean (GM) and heat tolerance index have been used for comparing genotypic performance across years or environments. HTI was developed to identify genotypes that perform well under both stress and non-stress conditions (Fernandez, 1993). Stress indices are determined based on either grain yield (Hansan et al, 2007) or on thousand kernel weight (Sareen et al, 2012). They are calculated using specific formulae.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Location

The study was conducted at the field station at the University of Zambia, School of Agricultural Sciences in Lusaka. The station is at an altitude of approximately 1 250m above sea level at latitude 15°22' South and longitude 28°20' East. The site falls under agro ecological region II of Zambia and receives an annual rainfall of between 800mm and 1200mm. The site has a perennial water source for irrigation and an automated meteorological station for recording weather data. The average maximum and minimum monthly temperatures for the site during the experimental period are shown in Table 1.

Table 1 Average Maximum and Minimum Monthly Temperatures of the Site

Month	May	June	July	August	September	October	November
Max. Temp.^{0} C	24.24	23.24	23.84	26.47	30.76	32.14	30.64
Min. Temp.^{0} C	10.34	10.00	9.45	12.11	15.34	18.50	18.40

3.2 Experimental Materials

The study consisted of eighteen spring wheat (*Triticum aestivum* L) genotypes obtained from different sources. Lorrie II was included as a check genotype. The genotypes were obtained from Seed-co, Zambia Seed Company (Zamseed), Zambia Agriculture Research Institute (ZARI), University of Zambia and from CIMMYT, heat tolerance screening nursery of 1998 cycle (Table 1).

Table 2: Wheat genotypes used in the study

Genotype	Source
Entry 10 HTN, 1998	CIMMYT
Entry 11 HTN, 1998	CIMMYT
Entry 13 HTN, 1998	CIMMYT
Entry 15 HTN, 1998	CIMMYT
Entry 20 HTN, 1998	CIMMYT
Entry 27 HTN, 1998	CIMMYT
Entry 41 HTN, 1998	CIMMYT
Entry 44 HTN, 1998	CIMMYT
Entry 45 HTN, 1998	CIMMYT
Entry 46 HTN, 1998	CIMMYT
Entry 47 HTN, 1998	CIMMYT
Nduna	SEED-CO
Sahai I	SEED-CO
UNZA WV I	UNZA
UNZA WV II	UNZA
Loerie II	ZAMSEED
Pungwa	ZAMSEED
Mampolyo	ZARI

3.3 Experimental Arrangement

The study was conducted in the field at two dates of sowing. These were the normal sown or optimal environment (E_1) and the late sown or heat stress environment (E_2). Thus, these environments were created by separation of planting dates. The field experiment was arranged in a Randomised Complete Block Design with four replications per treatment. There were 72 experimental units in each environment. The study also involved laboratory analysis to determine two physiological parameters, membrane thermostability and chlorophyll stability index.

3.4 Agronomic Practices

Eighteen spring wheat genotypes were evaluated from May to November, 2012 based on two planting dates. Under the optimal environment planting was done on 21st May, 2012 while under the late sown environment planting was done on 21st July, 2012. The purpose of adjusting the planting date was to coincide tillering and reproductive phases that require cool environment to elevated temperatures. Each experimental plot was 5m long with four rows spaced at 0.2m apart. Cultural practices were performed according to recommendations prescribed for wheat production in Zambia. These practises included land preparation, fertiliser application, planting, irrigation, weeding bird scaring and harvesting. Thus, the seed rate used was 100 kg/ha. 500 Kg/ha of compound D (10N: 20P: 10K) was used as basal dressing and 300 kg/ ha of urea (46%N) was applied as top dressing. Planting, weeding and harvesting operations were done manually.

3.5 Morpho-physiological Traits Measured

Data on various morpho-physiological traits were collected from randomly selected plants from two central rows of each experimental unit. The average was taken as trait measure for each experimental plot. Data for grain yield and yield components was collected at harvest. The following parameters were measured;

a) Canopy Temperature (CT) and Canopy Temperature Depression (CTD)

Canopy temperature measurements were made by an infrared thermometer (Model MT6, India) on cloudless periods in the afternoon (12:00-13:00 hours). As similar to the method of Bahar et al (2011), the data for each plot were the means of four readings taken from the same side of each plot at an angle of approximately 45° in the range of directions such that they covered different regions of the plot and integrated many leaves. Measurements were taken three days after irrigation and during grain filling period in both experiments. In the optimal environment measurements were taken on 21st August and on 19th October, 2012 in the heat stress environment. Air temperatures were also measured for computation of canopy temperature depression measurements (CTD). CTD was calculated using the formula: CTD=Air Temperature – Canopy Temperature.

b) Membrane Thermostability

Membrane thermostability (MTS) was determined by measuring the electrical conductivity of flag leaf leachate in deionised water during grain filling period. The electrolytes were measured using a conductivity meter (Model HI 98311, Hanna Instruments; Mauritius). MTS was only done in the experiment under the stress environment following the method described by Fokar et al (1998) which was also used by Islam et al (2011).

This method involved selecting and cutting eight fully expanded flag leaves of each genotype from each replication. Each leaf was divided into two parts to use as control and as heat treatment. Samples from halved leaves were placed into two different test tubes containing 10 ml deionised water and were then held in a cold room at 10°C. Thereafter leaf samples were thoroughly washed with deionised water and 15ml of deionised water were added. Then, one half of the test tubes were kept at 25°C and the other half at 45°C for 1 hour in a water bath (Yildirim et al, 2009).

Thereafter both the control and heat treated samples were kept for 18 hours in a cold room at 10°C to stabilise the contents of the liquid after treatment period. Conductivity readings were taken at 25°C using an electrical conductivity meter for control (C_2) and heat treated tubes (T_1). The samples were then boiled for 1hour. A second conductivity reading of the aqueous phase (C_2 and T_2) was taken at 25°C after samples were cooled. Leaf membrane thermostability was estimated using the equation proposed by Fokar et al (1998):

$$\textbf{Membrane Thermostability, MTS (\%)} = [1-(T_1/T_2)] \times 100$$

Where C and T refer to electrical conductivity of control and heat treated samples, and subscript 1 and 2 refer to electric conductivity readings before and after boiling, respectively.

c) Chlorophyll Stability Index

Chlorophyll Stability Index (CSI) was determined by getting the ratio of total chlorophyll under stress to total chlorophyll in the control. The procedure of Murty and Majumdar (1979) was followed and dimethylsulfoxide (DMSO) was used as a chlorophyll extracting solvent. The absorbance (A) of clear solution was determined at wave lengths 648.2 and 664.9 nm (Barnes et al, 1992) using a spectrophotometer (Jenway 6305; Barloworld, UK). Chlorophyll a and b, and total chlorophyll were calculated using the following well known equations;

Chlorophyll (Chl) a = $14.85\ A_{664.9} - 5.14\ A_{648.2}$

Chl b = $25.48\ A_{648.2} - 7.36\ A_{664.9}$ **Total Chl (a+b)** = $7.49\ A_{664.9} + 20.3\ A_{648.2}$

Where: Chl a is chlorophyll a; Chl b is chlorophyll b; Total Chl is total chlorophyll; 664.9 and 668.2 are wave lengths in nm; A is the absorbance of solution; 14.85, 5.14, 25.48, 7.36, 7.49 and 20.3 are constants.

Chlorophyll stability index (CSI) was then determined according to Sairam et al, (1997) and calculated using the equation:

CSI (%) = [Total Chl heated/ Total Chl control] x 100

d) Stay Green Duration

Stay green duration (SGD) was calculated in day units as a period between sowing and physiological maturity of wheat plants in each replication. It was done when plants lost about 95% of green colour following the procedure of Fokar et al (1998).

e) Tillers per Square metre

The number of tillers per square metre was determined through physical counting of tillers in a quadrant.

f) Flag Leaf Area

This was measured at physiological maturity stage following the method of Muller (1991). The determination involved collecting ten randomly selected samples from each plot and the length and width of each leaf were measured. Obtaining these leaf dimensions led to the calculation of leaf area using the formula suggested by Muller and used by other researchers over the years like Ubaidullah et al (2006).

Flag Leaf Area= [Maximum Width x Length x 0.74] cm^2.

g) Other Traits Measured

The following traits were also measured in both experiments;

i**. Plant height (cm**) - It was measured from the soil surface to spike tip in the middle stem excluding awns.

ii. **Days to 50% flowering**- considered as the number of days from planting to heading.

iii. **Spike** (**Ear**) **length** (**cm**) - This was determined by measuring the lengths of ten randomly selected ears in each replication followed by getting the average.

iv. Number of spikelets per spike (ear). This involved counting the number of spikelets per spike from ten randomly selected ears in each experimental unit.

v. **Above ground biomass** – Obtained through cutting twenty plants at harvest from ground level in each replication and weighing these plants in grams.

vi. **Grain Yield** - Four rows in each plot were harvested manually, hand threshed and winnowed. Grains were weighed according to their experimental units and the moisture content of grains in each unit was determined. These harvested grains were converted to kg/ha at 12% moisture content.

vii. **Harvest Index (%)** – This was determined as a ratio of grain yield to above ground biomass in each experimental unit basing on the plants used for biomass determination. This ratio is expressed as percentage.

viii. **One thousand kernel weight** (**TKW**). This was measured in grams and it involved randomly selecting seeds from grain yield of each experimental unit, counting and weighing them.

3.6 Data Analyses

The collected data was subjected to statistical analysis using GENSTAT statistical package; Fourteenth Edition. Means were separated using Fisher LSD method. Simple correlation analyses between traits were also done, and stress indices were calculated using proposed equations. A Stepwise multiple regressions analysis was done using SPSS version 16 to determine the cause and effect relationships of parameters under heat stress environment.

CHAPTER FOUR

4.0 RESULTS

4.1 Temperature Regime

The temperatures under the two environments differed considerably during different phenological stages of the crop. The genotypes planted in May (E1) were exposed to temperatures of less than 26°C during heading(25.37°C) and 25.71°C during grain filling period which is within normal temperatures of 22-26°C required for reproductive growth (Al-Khatib and Paulsen, 1990; Hansen et al, 2007), and was considered as normal or optimal environment (E_1). However, genotypes planted on 21st July, 2012 experienced temperatures of above 32°C during reproductive growth in October (Table 1) and were subjected to heat stress environment (E2).

The average maximum temperature during grain filling period under E_2 (32.15°C) was about 6.44°C higher than in E_1 (25.71°C); and grain filling is a phase of active and rapid dry matter accumulation in the grains. The maximum and minimum air temperatures during canopy temperatures measurements dates were 25.57 °C/ 13.86°C for E_1 (21st August) and 34.55°C / 21.17 °C for E_2 (19th October, 2012).

4.2 Performance of Genotypes in Optimal Environment

The analysis of variance for all the characters under optimal sowing or environment revealed significant differences among wheat genotypes involved in the study at $P< 0.001$ for grain yield, spike length, spikelets per spike, days to 50% flowering, tillers per square metre, flag leaf area, plant height, above ground biomass, canopy temperature depression, canopy temperature, harvest index and stay green duration. This therefore indicated the presence of substantial genetic variation among wheat genotypes for all morpho-physiological parameters measured under optimal sowing environment (Table 3).

Table 3: Summary of Analysis of Variance for all Traits in Optimal Sowing date (E_1)

Source of Variation	Df	GY	SS	SL	DF	TIL	FLA	CT	CTD	PHT	SGD	BM	HI
Replication	3	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Genotype	17	**	**	**	**	**	**	**	**	**	**	**	**
Error	51												
CV (%)		2.3	2.7	2.7	1.4	3.6	5.1	2.9	3.6	1.2	1.8	2.2	2.1

KEY: SL: Spike length; SS: Spikelets/spike; DF: Days to 50% flowering; TIL: Number of tillers/m²; FLA: Flag leaf area; CT: Canopy temperature; CTD: Canopy temperature depression; PHT= Plant height; SGD: Stay green duration; BM: above ground biomass; HI: harvest index; NS: Non-significant and **: Significant at 1% probability.

4.2.1 Grain Yield

Table 4 shows means of traits for optimal sowing date. The highest grain yield was obtained in the check variety Lorrie II (12 204 Kg/ha) giving a yield advantage of 30.57% above the overall mean of 9 345 Kg/ha. This check variety was followed by Entry 13, a genotype that was selected for heat tolerance, with grain yield of 11 221 Kg/ha. Entry 13 was followed by Nduna, a commercially grown variety that has not been selected for heat tolerance with an average grain yield 10 948 Kg/ha. Entry 11 produced the lowest grain yield of 6 486 Kg/ha which was 30.59% lower than the overall mean (9 345 Kg/ha).

4.2.2 Spike length and Spikelets per Spike

The spike length and the number of spikelets per spike are important yield components. In this study the maximum spike length of 12.29 cm was recorded in one of the genotypes that have been selected for heat stress tolerance, Entry 10. It was followed by two genotypes that have not been selected for heat tolerance, Mampolyo (11.86 cm) and Nduna (11.76 cm). On the other hand Entry 15 showed the shortest spike length of 9.99 cm.

In this study the check variety Lorrie II exhibited the highest number of spikelets per spike (21.9) under the optimal environment while Entry 27 recorded the lowest number (17.60) of spikelets. The highest number of spikelets in Lorrie II could be one of the reasons why it produced the highest grain yield under this environment.

