UNIVERSITY OF KWAZULU-NATAL

GENETIC ANALYSIS OF DROUGHT TOLERANCE IN SELECTED BREAD WHEAT (*Triticum aestivum* L.*)* GENOTYPES

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Genetic Analysis of Drought Tolerance in Selected Bread Wheat (*Triticum aestivum* L.*)* Genotypes

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THESIS ABSTRACT

Wheat (*Triticum aestivum* L.) is an important food security crop globally. The production and productivity of wheat is threatened by recurrent drought that is associated with global climate change. Breeding for drought tolerance using promising genetic resources and efficient phenotypic, biochemical and genomic technologies and methodologies is one of the novel strategies to enhance wheat yield. Therefore, the objectives of this study were: (1) to screen bread wheat genotypes for drought tolerance using phenotypic and proline analyses, (2) to estimate the variance components and heritability of yield and yield components of wheat under drought-stressed and non-stressed conditions, (3) to quantify genome-wide association of agronomic traits in wheat under drought-stressed and non-stressed conditions, and (4) to determine the combining ability and gene action controlling yield and yield components in wheat under drought stressed and well-watered conditions.

In the first study, 96 genotypes of diverse pedigrees including 88 lines from the International Maize and Wheat Improvement Center (CIMMYT)'s heat and drought tolerance nurseries, and eight local checks were evaluated under greenhouse and field conditions during 2014/15 and 2015/16 making four testing environments. The following phenotypic traits were collected after stress was imposed during the heading to anthesis period: days to heading (DTH), days to maturity (DTM), number of productive tillers (TN), plant height (PH), spike length (SL), number of spikelets per spike (SPS), number of kernels per spike (KPS), thousand seed weight (TSW), grain yield (GY) and proline content (PC). Analysis of variance, Pearson's correlation coefficient, principal component analysis, and stress tolerance index were calculated. Genotypes with high yield performance under stressed and optimum conditions maintained high values for yield components. Proline content significantly increased under stress, but was weakly correlated with agronomic traits under both optimal and water limited conditions. The positive correlation observed between grain yield and proline content under-drought stress conditions provides evidence that proline accumulation might ultimately be considered as a tool for effective selection of drought tolerant genotypes. The study selected 12 genotypes with high grain yield under drought stressed conditions and favorable adaptive traits useful for breeding.

The second study determined variance components and heritability of yield and yield related traits of a population of the 96 genotypes evaluated above. High levels of genotypic variance (σ^2_g) were estimated for spike length (73%), number of spikelets per spike (44.19%), plant height (51.26%), number of kennels per spike (32.98%), number of days to heading (44.24%) and thousand seed weight (22.98%), resulting in high broad-sense heritability estimates of > 0.50. Conversely, genotypic variation was relatively moderate for the number of days to maturity, grain yield and number of productive tillers per plant, accounting for 15.03%, 8.46% and 6.13% of the total variation, respectively. The heritability estimates of the later traits were low, 20% ≤ H^2 < 50%, which may limit their selection gains under drought-stressed environments. Further, quantitative trait loci analysis and progeny testing were recommended to discern the number of genes and associated genetic effect and to pinpoint genomic regions in the tested wheat genetic resources for effective drought tolerance breeding.

The third study performed genome-wide marker-trait association analysis of agronomic traits in wheat under drought-stressed and non-stressed conditions. A population of 93 genotypes selected from the first study was genotyped using the Diversity Arrays Technology sequencing (DArTseq) protocol. The following agronomic traits, assessed under drought-stressed and non-stressed conditions, were considered for the study: DTH, DTM, PH, SL, KPS, TSW and GY. Population structure analysis and genome-wide association mapping were undertaken based on 16,383 silico DArTs with < 10% missing data. The population evaluated was grouped into nine distinct genetic structures. Inter-chromosomal linkage disequilibrium showed the existence of linkage decay as physical distance increased. A total of 62 significant (P < 0.001) marker-trait associations (MTAs) explaining more than 20% of the phenotypic variation were detected under both drought-stressed and non-stressed conditions. Significant (P < 0.001) MTA events were observed for DTH, PH, SL, SPS, and KPS under both stressed and non-stressed conditions, while additional significant (P < 0.05) associations were considered for TSW, DTM and GY under non-stressed condition. The MTAs reported in this population could be useful to initiate marker-assisted selection and targeted trait introgression in wheat under drought-stressed and non-stressed conditions, and for fine mapping and cloning of the underlying genes.