4.2.3 Days to 50% flowering and Flag leaf area

Results also showed that Entry 41 had the shortest period to 50% flowering (67 days) while Entry 15 had the longest duration to heading of 83 days. Entry 41 is also one of the lines that have been selected for heat tolerance. Entry 45 recorded smallest flag leaf area of 19.14 cm² while Entry 10 exhibited the largest flag leaf area of 33.87 cm² giving an area advantage of 39.32% over the overall mean of 24.31cm². Entry 10 also showed high grain yield and its flag leaf area could be one of contributing factors to this yield.

4.2.4 Plant height and above ground biomass

Entry 10 and Entry 15 were the tallest genotypes with plant height of 107.37cm and were followed by Entry 20 and Pungwa with plant height of 102.57 cm. The check variety Lorrie II recorded the height of 99.60 cm while Entry 44 was the shortest genotype with plant height of 92.70 cm. Genotypes under study produced varying quantities of above ground biomass. Accordingly, Entry 13 recorded the highest amount of biomass of 100.58 g while Entry 46 produced the lowest amount of above ground biomass of 59.73 g. This could be one of the reasons why Entry 13 produced one of highest grain yields under the optimal environment.

4.2.5 Tillers per square metre and Harvest Index

Both the number of tillers per unit area and the harvest index are important yield components. In this study, Entry 15 and Entry 20 recorded the highest number of tillers per unit area of up to 638.8 under the optimal environment. These heat tolerant genotypes were followed by a check variety Lorrie with 616.8 tillers per square metre.

In this study, Entry 20 showed the highest ratio of economic yield to biological yield of 54.79% and was followed by Sahai I (51.15%), Entry 46 (50.82%), Entry 44 (50.42%) and the check genotype Lorrie II (49.76%). The high harvest index in Lorrie II could imply that it was able to convert photosynthates into economic yield. This high harvest in Lorrie II coupled with high number of tillers could have been one of the reasons that may have contributed to its highest recorded grain yield (12 204 Kg/ ha). Entry 27 recorded the lowest harvest index of 38.22%.

4.2.6 Canopy temperature and Canopy Temperature depression

Wheat genotypes used in this study exhibited significant differences for canopy temperature and canopy temperature depression under optimal sowing environment. Entry 20, Lorrie II and Pungwa showed the highest canopy temperature depression of up to 3.43°C and Entry 45 recorded the lowest CTD of 1.28°C. High canopy temperature depression could have contributed to high grain yield obtained in Entry 20, Pungwa and Lorrie II. These genotypes exhibited cooler plant canopies which have been reported to be associated with high grain yield under heat stress (Bahar et al, 2008). In this study canopy temperatures ranged from 22.20°C in Pungwa to a maximum of 24.60 °C in Entry 45 (Table 4).

Table 4: Means of Traits of wheat genotypes in Optimal Environment

Genotype	Yield (Kg/ha)	SS	SL (cm)	DF (days)	TIL	FLA (cm²)	CT (°C)	CTD (°C)	PHT (cm)	BM (g)	HI (%)
Entry 10	8 782	20.32	12.29	79.25	458.0	33.87	23.42	2.23	107.07	79.23	44.73
Entry 11	6 486	20.28	10.19	78.25	542.8	23.51	24.36	1.56	95.80	64.97	47.95
Entry 13	11 221	17.70	10.34	79.00	489.2	25.08	23.86	2.10	98.07	100.58	44.23
Entry 15	7 589	21.08	9.99	83.00	638.8	22.55	24.08	1.77	107.37	66.27	35.72
Entry 20	10 715	20.28	10.47	76.50	630.8	24.13	22.30	3.43	102.15	71.68	54.79
Entry 27	8 551	17.60	10.99	79.75	460.2	23.98	23.78	2.11	101.92	77.26	38.22
Entry 41	10 279	18.22	10.52	67.00	555.0	23.57	23.52	2.33	100.07	75.05	47.17
Entry 44	9 996	20.00	10.45	69.50	474.0	22.76	23.86	2.03	92.70	74.51	50.12
Entry 45	7 619	20.48	10.18	77.00	460.0	19.14	24.60	1.28	95.22	76.97	47.08
Entry 46	7 141	20.80	10.12	75.50	470.0	20.53	24.47	1.35	93.10	59.73	50.82
Entry 47	7 969	20.82	10.21	75.50	423.0	21.55	22.89	2.84	97.62	74.25	49.03
Mampo	9 352	20.05	11.86	73.00	528.5	25.90	23.34	2.52	97.50	80.38	48.15
Nduna	10 948	20.35	11.76	73.00	599.2	24.87	23.50	2.40	98.92	84.65	44.48
Pungwa	10 409	20.82	11.16	73.00	557.0	25.08	22.20	3.34	102.57	86.75	45.14
Sahai I	8 552	20.85	10.55	76.25	462.2	25.12	24.37	1.54	98.60	72.27	51.15
Unza I	10 195	20.55	10.86	76.75	488.0	24.49	23.36	2.55	98.90	67.99	46.70
Unza II	10 202	20.42	10.40	75.50	559.2	25.06	23.84	2.05	96.82	74.52	48.79
Lorrie II	12 204	21.90	11.20	75.75	616.8	26.32	22.63	3.30	99.60	84.03	49.76
LSD (5%)	**305.9**	**0.76**	**0.41**	**1.48**	**27.02**	**1.76**	**0.98**	**0.86**	**1.74**	**2.34**	**1.38**

KEY: SL: Spike length; SS: Spikelets/spike; DF: Days to 50% flowering; TIL: Number of tillers/m²; FLA: Flag leaf area; CT: Canopy temperature; CTD: Canopy temperature depression; PHT: Plant height; BM: Above ground biomass; HI: Harvest index

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4.3 Performance of Genotypes in Stress Environment

The summary statistics under heat stress environment are shown in Table 5. The results indicate that wheat genotypes in this study were significantly different (P<0.001) in their response to heat stress. Significant differences were revealed among wheat genotypes in stress environment for grain yield, spike length, spikelets /spike, days to 50% flowering, tillers/m^2, canopy temperature, canopy temperature depression, membrane thermostability, CSI, plant height, flag leaf area, above ground biomass and harvest index (Table 5).

Table 5: Summary of Analysis of Variance for all Traits in Stress Environment (E_2)

Source of Variation	Df	GY	SS	SL	DF	TIL	FLA	CT	CTD	PHT	SGD	MTS	CSI	BM	HI
Replication	3	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Genotype	17	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Error	51														
CV (%)		**2.6**	**3.0**	**2.8**	**2.2**	**2.4**	**3.1**	**1.9**	**2.1**	**1.2**	**2.0**	**2.9**	**1.8**	**5.2**	**2.3**

KEY: **SL**: Spike length; **SS**: Spikelets/spike; **DF**: Days to 50% flowering; **TIL**: Number of tillers/m^2; **FLA**: Flag leaf area; **CT**: Canopy temperature; **CTD**: Canopy temperature depression; **PHT**: Plant height; **SGD**: Stay green duration; **MTS**: Membrane thermostability; **CSI**: Chlorophyll stability index; **BM**: above ground biomass; **HI**: harvest index; **NS**: Non-significant and **: Significant at 1% probability

4.3.1 Grain Yield

The performances of eighteen wheat genotypes are shown in Table 6. Entry 20 recorded the highest grain yield of 9 120 Kg/ha giving a yield advantage of 24.56 % over the overall mean (7 322 Kg/ ha), and was followed by Entry 44, Pungwa and Entry 10. Entry 20, Entry 44 and Entry 10 are genotypes that have been selected for heat stress tolerance and have recorded very high grain yields under heat stressed environment. Pungwa exhibited high grain yield despite not being selected for heat tolerance. Lorrie II the check variety recorded the highest yield reductions under stress environment. Entry 15 produced the lowest grain yield of 5 674 Kg/ ha.

4.3.2 Spike length and Spikelets per spike

The minimum (9.60 cm) spike length was recorded for Entry 46 and Entry 11 while the highest was recorded in Entry 10 (11.79 cm). Entry 10 also recorded the highest spike length even under optimal environment. This could be one of the reasons why Entry 10 produced very high grain yields under both environments.

Pungwa, the check variety Lorrie II and Unza II produced the highest number of spikelets/ spike of up to (20.03). Entry 13 recorded the lowest number spikelets per spike (16.50) and this could have been one of the reasons which may have contributed to its low grain yield under stress environment.

4.3.3 Days to 50% flowering and Tillers/m^2

Entry 41 took the shortest period from planting to reach 50% flowering (56.25 days) and this was the same genotype which recorded very early flowering under optimal environment. Entry 15 took the longest period of 69.5 days to reach 50% flowering from the date of planting. The number of tillers per unit area also varied among wheat genotypes used in the study. The results in Table 6 indicate that the maximum number of tillers was recorded in heat tolerant genotype Entry 20 (499). Since the number of tillers is a yield component, Entry 20 could have produced the highest grain yield due to the highest number of tillers per unit area recorded. On the other hand, Entry 15 produced the lowest number of tillers and produced the lowest grain yield o under heat stress environment. The check variety Lorrie II produced 307.2 tillers per square metre which was 20.74% lower than the overall mean of 387.6 tillers. This could have resulted in its great yield decline under stress environment.

4.3.4 Flag leaf area and other parameters

Like under optimal environment Entry 10 recorded the largest flag leaf area under stress environment of 26.54 cm². It was followed by Nduna (24.68 cm²) and Unza I with flag leaf area of 23.60 cm². Nduna has not been selected for heat tolerance while Unza I has been selected. Sahai I showed the smallest leaf area of 17.27cm².

Entry 10 and Entry 41 were also the tallest genotypes even under heat stress environment with a plant height of 94.84 cm. On the other hand Entry 47 recorded the shortest plant height of 79.10 cm.

The check variety Lorrie II produced the lowest above ground biomass of 41.84 g while Entry 13 and Entry 10 produced the highest above ground biomass of 63.62 g and 63.46 g respectively. The high grain yield in Entry 10 could have been associated with its high above ground biomass.

The highest harvest index under the heat stress environment was recorded in Unza II (45.72%) and the lowest was obtained in Entry 15 (32.24%). Thousand kernel weight ranged from 36.46 g to 48.89 g. Accordingly, Entry 11 showed the lowest value while Entry 13 recorded the highest kernel weight (Table 6).

4.3.5 Physiological Traits under Stress Environment

The wheat genotypes that were used in this study expressed differential response to heat stress with respect to canopy temperature, canopy temperature depression (CTD), Stay green duration, chlorophyll stability index and membrane thermostability (Table 7).

a) Stay green duration

This was the period from planting to physiological maturity when wheat plants lost about 95% of the green colour. It is also referred to as green leaf duration. Wheat genotypes used in this study exhibited statistical differences for stay green duration measurements. These measurements ranged from 90.5 to 97.75 days. In this connection, Entry 10, Entry 27, Unza I, Pungwa and Entry 47 showed the longest stay green period. This means that these genotypes had longer period of photosynthesis which could have positively contributed to grain yield. On the other hand Unza II and Entry 45 recorded the shortest stay green duration and this presents genetic diversity for this trait under high ambient temperatures (Table 7).

Table 6: Means of Traits of wheat genotypes in Stress Environment

Genotype	Yield (Kg/ha)	SS	SL (cm)	DF (days)	TIL	FLA (cm^2)	PHT (cm)	BM (g)	HI (%)	TKW (g)
Entry 10	8528	19.80	11.79	65.75	428.9	26.54	94.82	63.46	42.21	45.38
Entry 11	5674	19.43	9.66	66.50	315.4	19.20	80.12	43.72	43.45	36.46
Entry 13	6253	16.50	10.03	65.00	347.5	18.55	80.67	63.62	34.10	48.89
Entry 15	5209	18.40	9.71	69.50	263.5	22.83	91.87	58.43	32.24	38.26
Entry 20	9120	19.63	9.86	68.25	499.0	22.30	85.80	49.30	45.18	39.43
Entry 27	7620	17.20	10.91	67.50	411.0	22.30	89.0	58.86	40.33	48.23
Entry 41	8509	17.90	9.82	56.25	452.2	21.62	94.47	60.91	45.23	43.40
Entry 44	8586	19.63	10.05	58.00	412.8	21.45	79.92	55.87	43.60	41.06
Entry 45	7157	19.10	9.81	64.25	367.8	19.35	82.12	55.14	43.81	38.33
Entry 46	6621	19.43	9.60	63.00	341.5	22.79	82.90	52.49	43.71	38.48
Entry 47	6919	18.97	9.75	64.00	357.2	18.51	79.10	52.70	43.51	36.89
Mampo	7339	19.15	10.50	59.75	426.0	18.56	88.47	55.44	44.16	39.10
Nduna	8146	19.25	10.60	59.00	442.8	24.86	83.05	50.44	35.99	39.78
Pungwa	8563	20.03	10.29	59.75	448.5	22.00	85.70	52.77	43.71	40.90
Sahai	6796	17.68	9.75	60.75	397.0	17.29	90.0	53.56	38.23	44.25
Unza I	7064	16.95	10.45	62.00	348.0	23.60	87.0	55.86	42.47	42.19
Unza II	7159	19.88	10.14	61.50	411.2	18.31	84.57	52.35	45.72	43.10
Lorrie II	6528	19.88	10.50	60.50	307.2	19.44	87.65	41.84	42.80	40.44
LSD (5%)	**272.7**	**0.80**	**0.41**	**2.00**	**13.45**	**0.93**	**3.31**	**4.04**	**1.36**	**1.82**

KEY: SL: spike length; SS: spikelets/spike; DF: Days to 50% flowering; TIL: number of tillers/m^2; FLA: flag leaf area; PHT= plant height; BM: above ground biomass; HI: harvest index; TKW: Kernel weight

Table 7: Means of Physiological Traits of wheat in Stress Environment

Genotype	Yield (Kg/ha)	SGD (days)	CT (^oC)	CTD (^oC)	CSI (%)	MTS (%)
Entry 10	8528	97.25	26.53	7.39	62.28	16.80
Entry 11	5674	96.00	27.34	5.07	52.56	9.56
Entry 13	6253	93.25	27.73	5.48	53.17	10.73
Entry 15	5209	95.50	26.96	5.23	48.78	9.94
Entry 20	9120	96.50	26.64	7.48	65.76	16.51
Entry 27	7620	97.75	27.05	6.03	65.34	13.68
Entry 41	8509	91.25	27.69	6.90	64.88	14.32
Entry 44	8586	90.75	26.98	7.07	69.33	17.86
Entry 45	7157	96.75	25.94	6.99	63.80	12.79
Entry 46	6621	94.00	26.68	7.18	64.86	12.79
Entry 47	6919	97.00	25.76	7.22	57.63	11.74
Mampo	7339	91.00	27.95	6.12	57.53	12.79
Nduna	8146	94.75	26.17	6.62	56.57	14.21
Pungwa	8563	97.00	26.33	7.18	62.70	14.51
Sahai I	6793	96.00	26.36	5.86	52.49	10.48
Unza I	7064	96.25	27.21	6.90	59.93	13.64
Unza II	7159	90.50	27.27	6.38	61.50	12.55
Lorrie II	6528	94.00	27.57	5.69	49.87	10.23
LSD (5%)	**272.7**	**2.72**	**0.71**	**1.63**	**2.15**	**0.54**
CV (%)	**2.6**	**2.0**	**1.90**	**2.11**	**1.82**	**2.90**

Key: **SGD**: Stay green duration; **CT**: Canopy temperature; **CTD**: Canopy temperature depression
CSI: Chlorophyll stability index; **MTS**: Membrane thermostability.

b) Canopy temperature and Canopy temperature depression

Genotypes showed statistically significant differences for canopy temperature (CT) measurements during grain filling period. The highest canopy temperature measurements were observed in Mampolyo, Entry 13 and Entry 41 of up to 27.95°C. The lowest CT values were noticed in Entry 45 and Entry 47 of 25.94°C and 25.76°C respectively. Canopy temperature depression measurements also showed significant variations among wheat genotypes with a range of 2.41°C. The minimum (5.09°C) CTD value was recorded in Entry 11, while the highest measurement was noticed in Entry 10 and Entry 20 of up to 7.48°C. The high CTD in Entry 10 and Entry 20 may have contributed to high grain yield obtained in these two genotypes that have been selected for heat tolerance.

c) Membrane thermostability and Chlorophyll stability index

Wheat genotypes under study also expressed variability to high ambient temperatures with respect to membrane thermostability. Membrane thermostability measurements ranged from 9.56% in Entry 11 to 17.86% in Entry 44. Heat stress also affected the availability of chlorophyll which is an essential element in the process of photosynthesis. Wheat genotypes used in this study revealed significant differences for chlorophyll stability index (CSI) measurements. The range for CSI was 20.55% with the highest measurement recorded in Entry 44 (69.33%) and the lowest value exhibited in Entry 15 (48.78%) followed by the check variety Lorrie II with CSI value of 49.87% (Table 7).

4.4 The Combined Analysis of Variance

The summary for combined analysis of variance for all traits in two environments is shown in Table 8. The results of this study revealed highly significant differences for all traits investigated among wheat genotypes. The environment also exhibited statistically significant effects for most of traits measured except for spike length and number of spikelets per spike at $P<0.001$. The genotype x environment interaction also showed significant effects for most of traits except for spike length.

Table 8: Summary of Combined Analysis of Variance for all Traits in two Environments

Source of Variation	Df	GY	SS	SL	DF	TIL	FLA	CT	CTD	PHT	SGD	BM	HI
Replication	6	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Environment	1	**	NS	NS	**	**	**	**	**	**	**	**	**
Genotype	17	**	**	**	**	**	**	**	**	**	**	**	**
G x E	17	**	**	NS	**	**	**	**	**	**	**	**	**
Error	102												
CV (%)		**2.4**	**2.0**	**2.7**	**1.8**	**3.3**	**4.4**	**2.0**	**2.7**	**2.0**	**1.9**	**3.6**	**2.2**

KEY: **SL**: Spike length; **SS**: Spikelets/spike; DF: Days to 50% flowering; **TIL**: Number of tillers/m²; **FLA**: Flag leaf area; **CT**: Canopy temperature; **CTD**: Canopy temperature depression; **PHT**= Plant height; **SGD**: Stay green duration; **BM**: above ground biomass; **HI**: harvest index; **NS**: Non-significant ******: Significant at 1% probability; **GXE**: Genotype x Environment

In Table 8 the environment showed highly significant influence at 0.001 probability level for grain yield; suggesting that high yield potential under optimal environment (E1) does not necessarily result in improved grain yield under stress environment (E2). Thus, an indirect selection for heat stress environment based on optimum environment will not be sufficient as plant responses for heat tolerance are highly expressed under stress conditions. These findings corroborate the earlier reports of Kumar et al, (2003); Shankarrao et al, (2010); and Sareen et al, (2012).

4.5 Means of Traits for Two Environments.

Table 9 presents means of traits of eighteen wheat genotypes for two environments. The check variety Lorrie II produced the highest grain yield of 12 204 Kg/ha and gave a yield advantage of 30.57% above the overall mean of 9 345 Kg /ha. It was followed by Entry 13 which has been selected for heat stress tolerance and Nduna with grain yields of 11 221 Kg/ ha respectively. Entry 11 produced the lowest grain yield of 6 486 Kg/ ha.

Table 9: Means of Traits of 18 wheat genotypes in two Environments

Genotype	GY (Kg/ha)		SS		SL (cm)		DF (days)		TIL		FLA	
	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2
Entry 10	8 782	8528	20.32	19.80	12.29	11.79	79.25	65.75	458.0	428.9	33.87	26.54
Entry 11	6 486	5674	20.28	19.43	10.19	9.66	78.25	66.50	542.8	315.4	23.51	19.20
Entry 13	11 221	6253	17.70	16.50	10.34	10.03	79.00	65.00	489.2	347.5	25.08	18.55
Entry 15	7 589	5209	21.08	18.40	9.99	9.71	83.00	69.50	638.8	263.5	22.55	22.83
Entry 20	10 715	9120	20.28	19.63	10.47	9.86	76.50	68.25	630.8	499.0	24.13	22.30
Entry 27	8 551	7620	17.60	17.20	10.99	10.91	79.75	67.50	460.2	411.0	23.98	22.30
Entry 41	10 279	8509	18.22	17.90	10.52	9.82	67.00	56.25	555.0	452.2	23.57	21.62
Entry 44	9 996	8586	20.00	19.63	10.45	10.05	69.50	58.00	474.0	412.8	22.76	21.45
Entry 45	7 619	7157	20.48	19.10	10.18	9.81	77.00	64.25	460.0	367.8	19.14	19.35
Entry 46	7 141	6621	20.80	19.43	10.12	9.60	75.50	63.00	470.0	341.5	20.53	22.79
Entry 47	7 969	6919	20.82	18.97	10.21	9.75	75.50	64.00	423.0	357.2	21.55	18.51
Mampo	9 352	7339	20.05	19.15	11.86	10.50	73.00	59.75	528.5	426.0	25.90	18.56
Nduna	10 948	8146	20.35	19.25	11.76	10.60	73.00	59.00	599.2	442.8	24.87	24.86
Pungwa	10 409	8563	20.82	20.03	11.16	10.29	73.00	59.75	557.0	448.5	25.08	22.00
Sahai I	8 552	6796	20.85	17.68	10.55	9.75	76.25	60.75	462.2	397.0	25.12	17.29
Unza I	10 195	7064	20.55	16.95	10.86	10.45	76.75	62.00	488.0	348.0	24.49	23.60
Unza II	10 202	7159	20.42	19.88	10.40	10.14	75.50	61.50	559.2	411.2	25.06	18.3
Lorrie II	12 204	6528	21.90	19.88	11.20	10.50	75.75	60.50	616.8	307.2	26.32	19.44
LSD (5%)	**305.9**	**272.7**	**0.41**	**0.80**	**0.42**	**0.41**	**1.48**	**2.00**	**27.02**	**13.45**	**1.76**	**0.93**

KEY: GY: Grain yield; SL: Spike length; SS: Spikelets/spike; DF: Days to 50% flowering; TIL: Number of tillers/m²; FLA: Flag leaf area; E_1 : optimal environment; E_2 : Stress environment

Table 9: Continued: Means of Traits of wheat genotypes in two Environments

Genotype	CT (^0C)		CTD (^0C)		PHT (cm)		BM (g)		HI (%)	
	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2
Entry 10	23.42	26.53	2.23	7.39	107.07	94.82	79.23	63.46	44.73	42.21
Entry 11	24.36	27.34	1.56	5.07	95.80	80.12	64.97	43.72	47.95	43.45
Entry 13	23.86	27.73	2.10	5.48	98.07	80.67	100.58	63.62	44.23	34.10
Entry 15	24.08	26.96	1.77	5.23	107.37	91.87	66.27	58.43	35.72	32.24
Entry 20	22.30	26.64	3.43	7.48	102.15	85.80	71.68	49.30	54.79	45.18
Entry 27	23.78	27.05	2.11	6.03	101.92	89.00	77.26	58.86	38.22	40.33
Entry 41	23.52	27.69	2.33	6.90	100.07	94.47	75.05	60.91	47.17	45.23
Entry 44	23.86	26.98	2.03	7.07	92.70	79.92	74.51	55.87	50.12	43.60
Entry 45	24.60	25.94	1.28	6.99	95.22	82.12	76.97	55.14	47.08	43.81
Entry 46	24.47	26.68	1.35	7.18	93.10	82.90	59.73	52.49	50.82	43.71
Entry 47	22.89	25.76	2.84	7.22	97.62	79.10	74.25	52.70	49.03	43.51
Mampo	23.34	27.95	2.52	6.12	97.50	88.47	80.38	55.44	48.15	44.16
Nduna	23.50	26.17	2.40	6.62	98.92	83.05	84.65	50.44	44.48	35.99
Pungwa	22.20	26.33	3.34	7.18	102.57	85.70	86.75	52.77	45.14	43.71
Sahai I	24.37	26.36	1.54	5.86	98.60	90.00	72.27	53.56	51.15	38.23
Unza I	23.36	27.21	2.55	6.90	98.90	87.00	67.99	55.86	46.70	42.47
Unza II	23.84	27.27	2.05	6.38	96.82	84.57	74.52	52.35	48.79	45.72
Lorrie II	22.63	27.57	3.30	5.69	99.60	87.65	84.03	41.84	49.76	42.80
LSD (5%)	**0.98**	**0.71**	**0.86**	**1.63**	**1.74**	**3.31**	**2.34**	**4.04**	**1.38**	**1.36**

KEY; CT: Canopy temperature; CTD: Canopy temperature depression; PHT: Plant height; BM: Above ground biomass; HI: Harvest index; E_1 : Optimal Environment; E_2; Stress Environment

Under heat stress environment the average grain yield was 7 322 Kg/ha and thereby recording 21.65% yield reduction as compared to optimal environment. Entry 20 was the most outstanding genotype with the highest grain yield of 9 120 Kg/ha giving a yield advantage of 24.56% above the overall mean of 7 322 Kg/ha and was followed by Entry 44, Pungwa and Entry 10. Entry 15 produced the lowest grain yield of 5 674 Kg/ ha.

The check variety Lorrie II and Entry 13 showed very high grain yield reduction of up to 46.51% under heat stress environment while Entry 10 exhibited the least yield reduction of up to 2.89%. Based on percentage yield reduction under stress, Lorrie II and Entry 13 were heat sensitive genotypes while Entry 10 and Entry 20 were heat tolerant. Entry 10 and Entry 20 showed high number of tillers and high canopy temperature depression and these could be some of the factors contributing to their superior performance under heat stress. These two genotypes have also been selected for heat tolerance. In this study, Pungwa and Nduna have also recorded high grain yields under heat stress even though they have not been selected for heat stress tolerance. These yield reductions among wheat genotypes under heat stress indicate that the environment exhibited significant influence on grain yield and other parameters.

4.5.1 Effect of Environment on Yield and other parameters

Table 10 shows the depressing effect of delayed planting (heat stress environment) on yield and other parameters. In this study the planting date or environment had a significant influence on yield and most of parameters except for spike length and number of spikelets. These parameters exhibited differential depression in response to heat stress environment emanating from delayed planting. Accordingly, wheat genotypes showed 21.65% grain yield reduction under heat stress and this translated to a yield decrease of 2023 Kg/ha.