The fourth study determined the combining ability and gene action controlling yield and yield related traits under drought-stressed and non-stressed conditions involving 12 wheat parents and their 66 half diallel crosses. The materials were evaluated using a 6 x 13 lattice design with two replications under field and greenhouse conditions during April to October 2016. Plant height, productive tiller number, kernels per spike, thousand seed weight and grain yield were recorded. Significant effects of genotypes, water regimes and test environments were observed. Significant general combining ability (GCA) effects for all traits and significant specific combining ability (SCA) effects for PH, KPS, TSW and GY were recorded, revealing the influence of both additive and non-additive gene effects in that order. For most traits, the ratios of GCA to SCA variances were less than a unity, indicating the predominance of non-additive gene effect. Parents LM17 and LM21 had consistent negative GCA effects for PH, hence, these could be selected for breeding for reduced plant height. Consistently high GCA effects were observed on LM02 for GY; LM02 and LM23 for KPS; and LM04 and LM09 for TSW suggesting the presence of additive genes. Crosses LM17 x LM23, LM04 x LM45, LM29 x LM45 and LM09 x LM85 had negative SCA values for plant height; hence, could be selected for reduced PH. Further, the following crosses: LM02 x LM45, LM29 x LM85 and LM21 x LM23; LM13 x LM23; and LM09 x LM21 were better specific combiners for drought stressed KPS, TSW and GY, respectively, and are useful for further selection. Positive correlation was observed between grain yield and proline content under-drought stressed conditions, hence it can be a useful biomarker for drought tolerance breeding. The high heritability estimates of spike length (94.61%), number of spikelets per spike (87.28%), plant height (86.33%), number of kennels per spike (78.43%), number of days-to-heading (76.26%) and thousand seed weight (68.15%) may suggest the effect of some major genes on these traits under both water-stressed and non-stressed conditions.

The present study identified a total of 65 highly significant marker trait associations under contrasting water regimes. These markers are useful genomic resources to initiate marker-assisted selection and trait introgression of wheat under drought-stressed and non-stressed conditions, and for fine mapping and cloning of the underlying genes.

DECLARATION

I, Learnmore Mwadzingeni, declare the following

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other University.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a) Their words have been re-written but the general information attributed to them has been referenced.

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Signed:

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Learnmore Mwadzingeni

As the candidate's supervisor, I agree to the submission of this dissertation

…….

Prof. H. Shimelis (Supervisor)

……..

Prof. T.J. Tsilo (Co-supervisor)

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DEDICATION

This thesis is dedicated to everyone who keeps advising me to "*learn more*" by consistently calling me "*Learnmore"*.

TABLE OF CONTENTS

ABBREVIATIONS AND ACRONYMS

CIMMYT	International Maize and Wheat Improvement Center
CV	Coefficient of variation
DArTseq	Diversity Arrays Technology sequencing
DH	Doubled haploid
DF	Degrees of freedom
DTH	Number of days to heading
DTM	Number of days to maturity
Eo	Evaporation from a class A pan
FC	Field capacity
GBS	Genotyping-by-sequencing
GCA	General combining ability
GWAS	Genome-wide association study
GY	Grain yield
H^2	Broad sense heritability
ICARDA	International Centre for Agricultural Research in the Dry Areas
KPS	Kernels per spike
LSD	Least significant differences
MAS	Marker-assisted selection
MTA	Marker-trait association
NGS	Next generation sequencing
PC	Proline content
PCA	Principal component analysis
PH	Plant height
QTL	Quantitative trait loci
SCA	Specific combining ability
SED	Standard error of differences
SHW	Synthetic hexaploid wheat
SL	Spike length
SNP	single nucleotide polymorphism
SPS	Spikelet per spike

STI	Stress tolerance index
TSW	Thousand seed weight
TN	Productive tiller number
UKZN	University of KwaZulu-Natal

INTRODUCTION TO THESIS

Background

Wheat is among the world's major food crops in terms of area under cultivation, production volume and the proportion of the world's population relying on it as a staple diet (Alexandratos and Bruinsma, 2012). It contains about 55% starch that contributes up to 20% of the global energy demand, about 12.1% protein, as well as some dietary fats, vitamin B, zinc, calcium, and iron (Šramková et al., 2009). Major world producers of the crop include Russia, China, Ukraine, Belarus and Moldova. In sub-Saharan Africa, South Africa, Ethiopia, Sudan, Kenya, Zimbabwe, Tanzania, and Zambia are the main producers contributing about 99% of the wheat grown in the continent (Mason et al., 2012). South Africa is the largest regional producer of wheat using about 505 500 ha of land. South Africa produces about 1,791 million tons of wheat per year. However, the country is importing more than 1.4 million tons per annum due to several wheat production constraints (Department of Agriculture, Forestry and Fisheries, RSA, 2013).