The above ground biomass was the most sensitive parameter to heat stress presenting the highest trait reduction of 28.75%. This was followed by the number of tillers per unit area which recorded a decrease of 25.87%. The above ground biomass and tillers are important yield components whose decrease could have impacted negatively on grain yield. Spike length exhibited the lowest percentage reduction of 0.57% under stress conditions. This would imply that the spike length was less sensitive to heat stress (Table 10).

Table 10: Effect of Environment on yield and other parameters

Parameter	Normal	Stress	% Reduction
Grain Yield (Kg/ha)	9 345	7322	21.65
Days to Flowering	75.75	62.82	12.90
Spike Length (cm)	10.75	10.18	0.57
Spikelets/spike	20.14	18.82	6.55
Tillers/m²	522.9	387.6	25.87
Stay green duration	107.83	94.74	13.08
Flag Leaf Area	24.31	21.08	3.29
Plant Height (cm)	99.11	85.99	13.24
Biomass (g)	76.17	54.27	28.75
Harvest Index (%)	46.89	41.69	11.09
Thousand Kernel Wt (g)	50.22	41.37	17.62

4.6 Strength of Association among Traits

The strength of association between grain yield and morpho-physiological traits in a heat stressed environment was determined using simple correlation analysis. The results are shown in Table 11. The results of this study showed positive and significant correlation between grain yield and other traits. Thus, strong and positive correlation were revealed for grain yield with canopy temperature depression (r = 0.79**); chlorophyll stability index (r = 0.71**); membrane thermostability (r = 0.682**); tillers/m² (r = 0.78**); harvest index (r = 0.49**); flag leaf area (r = 0.41*); spikelets per spike (r = 0.46**); plant height (r = 0.53**); biomass (r = 0.38**). The chlorophyll stability, canopy temperature depression, membrane thermostability and number of tiller per square showed very strong and positive association with grain yield in this study and could be considered as indirect selection criteria for yield under heat stress.

Table 11: Simple Correlation between Yield and Morpho-physiological Traits

Trait	Correlation
Spike Length	0.48**
Plant Height	0.53**
Canopy Temperature Depression	0.79**
Canopy Temperature	-0.16*
Membrane Thermostability	0.68**
Flag Leaf Area	0.41 **
Days to 50% Flowering	0.35**
Tillers/ m²	0.78**
Spikelets/ spike	0.46*
Biomass	0.38**
Harvest Index	0.49**

*= Significant at P≤ 0.05 ** = Significant at P≤ 0.01

However, canopy temperature showed negative and significant correlation with grain yield. This means that grain yield under stress in this study was greatly influenced by these morpho-physiological traits.

There were also strong correlations among traits observed in this study. Canopy temperature depression was positively correlated with CSI (r= 0.671**); MTS (r=0.739**); flag leaf area (r= 0.426*); tillers/m² (r= 0.596**); harvest index (r= 0.524**); spikelet/spike (r = 0.294*). Positive correlations were observed between CSI and MTS (r = 0.737**); CSI and harvest index (r=0.599**). Correlation coefficients are presented in the Table 12.

Table 12: Association among Traits under heat stress environment

Par	GY	CTD	CT	CSI	MTS	FLA	TIL	SS	HI
GY	1	0.792**	- 0.211*	0.712**	0.682**	0.412*	0.783**	0.276*	0.485**
CTD		1	-0.407*	0.671**	0.739**	0.426*	0.596**	0.296*	0.524**
CT			1	-0.159	-0.311*	- 0.165	- 0.149	-0.245*	-0.012
CSI				1	0.737**	0.30*	0.567**	0.141	0.599**
MTS					1	0.34*	0.734**	0.189	0.431*
FLA						1	0.226*	0.108	0.123
TIL							1	0.198	0.397*
SS								1	0.426*
HI									1

* = Significant (P≤0.05), **=Significant (P≤0.01)

KEY: **GY**: Grain yield; **CTD**: canopy temperature depression **CT**: canopy temperature; **CSI**: Chlorophyll stability index; **MTS**: Membrane thermostability; **FLA**: Flag leaf area; **TIL**: Tillers/m²; **SS**: Spikelets/spike; **HI**: Harvest Index. **Par**: Parameter.

The simple correlation between grain yield and morphological traits also indicated positive and significant relationship apart from canopy temperature which exhibited a negative and significant correlation with yield even under combined analysis (Table13).

Table 13: Correlation between Grain yield and other traits across Environments

Trait	Correlation
Plant Height	0.51**
Canopy Temperature Depression	0.76**
Canopy Temperature	- 0.21*
Days to 50% Flowering	0.35**
Harvest Index	0.49**
Flag Leaf Area	0.41*
Tillers/ m	0.74**
Spike Length	0.30*
Spikelets / spike	0.28*
Biomass	0.38**

* = Significant (P≤ 0.05) ** = Significant (P≤ 0.01)

4.7 Relationship of Yield and Morpho - physiological Traits

Correlation is a measure of association and does not provide the cause and effect relationship. The cause and effect relationship identifies the components that explain most of variation in the yield. Once these components are identified they could be used as indirect selection criteria to improve the performance of wheat under high ambient temperatures. In order to identify such components a stepwise multiple regression analysis was used, taking yield as a dependent variable and morpho-physiological traits as independent variables under heat stressed environment.

This analysis identified the component that explained most of variations in yield. According to this model membrane thermostability explained most of the variation in yield with a multiple coefficient of determination $R^2 = 80.7\%$. The addition of the number of tillers per square metre to the model contributed 90.9% of variation in grain yield. When canopy temperature depression was considered as the third parameter they collectively contributed 93.3% of variation in grain yield (Table 14).

Table 14: Coefficients of Determination of Yield

Variable	Coefficient of Determination (R^2)
MTS	80.7%
MTS; TIL	90.9%
MTS; TIL; CTD	93.3%
MTS; TIL; CTD; DF	93.9%
MTS; TIL; CTD; DF; SGD	94.8%

KEY: **MTS**: Membrane thermostability; **TIL**: Tillers/m^2; **CTD**: canopy temperature depression; **DF**: Days to 50% Flowering. **SGD**: Stay green duration;

Addition of a fourth parameter to the model (days to 50% flowering) they contributed 93.9% of the variation in yield. Inclusion of stay green as a fifth parameter explained 94.8% of variations in grain yield. Further addition of other traits did not amount to significant contribution to the total variation and were not included in the model (Table 14).

Considering the contribution of each trait to grain yield under heat stress environment, the number of tillers per square metre explained 10.2% of the variation; canopy temperature depression showed 2.4%; days to 50% flowering indicated 0.6% of variation and 0.8% emanated from stay green duration. Therefore, membrane thermostability, tillers per square metre and canopy temperature depression were the most important parameters explaining variation in grain yield and could be used as indirect selection under heat stress (Table 15).

Table 15: Stepwise multiple Regression of Yield on Morpho-physiological Traits

Variable	Partial Square	R-Model Square	R-F-Value	Pr > F
MTS	0.807	0.807	292.27	0.000
TIL	0.102	0.909	77.89	0.000
CTD	0.024	0.933	24.341	0.000
DF	0.006	0.939	6.572	0.000
SGD	0.008	0.948	10.575	0.000

KEY: **MTS**: Membrane thermostability; **TIL**: Tillers/m²; **CTD**: canopy temperature depression;

DF: Days to 50% Flowering. **SGD**: Stay green duration;

4.8 Heat Stress Indices

Table 16 shows heat indices for eighteen which have been calculated based on grain yields obtained in two environments. The eighteen wheat genotypes used in this study showed significant differences (P<0.001) for grain yield in two environments. The stress susceptibility index (SSI) was proposed by Fisher and Maurer (1978). The geometric mean and stress tolerance index were proposed by Fernandez (1992). According to Sareen et al, 2012, these indices are still important in differentiating genotypes with stress tolerance and susceptibility.

In this study Lorrie II recorded the highest grain yield of 12 204 Kg/ha under optimal environment followed by genotypes Entry 13, Nduna and Entry 20; while Entry 11 produced the lowest yield (6 486 kg/ha). Under stress conditions Entry 20 had the highest yield of 9120 Kg/ha and was followed by genotypes Entry 44, Pungwa and Entry 10. The lowest grain yield in stress environment was recorded in Entry 15 of 5 209 Kg/ ha (Table 16).

Wheat genotypes expressed variability in stress indices. Accordingly, the highest heat susceptibility index (HSI) was recorded in the check Lorrie II (2.15) and this means that it was a very sensitive genotype to heat stress. This assertion was reflected by the check variety's greatest yield reduction under stress environment. Lorrie II was followed by Entry 13 with a stress susceptibility index of 2.04. On the other hand, Entry 10 was regarded as a less sensitive genotype to heat stress with the lowest stress susceptibility index of 0.13. Entry 20 recorded the highest mean production (MP) across the environments and provided a yield advantage of 15.97% above the overall mean. Entry 20 also exhibited the highest heat tolerant index of 1.12 portraying a suggestion that it was the most heat tolerant genotype (Table 16).

Table 16: Heat Stress Indices of Wheat genotypes

Genotype	Yield (Kg/ha)		Heat Stress Indices			
	Normal	Stress	MP	GMP	SSI	HTI
Entry 10	8 782	8 528	8655	8654	0.13	0.86
Entry 11	6 486	5 674	6080	6066	0.58	0.42
Entry 13	11 221	6 253	8737	8376	2.04	0.80
Entry 15	7 589	5 209	6399	6287	1.45	0.45
Entry 20	10 715	9 120	9917	9885	0.69	1.12
Entry 27	8 551	7 620	8085	8072	0.50	0.75
Entry 41	10 279	8 509	9394	9352	0.80	1.00
Entry 44	9 996	8 586	9291	9264	0.65	0.98
Entry 45	7 619	7 157	7388	7384	0.28	0.62
Entry 46	7 141	6 621	6881	6876	0.34	0.51
Entry 47	7 969	6 919	7444	7425	0.61	0.63
Mampo	9 352	7 339	8346	8285	0.99	0.79
Nduna	10 948	8 146	9547	9444	1.18	1.02
Pungwa	10 409	8 563	9486	9441	0.82	1.02
Sahai I	8 552	6 796	7674	7624	0.95	0.67
Unza I	10 195	7 064	8630	8486	1.42	0.82
Unza II	10 202	7 159	8680	8546	1.38	0.84
Lorrie II	12 204	6 528	9365	8925	2.15	0.92

Key: **MP**: Mean Productivity= (xp $_+$ xs)/2; **GMP**: Geometric Mean Productivity= √ (xp x xs)
HSI: Heat Susceptibility Index = [1- (xs/xp)] / [1-(Y_S/Y_P); **HTI**: Heat Tolerance index= (xs x xp)/ $(Y_p)^2$

Where, **xs** and **xp** indicate genotypic yield under stress and optimal conditions (respectively);
Ys and **Yp** are mean yields of all the genotypes under stress and non-stress conditions.

4.9 Comparative Performance of Heat Tolerant Versus Other Genotypes

This study comprised two categories of genotypes. These were the heat tolerant and non heat tolerant selection. The heat tolerant category included Unza I and Unza II, while the non-heat tolerant included the check variety Lorrie II. Figure 2 illustrates the comparative performance of these two groups of genotypes used in this study. From this Figure, the heat tolerant genotypes had a slight edge over the other genotypes under the depressing effect of the environment. The genotype Pungwa exhibited some attributes of heat tolerance though it has not been specifically selected for heat stress tolerance. The performance of the check variety Lorrie II was negatively affected under heat stress environment.

The difference in performance between these classes of genotypes could be attributed to morpho-physiological parameters which were superior in heat tolerant category. Notably, the heat tolerant genotype recorded an average canopy temperature depression of 6.56°C while the other class recorded 5.16°C. The heat tolerant genotypes also registered an average chlorophyll stability index of 60.76%, while the non-heat tolerant genotypes recorded an average of 55.32% (Table 17).

Table 17: Averages for Three Parameters under heat stress

Parameter	HTG	NHT
CTD	6.56° C	5.16 °C
BM	55.59 g	50.81 g
CSI	60.76%	55.32%

KEY: CTD: Canopy temperature depression**; BM:** Above ground biomass**; CSI:** Chlorophyll stability index **HTG:** Heat tolerant genotypes**; NHG:** Non-Heat tolerant genotypes

These attributes suggest that the heat tolerant genotypes had cooler canopies and had more chlorophyll under heat stress which were important for the process of photosynthesis and ultimately grain yield.

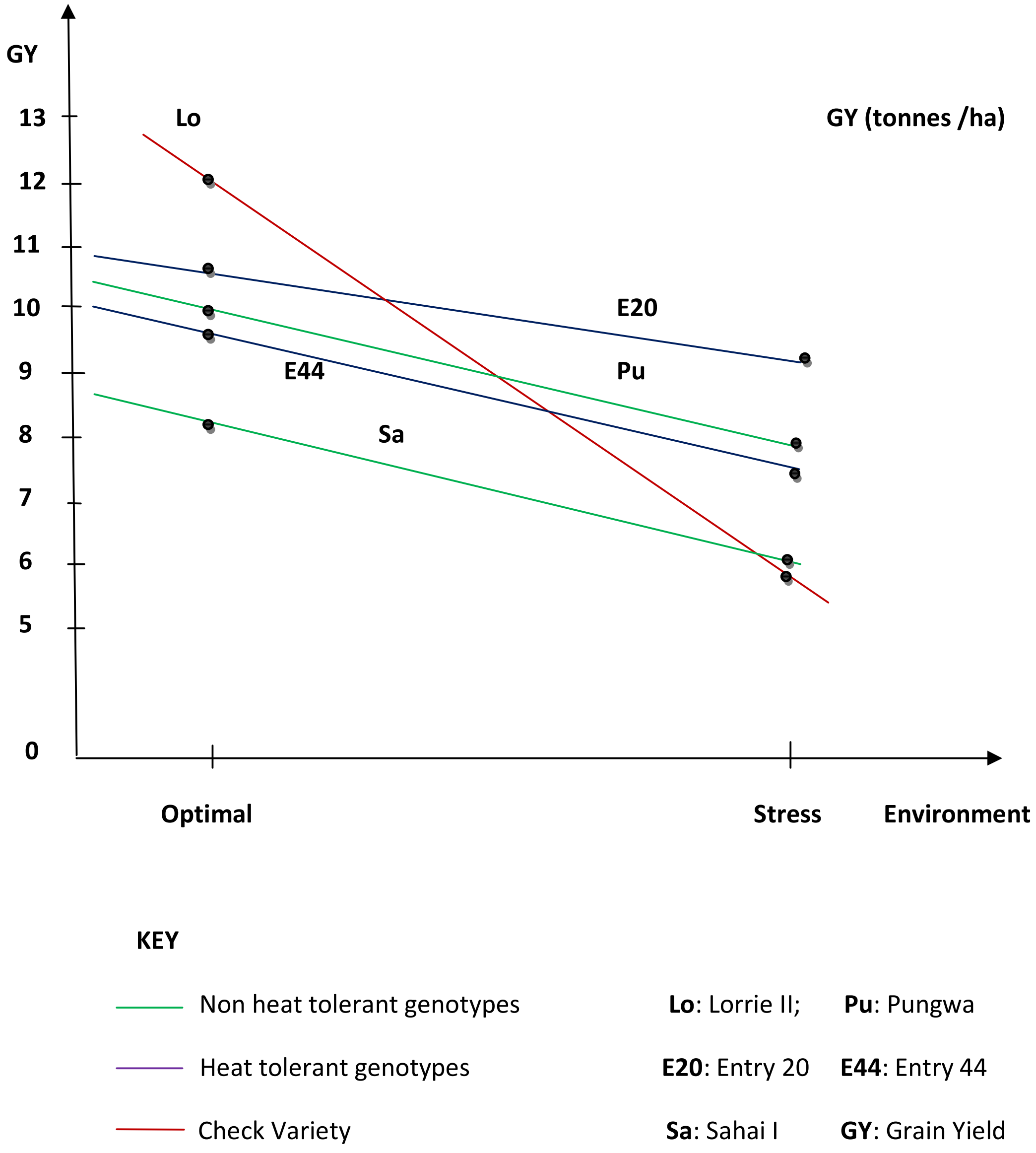

Figure 2: Comparison of Heat Tolerant versus Non Heat Tolerant Wheat genotypes

CHAPTER FIVE

5.0 DISCUSSION

Heat stress is a major limitation to wheat productivity in environments subjected to high ambient temperatures. Exposure of wheat to temperatures higher than the optimum reduces grain yield and quality (Ashraf and Harris, 2005). This reduction in grain yield is happening at a time when the demand for wheat products is increasing. Consequently the development of heat tolerant cultivars is of major concern in wheat breeding programmes. The success of this undertaking requires a detailed understanding of morpho-physiological traits associated with heat tolerance and provide information upon which selection would be based on. It is against this background that this study was conducted.

Based on this premise, the analysis of variance in this present study revealed significant differences for all parameters under investigation thereby indicating presence of substantial genetic variation among eighteen wheat genotypes for all traits measured in both environments. These findings corroborate the earlier reports of Kumar et al, 2003; Shankarrao et al, 2010; and Sareen et al; 2012. The average performances of genotypes for all parameters under study exhibited a general decline in stress environment. This decline is attributed to heat (high temperature) stress which imposed substantial effects on all the characters studied, varying with the genotypes and characters. These differences are essential in wheat improvement programmes under high ambient temperatures. Similarly, the decline in average performances of wheat genotypes under heat stress has been reported by Ubaidullah et al, (2006); Bahar et al, (2011) and Kumar et al, (2012).

Plant height and above ground biomass were considerably reduced under heat stress environment by up to 28.75%. Thus, all genotypes used in the present study showed shorter heights and produced less biomass in comparison to their optimal environment. The decrease in plant height may have occurred due to shortening of growth and photosynthetic period imposed by heat stress in a late sown environment. These results coincide with the findings of Mishra et al, (2000); Ubaidullah et al, (2006) who observed significant negative effects of delayed planting on plant height and biomass in wheat. Interestingly, the taller genotypes in this study like Entry 10 and Entry 41 under stress environment have been associated with production of high above ground biomass and high grain yield. The correlation coefficient between grain yield and above ground biomass was weak (r = 0.36) but positive and significant. This provides an indication that above ground biomass and plant height could be used together with other parameters that exhibit a strong and positive correlation with grain yield when breeding programmes for heat tolerant wheat cultivars.

Studies have revealed that tillering capacity and leaf area are determined by wheat genetic constitution and growing conditions (Reynolds et al, 2007), and are strongly influenced by environmental conditions like heat stress (Ashraf and Harris, 2005). In this study, a reduction in tillers per square metre of 25.87% and a 13.29% decline in leaf area were observed. A decrease in tillers implied a reduction in the number of wheat heads produced which had a negative subsequent effect on grain yield. In this study, genotypes with high number of tillers per unit area and large leaf area like Entry 20 and Entry 10 produced high grain yields under high ambient temperatures. Conversely, Entry 15 recorded the lowest number of tillers per square metre (263.5) and produced the lowest grain yield among wheat genotypes under evaluation (Table 6). The correlation coefficient values of these traits with grain yield were positive and significant. The relationship with grain yield was weak for flag leaf area (r = 0.412) and strong for tillers per square metre (r = 0.78) under stress environment. This strong association of the number of tillers per unit area with grain yield suggest that the number of tillers/m² could be used as indirect selection criteria for grain yield under heat stress conditions.

According to Hansan et al, (2007) heat stress speeds up the development of spikes and thereby reducing spike length and the number of spikelets per spike. In this study the spike length and the number of spikelets per spike varied with the genotype in each environment. The environment imposed non significant influence on spike length and on the number of spikelets per spike. On the other hand the genotype x environment interaction showed non-significant effect only on spike length. A reduction in these two yield components has been observed with spike length recording a mean reduction of 0.57% while spikelets per spike showed 6.55% reduction. These yield components showed positive and significant correlation with grain yield. This study observed that Entry 10 had the maximum spike length of 11.79 cm under stress conditions and also recorded the lowest stress susceptibility index of 0.13 and high grain yield. These results suggest that spike length and spikelets per spike could be used as indirect selection criteria for yield with other parameters like number of tillers per unit area even under high ambient temperatures.

Harvest index has been reported as a key parameter for crop yield predictions since it determines the physiological efficiency of the crop to mobilise photosynthates and transport it to organs of economic value (Mushtaq et al, 2011). It is influenced by genotypic and environmental conditions. In this study heat stress declined the mean value of harvest index by 11.09% with Unza II recording the highest harvest index of 45.27% and was followed by Entry 41 and Entry 20 under heat stress. On the other hand Entry 15 recorded the lowest harvest index and grain yield. This present investigation also showed a highly positive significant correlation between grain yield and harvest index ($r = 0.485$; $P \leq 0.01$). These results are similar with the findings of Bahar et al, (2011). These results provide a suggestion that harvest index could be used as an indirect selection criterion for grain yield under heat stress environments.

The duration to 50% flowering and thousand kernel weight, under stress environment, also declined by 12.9% and 17.29% respectively. In this study all genotypes in stress environment recorded early heading due to accelerated plant growth in comparison to optimal environment. These results are in agreement with those of Ubaidullah et al, (2006) who reported genetic differences among wheat genotypes for days to heading and thousand kernel weight, and a reduction in these traits under heat stress.

Plant responses to heat stress also include physiological changes. In recent years researchers have linked physiological responses of plants to elevated temperatures with their tolerance mechanisms. This has paved way for physiological breeding approach which aims to combine traits associated with all three drivers of yield (light interception, radiation use efficiency and partitioning of assimilates) to result in a cumulative genetic effect on yield (Cossani and Reynolds, 2012). Traits for which there is reasonable evidence include cool plant canopy (Elbashier et al, 2012); membrane thermostability (Kumar et al, 2012); stay green duration (Yildirim et al, 2009) and high chlorophyll stability index (Sharifi et al, 2012). These physiological traits provide a gain in wheat productivity under high temperatures and could be used as indirect selection criteria for grain yield under heat stress environments.

Lopes and Reynolds (2012) have reported that the green area displayed by a crop is a good indicator of its photosynthetic capacity, while chlorophyll retention or ‘stay green’ is regarded as a key indicator of stress adaptation. Thus stay green genotypes are able to maintain grain filling under elevated temperatures and produce consistently high grain yield. In this study wheat genotypes have shown significant differences among them for stay green (P< 0.001) in both environments. The environment and genotype x environment interaction both exhibited significant effect on stay green duration character. Entry 10, Unza I, Entry 27 and Pungwa recorded the highest stay green duration of up to 97.75 days in stress conditions. This means that these genotypes with longer stay green capacity could have utilised available resources efficiently and had higher photosynthetic efficiency during grain filling period under heat stress condition. Furthermore wheat genotypes have shown a reduction in stay green duration value under stress of up to 13.08% with a positive and non significant correlation of r= 0.26 with grain yield. Similar observations were made by Bahar et al, (2011) who observed a positive non-significant correlation (r = 0.332) between grain yield and stay green duration among spring wheat genotypes in Turkey.

Genetic variability in canopy temperature among wheat genotypes under high ambient temperatures exists and cool canopy temperatures have been associated with high grain yield (Elbashier et al, 2012). In this study, genotypes exhibited significant differences with regards canopy temperatures under both environments. Contrary to other parameters, CT measurements showed an increase under heat stress conditions, and were negatively correlated with grain yield and other morpho-physiological traits. An increase in canopy temperature and its negative correlation association with other traits under heat stress has also been reported by other researchers (Bahar et al, 2011; Amani et al, 1996). Genotypes with cooler canopies such as Entry 10 and Entry 20 have been associated with high grain yields. Not only did these two genotypes with cooler canopy out performed in stress condition, but canopy temperature could have enhanced greater flag leaf chlorophyll content and higher photosynthetic activity. This may have enhanced dry matter accumulation and high grain yield in these genotypes.

Wheat genotypes in this study exhibited great genetic variability in canopy temperature depression (CTD) and cultivars with high CTD values have been generally associated with high grain yield. Canopy temperature depression measurements in this study have also shown a positive and highly significant correlation with grain yield and other physiological parameters under both environments. These relationships were also observed by other researchers (Bahar et al, 2008 and Elbashier et al, 2012). Entry 20 recorded the highest CTD (7.48°C) and grain yield (9120 Kg/ha) while Entry 11 produced the low grain yield of 5 674 Kg/ ha with lowest CTD (5.07°C) under heat stress environment. CTD showed a strong and positive significant correlation (r = 0.79) with grain yield. Its relationship with most of morpho-physiological traits such was positive. The superior performance of genotypes with cooler canopy like Entry 20 under heat stress could be due to increased stay green duration and high chlorophyll content that enhanced photosynthetic activity.

Canopy temperature depression values vary with crop growth stages. In support of this view, Reynolds et al (1994) reported that CTD average values of bread wheat genotypes were respectively 7.4, 9.0 and 6.5°C before heading, at heading and grain filling period. In this study, CTD measurements were done during grain filling period. Similar average CTD value of 6.49°C was observed for all genotypes under stress conditions. These results provide considerable evidence that CTD could be used as a rapid indirect selection tool for grain yield under heat stress conditions in Zambia.

The stability of chlorophyll under heat stress is very important and it gives an indication of availability of chlorophyll in plants. Thus, high chlorophyll stability index (CSI) helps the plants to withstand stress through availability of chlorophyll and thereby leading to increased photosynthetic rate and more dry matter production (Ananthi et al, 2013). The present investigation has shown significant differences among wheat genotypes for chlorophyll stability index, and genotypes with high CSI have been associated with high grain yield. Furthermore, CSI exhibited a positive significant correlation with grain yield ($r= 0.712$; $P\leq 0.01$). In support of this view, Sharifi et al, (2012) reported genetic variability among wheat genotypes with respect to chlorophyll stability index in stress environment and that genotypes with high CSI were positively associated with high grain yield. The genotype x environment interaction imposed a significant influence on CSI. Canopy temperature depression was strongly and positively correlated ($r = 0.671$) with chlorophyll stability index under heat stress. These present results provide sufficient evidence that chlorophyll stability index could also be used with CTD as an indirect selection criterion for grain yield under heat stress.

Membrane thermostability (MTS) is an important physiological mechanism of heat tolerance in wheat and is responsible for adaptation of plants to high ambient temperatures (Islam et al, 2011). Therefore the plant's ability to maintain membrane integrity and function is what determines tolerance towards heat stress (Abdulla et al, 2011). This is because cell membranes are primarily composed of proteins and lipids and any damages in these two components are likely to affect their structure and function. Membrane thermostability has a reasonable relationship to plant's performance under heat stressed environments and has therefore been considered as a possible selection criterion for grain yield under heat stress (Blum et al, 2001). Many authors have used membrane thermostability test to study the genetics of heat tolerance in wheat (Saadalla et al, 1990a; Balota et al, 1993; Reynolds et al, 1994; Ibrahim and Quick, 2001; Islam et al, 2011; Sharif et al, 2012).

In this study wheat genotypes exhibited significant differences for membrane thermostability under stress and genotypes with high MTS produced high grain yield. Heat tolerant genotypes like Entry10 and Entry 20 which suffered less membrane injury were also coupled with high CTD, CSI and higher grain yield compared to susceptible ones under stress conditions. Low grain yield in susceptible genotypes could be attributed to high temperatures which could have denatured membrane proteins and caused lipid phase transitions; and this did not sustain respiratory and photosynthetic performance in these genotypes (Blum et al, 2001; Yildirim et al, 2009; Islam et al, 2011).

Positive significant correlation between grain yield and membrane thermostability of 0.682 was observed in this study. Yildirim et al (2009) also observed a strong and positive correlation of 0.68 in wheat during grain filling period. The significantly positive correlation between MTS and grain yield during grain filling would be useful to detect intrinsic high yielding heat tolerant genotypes that could be used in wheat breeding programmes. Consistent with these results, Yildirim et al (2009) and Dhanda and Munjal (2012) reported significant positive correlation between MTS and grain yield during grain filling and at anthesis periods respectively. Still in agreement with the present results, Shanahan et al (1990) obtained a significant increase in yield of spring wheat in hot location by selection of membrane thermostable lines as determined by measurements on flag leaves at anthesis.

Heat stress in the present study was severe enough to cause tremendous reduction in grain yield of wheat genotypes. This could be due to the fact that under high temperature conditions plant life cycle was shortened and the duration of all phenological phases were reduced, so plants did not have enough assimilates to translocate to sinks. Similar results were also reported by others workers (Hassan et al, 2007; Dias and Lindon, 2009 and Shankarrao et al, 2010). The wheat genotypes in this study exhibited variable yield performances both under optimal and heat stress conditions (P<0.001). Under optimal conditions Lorrie II produced the highest grain yield while Entry 20 recorded the highest grain yield of 9120 Kg/ha (Table 13). Grain yield in this study exhibited significant positive correlation with most of morpho-physiological traits except for canopy temperature which showed a significant negative correlation.

Heat stress condition decreased grain yield significantly but the amount of reduction was not equal in different wheat genotypes. For instant, Lorrie II exhibited the highest grain yield decrease of up to 46.51 % while Entry 10 showed the lowest yield reduction of 2.89%. However, the average yield decrease for all genotypes under late sown (stress) environment was 21.65%. The present average yield reduction falls within the range of 20 - 30% reported by Sareen et al, 2012. These workers reported that heat stress resulting from a delay in sowing by one month can lead to about 20-30% loss in grain yield depending on climatic conditions.

In this study the heat tolerant genotypes like Entry 10, Entry 20 and Unza II exhibited a slight edge under heat stress conditions over heat sensitive genotypes like Lorrie II. The difference in performance between these two classes of genotypes could be attributed to morpho-physiological parameters which were superior in heat tolerant category. Notably, the heat tolerant genotypes recorded an average CTD of 6.56°C while the other class recorded 5.16°C. Also the heat tolerant genotypes registered an average chlorophyll stability index of 60.76% while the sensitive genotypes recorded an average of 55.32%. This implies that the heat tolerant genotypes had cooler canopies and more chlorophyll that enhanced photosynthetic efficiency and dry matter accumulation than heat sensitive genotypes (Barnabas et al, 2008). However, the genotype Pungwa exhibited some attributes of heat tolerance under stress conditions although it has not been specifically selected for heat tolerance.

To elucidate the genetics and physiology of the reaction to high temperature stress in wheat yield based indices have been proposed on heat tolerance. These include mean productivity (MP), geometric mean (GM), and stress susceptibility index (SSI) and stress tolerance index (STI). The heat tolerant index also referred as STI was developed to identify genotypes that perform well under both stress and non-stress conditions (Fernandez, 1992; Fisher and Maurer, 1978; Porch, 2006).

From this study Lorrie II had the highest SSI of 2.15 despite recording the highest grain yield under optimal conditions. This means that Lorrie II with Entry 13 was stress sensitive genotype and this was vindicated by its largest yield reduction of up to 46.51%. Accordingly, Entry 10 was considered as a highly stable and adapted genotype to unfavourable conditions as manifested by its lowest stress susceptibility index of 0.13. Like the latter genotype, Entry 20 was considered as the most heat tolerant genotype with the highest mean productivity, and HTI of 1.12. These results suggest that stress indices such as mean productivity and heat tolerant index could be used as selection criteria for screening wheat genotypes for heat tolerance (Sareen et al, 2012).

In this study, the stepwise multiple regression analysis identified membrane thermostability, number of tillers per square metre, canopy temperature depression, days to 50% flowering and stay green duration as traits with significant contribution to total variation in grain yield. Membrane thermostability explained most of variation in yield with a high coefficient of determination R^2 of 80.7%.

This explanation in yield variation entails that membrane thermostability affected plant performance in this study. This is because cell membranes which are dynamic structures formed essentially by lipids and proteins supports many biophysical and biochemical traits, with emphasis in regulation and transport of ions and enzymatic activity. These permeable selective barriers could have allowed the development of many biological responses, but they were also targets of heat stress (Dias et al, 2009; Islam et al, 2012).

Heat stress might have affected membrane stability in heat sensitive genotypes and thereby increasing the electrolyte leakage as a result of loss membrane selectivity. During grain filling, this effect could have been associated to increased levels of lipid peroxidation (Balota et al, 2004) coupled to higher ethylene synthesis (Dias et al, 2009). This could have subsequently adversely affected leaf chlorophyll content, stay green duration, photosynthetic efficiency and dry matter accumulation in heat sensitive genotypes like Lorrie II. In heat tolerant genotypes like Entry10, Entry 20 and Unza II lipid peroxidation and ethylene levels might not have changed and this could have supported the maintenance of membrane stability that promoted high chlorophyll content, photosynthetic efficiency and dry matter accumulation (Saadalla et al, 1990, Shanahan et al, 1990, Balota et al, 2004).

It is based on this understanding that some researchers have used membrane thermostability test to study heat tolerance in wheat (Ibrahim and Quick, 2001; Reynolds et al, 1994; Islam et al, 2011; Sharifi et al, 2012). In line with this view, Shanahan et al (1990) observed significant increase in yield of spring wheat in hot locations by selection of membrane thermal stable lines, as determined by measurements on flag leaves at anthesis. Also, Islam et al (2011) reported that direct selection for membrane thermostability improved biomass, grain yield, thousand grain weight, canopy temperature depression, grain filling rate and chlorophyll retention in spring wheat genotypes. In this backdrop, membrane thermostability is an important physiological trait that could be used as an indirect selection criterion for grain yield under heat stress conditions in Zambia.

Other traits that showed significant influence in explaining variation in grain yield were tillers per unit area (10.2%), canopy temperature depression (2.4%), days to 50% flowering and stay green duration. High tillering capacity generally results in high ground cover and in more tillers bearing the kernels, and thereby improving the grain yield. Canopy temperature depression showed a strong positive correlation with grain yield in this study. In support of this view, Balota et al, 2008 reported that wheat cultivars with high canopy temperature depression tend to have high grain yield under dry, hot conditions because such cultivars had cooler canopies that enhanced chlorophyll retention, photosynthetic efficiency and high dry matter accumulation. As a result of this, canopy temperature depression has been used as a selection criterion to improve adaptation to drought and heat in wheat.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

The study revealed that exposure of wheat to high ambient temperatures adversely affects morpho-physiological traits and grain yield. However, the negative effect of heat stress was statistically different among wheat genotypes and this suggests availability of genetic variability among wheat genotypes which is an essential component in plant breeding and selection for wheat crop improvement. Morpho-physiological traits in this study exhibited a decline under heat stress. These traits also provided varying reasonable evidence of association with grain yield.

Physiological traits such as canopy temperature, canopy temperature depression, membrane thermostability, chlorophyll stability index and stay green duration have also shown strong correlation with grain yield. Because of this association, these traits constitute the best available ‘handle’ for genetic improvement of wheat suitable for cultivation under heat stressed environments. Thus, they could be used as indirect selection criteria for developing heat tolerant wheat genotypes that would provide sufficient yields to meet the ever increasing wheat demand.

In this study, membrane thermostability, tillers per unit area and canopy temperature depression exhibited significant influence in explaining variations in grain yield. In practical plant breeding the number of tillers per unit area and canopy temperature depression would be the most appropriate and rapid indirect selection criteria for heat stress tolerance in wheat.

Based on heat tolerance indices of genotypes, Entry 20 was the most heat tolerant genotype. Other genotypes with high heat tolerance were Entry 10, Entry 41, Entry 44, Nduna, Pungwa, Unza I and Unza II. These results suggest that these genotypes could be used as sources of genetic material for wheat breeding programmes in heat stressed environments. Also, stress tolerance indices like heat tolerance index and mean productivity could be used as selection criteria to identify heat tolerant genotypes.

Furthermore, it is clear that the morpho-physiological parameters in this study considerably explained some mechanisms which indicate tolerance to heat stress in wheat, as their relevance in describing genotypic variability is significant. This has therefore generated information on mechanisms of heat stress in wheat that could be used in breeding for heat tolerant wheat.

Therefore, this study should be repeated in many locations including hot river valleys of Zambia that are endowed with abundant water resources for irrigation.

REFERENCES

Abdullah F., **F**. **Hareri**, **M**. **Naesan**, **M**.**A**.**Ammar and O**. **Zaherkabar**. **2011**. Effect of Drought on different Physiological Characters and yield components in different varieties of Syrian Durum wheat. Journal of Agricultural science; Vol.3 (3): 127-133.

Al-Khatib K. and Paulsen G.M. 1990. Photosynthesis and productivity during high temperature stress of wheat genotypes from major world regions. *Crop Science* 30:1127-1132.

Amani I., **Fischer**, **R**.**A**, **Reynolds**, **M**.**P**. **1996**. Canopy temperature depression association with yield of irrigated spring wheat cultivars in hot climate. J. *Agron*. *Crop*. Sci. 176(2): 119-129.

Ananthi K, Vijayaraghavan H, M. Karuppaiya and T. Anand. 2013. Drought induced changes in Chlorophyll Stability Index, Relative Water Content and Yield of Cotton Genotypes. Insight Botany, 3: 1-5.

Aquino P., F. **Carrion and** P.**Kosina**. 2009. Selected wheat Statistics. In: Dixon, J., H. Braun, P. Kosina and J. Crouch (Ed). Wheat facts and futures; 2009. Mexico. D.F CIMMYT.

Arnon D I (1949). Copper enzymes in isolated chloroplasts, polyphenoloxidase in *Beta vulgaris*. Plant Physiol., 24: 1-15.

Ashraf M. and, **A**.**S**. **Bhatti**. 1998. Effect of delayed sowing on some parameters of photosynthesis in wheat (*Triticum aestivum* L.). Wheat Information Services. pp 46-48.

Bahar B, **M**. **Yildirim**, **C**. **Barutcular**, **Genc I**. 2008. Effect of canopy temperature depression on grain yield and yield components in bread and durum wheat. Notulae *Botanicae Hort* Agrobotanici Cluj-Napoca, 36(1): 34-37.

Bahar B, Yildirim M, C.Yucel. 2011. Heat and drought resistance criteria in spring bread wheat (Triticum aestivum L.). Morpho-physiological parameters for heat tolerance. Scientific Research and Essays. Vol.(6) pp 2212-2220. Academic Journals.

Barnabas B, Jager K, Feher A. 2008. The effect of drought and heat on reproductive processes in cereals. *Plant Cell Environ* **31**: 11-38.

Barnes, J.D; Balaguer, L: Manrique, E.; Elvira, S. Davison, A.W. 1992. "A reappraisal of the use of DMSO for the extraction and determination of chlorophyll a and b in lichens and higher plants." *Environmental and Experimental Botany*, 32: 85-100.

Battisti D. S. and Naylor R. L 2009. Historical warning of future food insecurity with unprecedented seasonal heat. Science 9: 323 no. 5911 pp 240-244.

Bauer, A., D. Smika, and A. Black. 1983. Correlation of five wheat growth stage scales used in the Great Plains. USDA-ARS, Peoria, III.

Bita, C.E and T. Gerats, 2013. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress crops. Front Plant Sci 2013; 4: 273.

Blum A. **1988**. Breeding for stress environments. CRC. Press, Inc. Boca Raton, Florida

Blum A., N. Klueva and H.T. Nguwen.2001. Wheat Cellular thermo tolerance related to yield under heat stress. *Euphytica*. 117(2): 117-123.

Braun, H.J., Atlin, G. and T. Payne. 2010. Multi location testing as a tool to identify plant response to Global Climate Change. In: Reynolds, C.R.P (Ed). Climate change and crop production CABI, London, UK.

Cossani M.C. Reynolds, M.P. 2012. Physiological Traits for Improving Heat tolerance in wheat. Plant Physiology. Vol. 160(4):1710 – 1718

Curtis, **B**.**C**. **2002**. Wheat in the world. In: Curtis, B.C., Rajaram S., Maipherson H.G. (Eds). Bread wheat improvement and production. FAO Plant Production and Protection Series. p 30 Rome.

Dias A.S. and Lindon, F.C., 2009. Evaluation of grain filling rate and duration in bread wheat and durum wheat under heat stress after anthesis. J.Agron.Crop Sci.195: 137- 147

Dixon J, H.J. Braun, P.Kosina and J. Crouch (Eds). 2009. Wheat facts and future, 2009. Mexico. D.F. CIMMYT.

Easterling D.R and Apps, M. 2005. Assessing the consequences of climate change for food and forest resources: a view from the IPPC. Clim. Change 70: 165-189.

Elbashier E.M.E., I.A.S. Tahir, A.S.I. Saad, A.S. Ibrahim. 2012. Wheat genotypic variability in utilizing nitrogen fertiliser for cooler canopy under a heat stressed environment. African Journal of African Agricultural Research. Vol.7 (3) pp: 385-392.

Farooq M., H. Bramley, A. J. Palta, K.**H**.**M**. **Siddique**. **2011**. Heat Stress in Wheat during Reproductive and Grain-Filling Phases. *Critical Reviews in Plant Sciences*. 30: 1-17.

Fernandez, G.J. 1992. Effective Selection criteria for assessing plant stress tolerance. P 257-270. In: Proceedings of international symposium and Adaption of Vegetables and other food crops in Temperate and water stress. Taiwan.

Fischer R. A. and R. Maurere. 1977. Drought resistance in spring wheat cultivars. I. Grain Yield response. Australian Journal of Agricultural Res. 29: 897-912.

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

Guy C. 1999. Molecular responses of plants to cold shock and cold acclimation. *J. Mol. Microbiol. Biotechnol.* 1 231-242.

Hansan, M.A., Ahmed, J.U., M.M. Bahadur, M.M. Haque and S.Sikder. 2007. Effect of late planting heat stress on membrane thermostability, Proline content and heat susceptibility index of different wheat cultivars. J. Nam. Sci. Foundation Sri Lanka 35 (2):109 -117.

Hansen J., Sato M., R. Reudy. 2012. Perception of climate change. Proc Natl Acad Sci USA.

Harris K., P.K. Subudhi, A. Borrell, D. Jordan, D.Rosenow, H.T. Nguyen, R. Klein and J. Mullet. 2007. Sorghum stay green QTL individually reduce post flowering drought induced leaf senescence. Exp Bot. 58: 327- 338.

Haun, J.R. 1973. Visual quantification of wheat development. Agron. J. 65: 116 – 119.

Hays D.B., J.H. Do, R.E. Mason, G. Morgan, S.A. Finlayson. 2007. Heat Stress induced Ethylene Production in developing wheat grains induces kernel abortion and increased maturation in a susceptible cultivar. Plant Sci.172: 1113- 1123.

Hede A.R., B. Skovmand, M.P. Reynolds, J.Crossa, A. L. Vilhelmsen, O. Stolen. 1999. Evaluating genetic diversity of heat tolerant traits in Mexican Landraces. Genet. Resour. Crop Evol. 46: 37- 45.

Ibrahim, A.M.H., and Quick J.S. 2001. Heritability of heat tolerance in winter and wheat. Crop sci. 4101- 1405.

Islam M.A., Barma, N.D.C., A.M. Hakim and D.K.R. Sarker 2011. Genetic Variability and selection response of Heat Tolerance through Membrane thermostability in spring wheat (*Triticum aestivum* L.) Bangladesh J.Pl. breed. Genet., 23(2): 15-22.

Karimizadeh R. and Mohammadi R. 2011. Association of Canopy temperature depression with yield of durum wheat genotypes under supplementary irrigation and rain fed conditions. AJCS 5(2): 138-146.

Koc M., Baruticular, C. Genc I. 2003. Photosynthesis and Productivity of old and Modern durum wheats in Mediterranean Environment. Crop Sci. 43(6): 2089-2097.

Krattinger, S.G., E.S. Lagudah, W. Spielmeyer, Singh R.P., Huerta-Espino J, McFadden H., Bossolini E., Selter L.L., B.Keller. 2009. A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323: 1360-1363.

Kumar, Salendra, V.K., Dwivedi and N.K. Tyagi. 2003. Genetic variability in some metric traits and its contribution to yield in wheat (Triticum aestivum L.). Progressive Agriculture, 3(1/2): 152- 153.

Kumar U., A.K. Joshi, Kumari M., Paliwal R., S. Kumar, Roder M.S. 2010. Identification of QTLs for Stay green Trait in Wheat (*Triticum aestivum L.*) in the 'Chirya 3'x 'Sonalika' population. Euphytica. 174: 437-445.

Kumar R. R., S. Goswami, S.K. Sharma, K. Singh, K.A. Gagpayle, N.Kumar, G.K.Rai, M.Singh and R. D. Rai. 2012. Protection against heat stress in wheat involves change in cell Membrane stability, Antioxidant Enzymes, Osmolyte, H_2O_2 and Transcript of heat shock protein. International Journal of Plant Physiology and Biochemistry. Vol.4 (4): 83-91.

Large, E.G. 1954. Growth stages in cereals: Illustration of the Feeke's scale. Pl. Path.3: 128-129.

Larkidale J., Hall J.D., Knight M.R., Vierling E. 2005. Heat stress phenotypes of Arabidopsis mutants implicate multiple signalling pathways in the acquisition of thermotolerance. Plant Physiol. 138 882-897.10.1104/ pp. 105.062257.

Lim P .O, Kim H. J, Ham H.G. 2007. Leaf Senescence. *Annu Rev Plant Biol* **58**: 115-136.

Lopes M.S. and Reynolds P.M. 2012. Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. Funct. Plant Biol. 37: 147-156.

Lumpkin, A.T. 2011. Wheat- Global Alliance for improving food security and the livelihoods of the Resource poor in the developing world. Proposal submitted by CIMMYT and ICARDA to CGIAR consortium Board in collaboration with Biodiversity, ICRISAT, IFPRI, ILIRI and IWMI. CIMMYT.

MACO/CSO, 2011. National food balance for Zambia for 2011/2012 Agricultural Marketing Season. Ministry of Agriculture and Cooperatives (MACO)/ Central Statistics Office (CSO). Lusaka.

Maestri E., Klueva N., Perrotta C., Gulli M., Nguyen H.T., Marmiroli N. 2002. Molecular genetics of heat tolerance and heat shock proteins in cereals. *Plant Mol. Biol.*48 667-681.10.1023/A:1014826730024.

Maydup M.L., Antonietta M. Bulamet, J.J, Graciano C, Lopez J.R. Tambussi E.A. 2010.The contribution of ear photosynthesis to grain filling in bread wheat (*Triticum aestivum* L.). Field Crops Research; 119:48-58

Mishra, D.K., R.A. Khan and M.S. Baghel.2000. Stability of wheat varieties under various dates of sowing. Ann. Agric .Res. 21: 564-566.

Mitra, R. and Bhatia C.R. 2008. Biogenetic cost of heat tolerance in wheat crop. Curr. Sci. 94: 1049-1053.

Modhej, A. and B. Behdarvandi. 2006. Effect of heat stress after anthesis on source limitation of wheat and barley genotypes. 24th Annual Meeting of ESCB, Belgium. P28.

Mohammadi T.M., M. Amin, F.E.S. Khan and A.J. Khan. 2008. Identification of Traits in bread wheat genotypes (*Triticum aestivum* L.) contributing to grain yield through correlation and Path coefficient Analysis. Pak. Bot. 40(6): 2393- 2402.

Mohammadi M., Karimizadeh and M. Abdipour. 2011. Determination of Best Heat Stress Tolerant genotypes under supplementary irrigated and rain fed conditions. AJCS 5(2); 138-146.

Muleoki, P. 1997. Wheat (*Triticum aestivum* L.)and Barley (*Hordeum vulgare* L.) In: Muleokela, S.W.(Ed). Zambia Seed Technology Handbook. Ministry of Agriculture, Food and Fisheries; pp 154-158.

Muller, J., 1991. Determination of leaf area by means of linear measurements in wheat and triticale (Brief report). Arciv. Fuchtungsforschung, 21: 121-123.

Munjal, R. and R.K. Rena. 2003. Evaluation of Physiological Traits in wheat (*Triticum aestivum* L.) for terminal high temperature tolerance. In: Proceedings of the Tenth International Wheat Genetics Symposium, Classical and Molecular Breeding, Poestum, Italy, 2(3): 804-805.

Murthy, K.S. and Majumdar, 1962. Modification of techniques for determination of chlorophyll stability index in relation to drought studies in rice. Curr. Sci. 31: 470-471.

Mushtaq, T., S. Hussain, M.A. Bukhsh, J. Iqbal and T. Khaliq. 2011. Evaluation of two wheat genotypes performance of wheat under drought conditions at different growth stages. Crop and Environment 2(2): 20-27.

Nagarajan S. Jagadish S., Prasad A., Anand A., Pal M et al. 2010. Local climate affects growth, yield and grain quality of aromatic and non- aromatic rice in north-western India. *Agric. Ecosyst. Environ.*138 274-281.10.1016/ j.agee.2010.05.012.

Nikulopoulos, D., C. Korgiopoulos, K. Mavropous, G. Liakopoulos, G.Karabourniotis. **2008**. Leaf Anatomy affects the extraction of photosynthetic pigments by DMSO. Talanta, 76: 1265- 1268.

Nouri A., A. Etminan, A. Jaime, S. **Teixeira and M. Mohammadi. 2011**. Assessment of Yield, Yield-related traits and drought tolerance of durum wheat genotypes (*Triticum turjidum var durum Desf)* Aust. J. Crop Sci. 5; 8-16.

Pinto, R.S., M.P. Reynolds, K.L.Matthews, C.L.Mcintyre, J.Olivares-Villegas, S.C.Chapman. **2010.** Heat and drought adaptive QTL in wheat population designed to minimise confounding agronomic effects. Theor Appli. Genet 121: 1001-1021.

Porch, T. G. 2006. Application of Stress Indices for Heat Tolerance Screening of Common Bean. J. Agronomy and Crop Science 192, 390 – 394.

Rad, M.R.N, Kadir, M.A, Yusop, M.R. **2012**. Genetic behaviour for plant capacity to produce chlorophyll in wheat (Triticum aestivum L.) under drought stress. AJCS 6(3): 415-420.

Reynolds, M.P., Balota M, Delgado M.I.B, Amani, I. Fisher, R.A. 1994. Physiological and morphological traits associated with spring wheat yield under hot irrigated conditions. Aust J Plant Physiol.21: 717- 730.

Reynolds, M.P., S. Nagarajan. M.A.Razzaque and O. A. A. Ageeb. 2001. Heat Tolerance. In: Reynolds, M.P., Ortz-Monasterio, J.I., McNab, A. (Eds). Application of Physiology in wheat breeding. Mexico, D.F. CIMMYT pp. 124-135.

Reynolds, M.P., Pierre C.S., Saad A.S.I., M. Vargas, A.G. Condon. 2007. Evaluating potential genetic gains in wheat associated with stress adaptive trait expression in elite genetic resources under drought and heat stress. Crop Sci. 47: S-172-S- 189.

Reynolds, **M.P. and R. Tuberosa. 2008**. Translational research impacting on crop productivity in drought prone environments. Curr opin Plant Biol 11: 171- 179.

Reynolds, M.P., Y. Manes, A. Izanloo, Langridge, P. 2009. Phenotyping approaches for physiological breeding and gene discovering in wheat. Ann Appl Biol 155: 309-320.

Reynolds, M.P., Hays, **D. and S. Chapman. 2010**. Breeding for adaptation to heat and drought stress. In: Reynolds, CRP (Eds). Climate Change and crop production, CABI, London, UK.

Richards, R.A. and Lukas, Z. 2002. Seedling vigour in wheat: sources of variation for genetic and Agronomic improvement. Aust J. Agric Res 53: 41-50.

Saadalla, M.M.; J.S. Quick and J.F. Shanahan. 1990a. Heat tolerance in winter wheat: II. Membrane thermostability and field performance. Crop Science 30: 1243- 1247.

Saidi Y., Finka A., Muriset M., Bromberg Z., Weiss Y.G., Maathuis F. J.M., et al. 2009. The heat shock response in moss plants is regulated by specific calcium- permeable channels in plasma membrane. *Plant cell* 21 2829-2843.10.1105/ tpc.108.065318.

Sairam R.K., Srivastava, G.C. and Saxena, D.C. 2000. Increased antioxidant activity under elevated temperatures; a mechanism of heat tolerance in wheat genotypes. Biol. Plant 43: 245-251.

Sairam R K, Deshmukh P S, Shukla D S. 1997. Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. J. Agronomy Crop Sci. 178: 171- 178.

Sareen, S., B.S. Tyagi, V. Tiwari, and Sharma I. 2012. Response Estimation of Wheat Synthetic lines to Terminal Heat Stress using stress indices. Journal of Agricultural science. Vol. 4: 97-104.

Semenov, M.A. 2009. Impact of climate change on wheat in England and Wales. J.R. Soc. Interface 6: 343-350.

Semenov, M.A. and Halford, N.G. 2009. Identifying target traits and molecular mechanisms for wheat breeding under a changing climate. J Exper. Bot. 60:2791- 2804.

Shanahan,J.F, Edwards I.B., Quick J.S. and Fenwick J.R. 1990. Membrane Thermostability and heat tolerance of spring wheat. *Crop Sci*. **30**: 247-251

Shankarrao, B.C., S. Mukherjee, A.K. Pal and D.K. De. 2010. Estimation of variability for yield parameters in Bread wheat (Triticum aestivum L.) grown in Gangetic West Bengal. Electron Journal of plant breeding. 1(4): 764-768.

Sharifi P S, Amirnia R, Majidi E, H. Hadi, M. Roustaii, B. Nakhoda, H. M. Alipoor, and F. Moradi. 2012. Relationship between drought stress and some antioxidant enzymes with cell membrane and chlorophyll stability in wheat lines. African journal of microbiology research Vol. 6(3), pp. 617-623.

Singh P, Kanemasu E.T. 1983. Leaf and Canopy temperature of pearl millet genotypes under irrigated and non-irrigated conditions. Agron. J., 75: 497- 501.

Singha, P. Bhowmick J. and Chaudhury, B. 2006. Effect of Temperature on yield and yield components of fourteen wheat (*Triticum aestivum* L.) genotypes. Environ. Ecol 24: 550-554.

Stein C.E. and E.S. Braga. 2010. Chlorophyll a in macro – Vegetable as an indicator of the Environmental impacts in the Cananeia- Iguage Estuarine- Lagoon System-SP. Safety, Health and Environment World Congress. July 25-28, Sao Paulo, Brazil. Pp 84-87.

Streck, N.A. 2005. Climate change and agro ecosystems: the effect of elevated atmospheric CO_2 and temperature effects on crop growth, development and yield. *Ciencia Rural* 35: 730-740.

Tahir, I.S.A., Nakata, N., Ali, A.M., Mustafa, H. M., Saadi, A.S.I., Takata, K., Ishikawa, N., Abdalla O.S. 2006. Genetic and temperature effects on wheat grain yield and quality in a hot- irrigated environment. Plant Breed; 125:323- 330.

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

Tyagi, P.K., Pannu R.K., Sharma K.D., Chaudhary B.D. and Singh D.P. 2003. Response of different wheat (*Triticum aestivum* L.) cultivars to terminal heat stress. Tests Agrochem. Cultivars 24: 20-21.

Ubaidullah, Raziuddin, Tila Mohammad, Hafeezullah, Sardar Ali and Abdul wahab Nassimi, 2006. Screening of wheat (*Triticum aestivum* L.) Genotypes for some important Traits against Natural Terminal Heat Stress. *Pakistan Journal of Biological Sciences, 9: 2069- 2075*.

Vijayalanshmi, K., Fritz A. Paulsen G, Pandravada S, Gill, B. 2010. Modelling and Mapping QTL for senescence- related traits in winter wheat under high temperature. Mol. Breed 26: 163 - 175.

Wahid A., S. Gelan, Ashraf M. and Foolad M.R. 2007. Heat tolerance in plants; an overview. Environ. Exp. Bot.61:199-223.

Wahid A., Shabbir A. 2005. Induction of heat stress tolerance in barley seedlings by pre-sowing seed treatment with glycine betaine. *Plant Growth Regul.* 46 133-141.10.1007/ S10725-005-8379-5.

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

Xu, W., Zhang Y.H., and Wang Z.M. 2004. Effect of heat stress during grain filling on Phosphoenolpyruvate carboxylase and ribulose 1-5-biphosphate carboxylase/oxygenase activities of various green organs in winter wheat. *Photosynthetica 42: 317- 320.*

Yildirim, M., Bahar B., Koc, M. and Barutcular. 2009. Membrane Thermal stability at Different Developmental Stages: Tarim Bilimleri Dergisi, 15(4): 293 – 300.

Yildirim M., Kilic H., Kendal E., Karantia, T. 2011. Application of Chlorophyll meter readings as yield predictors in durum wheat. J. Plant Nutr. 34(2): 151- 300.

Zadoks, J.C., T.T. Chang, and C.F. Konzak. 1974. A decimal code for growth stages of cereals. Weed Res. 14: 415 – 421.

Zhao H., Dai T., Jiang D. and Cao W. 2008. Effect of high temperature on key enzymes involved in starch and protein formation in grains of two wheat cultivars. *J. Agron. Crop. Sci 194: 47-54.*

APPENDICES

Appendix 1: Means of Traits of wheat genotypes across Environments

Genotype	Yield (Kg/ha)	SS	SL (cm)	DF (days)	TIL	FLA (cm^{2})	CT (^{0}C)	CTD (^{0}C)	PHT (cm)	BM (g)	HI (%)
Entry 10	8 655	20.06	12.04	75.50	443.4	30.21	24.98	4.86	100.95	71.35	43.47
Entry 11	6 080	19.85	9.92	72.38	429.1	21.36	25.85	3.32	87.96	54.34	45.70
Entry 13	8 737	17.10	10.18	72.00	418.4	21.82	25.80	3.79	89.37	82.10	39.17
Entry 15	6 399	19.74	9.85	76.25	451.1	22.69	25.50	3.50	99.62	62.35	33.98
Entry 20	9 917	19.95	10.16	72.38	564.9	23.22	24.47	5.46	93.97	60.49	49.99
Entry 27	8 085	17.40	10.95	73.62	435.6	23.14	25.42	2.04	95.46	68.06	39.27
Entry 41	9 394	18.06	10.18	61.62	503.6	22.60	25.61	4.62	97.27	67.98	46.20
Entry 44	9 291	19.81	10.25	63.75	443.4	22.11	25.42	4.55	86.31	65.19	50.12
Entry 45	7 388	19.79	9.99	70.62	413.9	19.24	25.27	4.14	88.67	66.06	45.44
Entry 46	6 881	20.11	9.86	69.25	405.8	21.66	25.58	4.26	88.00	56.11	47.26
Entry 47	7 444	19.90	9.98	69.75	390.1	20.03	24.32	5.03	88.66	63.47	46.27
Mampo	8 346	19.60	11.18	66.38	477.2	22.23	25.64	4.32	92.99	67.91	46.15
Nduna	9 547	19.80	11.18	66.00	521.0	24.86	24.84	4.451	90.99	67.54	40.24
Pungwa	9 486	20.45	10.72	66.38	502.8	23.54	24.26	5.26	94.14	69.76	44.43
Sahai I	7 674	19.26	10.15	68.50	429.6	21.21	25.36	3.70	94.30	62.91	44.69
Unza I	8 630	18.75	10.66	69.38	418.0	24.05	25.28	4.72	92.95	61.93	44.58
Unza II	8 680	20.15	10.27	68.50	485.2	21.69	25.56	4.22	90.70	63.43	47.25
Lorrie II	9 366	20.89	10.85	68.12	462.0	22.88	25.10	4.50	93.62	62.94	46.28
LSD (5%)	**284.0**	**1.06**	**0.72**	**2.54**	**28.40**	**1.87**	**0.96**	**0.83**	**1.32**	**4.58**	**1.95**

KEY: SL: Spike length; SS: Spikelets/spike; DF: Days to 50% flowering; TIL: Number of tillers/m^{2}; FLA: Flag leaf area; CT: Canopy temperature; CTD: Canopy temperature depression; PHT: Plant height; BM: Above ground biomass; HI: Harvest index

Appendix II: ANOVA for Grain Yield under Optimal Environment

Source of Variation	df	Mean Square	v.r.	F pr
Replication	3	13 978	0.30	
Genotype	17	11 036 863	273.75	< 0.001
Error	51	46 422	-	
Total	71			

Appendix III: ANOVA for Grain Yield under Stress Environment

Source of Variation	df	Mean Square	v.r.	F pr
Replication	3	56 747	1.54	
Genotype	17	4 608 197	124.68	< 0.001
Error	51	36 909	-	
Total	71			

Appendix IV: Combined Analysis of Variance for grain yield

Source of Variation	df	Mean Square	v.r.	F pr
Replication	6	40 406	1.31	
Environment	1	131 908 173	4 358.49	< 0.001
Genotype	17	10 355 127	248.53	< 0.001
Genotype x Environment	17	5 283 633	126.81	< 0.001
Error	102	41 665	-	
Total	143			

Appendix V: ANOVA for Tillers / m ² under Optimal Environment

Source of Variation	df	Mean Square	v.r.	F pr
Replication	3	267.7	2.12	
Genotype	17	18 099.2	49.95	< 0.001
Error	51	362.4	-	
Total	71			

Appendix VI: ANOVA for Tillers / m² under Stress Environment

Source of Variation	df	Mean Square	v.r.	F pr
Replication	3	284.23	0.30	
Genotype	17	14 596.65	273.75	< 0.001
Error	51	4577.43	-	
Total	71			

Appendix VII: ANOVA for Days to 50% Flowering under Optimal Environment

Source of Variation	df	Mean Square	v.r.	F pr
Replication	3	3.352	3.08	
Genotype	17	56.353	51.84	< 0.001
Error	51	1.087	-	
Total	71			

Appendix: VIII: ANOVA for Days to 50% Flowering under Stress Environment

Source of Variation	df	Mean Square	v.r.	F pr
Replication	3	3.792	1.92	
Genotype	17	56.063	28.34	< 0.001
Error	51	1.978	-	
Total	71			

Appendix IX: ANOVA for Plant Height under Optimal Environment

Source of Variation	df	Mean Square	v.r.	F pr
Replication	3	3.851	2.58	
Genotype	17	65.125	43.56	< 0.001
Error	51	1.495	-	
Total	71			

Appendix X: ANOVA for Plant Height under Stress Environment

Source of Variation	df	Mean Square	v.r.	F pr
Replication	3	0.949	0.17	
Genotype	17	92.702	17.07	< 0.001
Error	51	5.430	-	
Total	71			

Appendix XI: ANOVA for Above Ground Biomass Optimal Stress Environment

Source of Variation	df	Mean Square	v.r.	F pr
Replication	3	0.475	0.17	
Genotype	17	347.566	127.80	< 0.001
Error	51	2.720	-	
Total	71			

Appendix XII: ANOVA for Above Ground Biomass under Stress Environment

Source of Variation	df	Mean Square	v.r.	F pr
Replication	3	0.949	0.17	
Genotype	17	92.702	17.07	< 0.001
Error	51	5.430	-	
Total	71			

Appendix XIII: ANOVA for Flag Leaf Area under optimal Environment

Source of Variation	df	Mean Square	v.r.	F pr
Replication	3	2.064	1.34	
Genotype	17	36.750	23.85	< 0.001
Error	51	1.541	-	
Total	71			

Appendix XIV: ANOVA for Flag Leaf Area under Stress Environment

Source of Variation	df	Mean Square	v.r.	F pr
Replication	3	0.210	0.49	
Genotype	17	26.1721	61.09	< 0.001
Error	51	42.84	-	
Total	71			

Appendix XV: ANOVA for Stay Green Duration under Optimal Environment

Source of Variation	df	Mean Square	v.r.	F pr
Replication	3	16.63	4.56	
Genotype	17	48.941	13.41	< 0.001
Error	51	3.649	-	
Total	71			

Appendix XVI: ANOVA for Stay Green Duration under Stress Environment

Source of Variation	df	Mean Square	v.r.	F pr
Replication	3	5.130	1.39	
Genotype	17	24.029	6.53	< 0.001
Error	51	3.649	-	
Total	71			

Appendix XVII: ANOVA for Harvest index under Stress Environment

Source of Variation	df	Mean Square	v.r.	F pr
Replication	3	0.3476	0.38	
Genotype	17	62.3612	67.73	< 0.001
Error	51	0.9208	-	
Total	71			

Appendix XVIII: ANOVA for Harvest index under Optimal Environment

Source of Variation	df	Mean Square	v.r.	F pr
Replication	3	4.1594	4.38	
Genotype	17	81.4469	85.83	< 0.001
Error	51	0.9489	-	
Total	71			

Appendix XIX: ANOVA for Membrane Thermostability under Stress Environment

Source of Variation	df	Mean Square	v.r.	F pr
Replication	3	0.0496	0.35	
Genotype	17	23.3431	163.38	< 0.001
Error	51	0.1429	-	
Total	71			

Appendix XX: ANOVA for Canopy temperature under Optimal Environment

Source of Variation	df	Mean Square	v.r.	F pr
Replication	3	0.1128	0.24	
Genotype	17	2.0343	4.25	< 0.001
Error	51	0.4781		
Total	71			

Appendix XXI: ANOVA for Canopy temperature under Stress Environment

Source of Variation	df	Mean Square	v.r.	F pr
Replication	3	1.1588	4.63	
Genotype	17	1.6326	6.52	< 0.001
Error	51	0.2506		
Total	71			

Appendix XXII: ANOVA for Ear length under Stress Environment

Source of Variation	df	Mean Square	v.r.	F pr
Replication	3	0.35443	4.29	
Genotype	17	1.21883	14.29	< 0.001
Error	51	0.08255		
Total	71			