Wheat Production Constraints and Breeding Objectives in South Africa

Wheat production in South Africa faces several biotic and abiotic constraints. Therefore, national and private breeding and biotechnology institutions striving to improve wheat yield and quality through targeted research and development. Key players in wheat breeding and biotechnology research in the country include the Department of Agriculture, Forestry and Fisheries (DAFF), the Agricultural Research Council-Small Grain Institute (ARC-SGI), Sensako, CenGen, Pannar Seed and various universities. Furthermore, several research and development collaborations exist between local institutions and leading international wheat research organisations such as the International Maize and Wheat Improvement Centre (CIMMYT) and the International Centre for Agricultural Research in the Dry Areas (ICARDA) (Lantican et al., 2016).

The main wheat improvement goals in the country include resistance breeding against the major pests and diseases such as the Russian wheat aphid, wheat rusts and *Fusarium* head blight (Smit et al., 2010; Figlan et al., 2014; Terefe et al., 2014; Tolmay et al., 2016). Thus far, several pre-breeding, conventional and marker-assisted breeding programs are underway in an effort to develop pest and disease resistant and high yielding cultivars. Apart from breeding for biotic

stress resistance, the ARC-SGI has a dedicated quality breeding program to improve the nutritional and end-use quality mainly through conventional breeding. Further, pre-harvest sprouting tolerance is among current research priorities aimed at retaining grain yield and quality in the event of prolonged rains after physiological maturity (Smit et al., 2010). Although wheat has received much attention in terms of breeding for improved productivity under optimal conditions and breeding for resistance to biotic stresses, much research effort is still needed to improve the crop's tolerance to abiotic factors, particularly heat and drought stress (Mason et al., 2012; Yildirim et al., 2013). Recurrent drought stress is among the major yield limiting factors of wheat production and productivity in South Africa and globally. This calls for a dedicated research effort towards development of improved cultivars with adaptability to marginal rainfall conditions. Figure 0.1 show wheat exposed to water stress of water stress.

Figure 0.1 Evaluation of drought tolerance in wheat during initial stages of water stress.

Drought and Selection for Drought Tolerance in Wheat

Drought occurs when moisture supply become insufficient and erratic to support optimal crop growth, development and production (Blum, 2010). Success in breeding drought tolerant wheat genotypes have been achieved through selection for or modification of physiological and morphological traits (Mir et al., 2012; Monneveux et al., 2012; Tardieu, 2012). In cases where drought occurs earlier during the season, wheat genotypes with the capacity to constitutively develop longer coleoptiles and vigorous seedlings with optimum growth rate will develop the potential number of productive tillers (Rebetzke et al., 2005; Rebetzke et al., 2007). If late season/terminal drought stress occurs, selection will be in favour of genotypes that reproduce and fill the grain before the onset of stress (Blum, 2010). Delayed senescence or stay green will be required to sustain grain filling during late season drought to maintain photo-assimilate production (Thomas and Howarth, 2000; Gong et al., 2005), or stem reserves mobilization after leaf senescence (Gupta et al., 2011). However, these two traits are often negatively correlated, hence may not be simultaneously selected for breeding. Other important physiological traits useful when phenotyping wheat for drought tolerance include high effective use of water (EUW), high stomatal conductance, and low canopy temperature. These traits indicate the genotype's ability to maintain high soil moisture extraction, photosynthesis and evaporative cooling due to transpiration maintenance (Fischer et al., 1998). Physiological processes are maintained in genotypes with high osmotic adjustment potential through accumulation compatible osmolytes such as proline, mannitol and trehalose that enhance water uptake under stress, and protects the plant tissue from being damaged through oxidation effects caused by stress (Zivcak et al., 2009). If deep soil moisture is not available, traits that favour high water use efficiency (WUE) such as reduced stomatal conductance and reduced transpiration will be considered, though they may be associated with reduced productivity. Slow wilting genotypes showing delayed leaf rolling should be selected as better adapters that maintains high leaf relative water content (Blum, 2010).

Selection can also target genotypes with short plant height (semi-dwarf), which are often associated with increased partitioning of assimilates to the grain under drought stress, hence stable yield and increased harvest index (Budak et al., 1995). Despite the challenges with root phenotyping, selecting for high root length density, deeper rooting system with good soil penetrating ability and high root hydraulic conductivity remains key when breeding wheat for

water limited environments (Paez-Garcia et al., 2015). Thus, high throughput root phenotyping techniques and marker assisted selection should be prioritized. Yield and yield components that directly contribute to the complex trait including the number of productive tillers per plant, number of kennels per ear and grain weight should be the primary target during selection because they are indicators of reproductive success (Mwadzingeni et al., 2016). In cases were yield is recorded under stressed and non-stressed conditions drought tolerance indices can be calculated and used as criteria for genotype selection and recommendation to allow selection for relatively high performance under either condition (Fernandez, 1992; Khakwani et al., 2012).

The genetic bases of morpho-physiological and adaptation mechanisms employed by plants should be understood so that breeding strategies can be revolutionized towards genomic selection. The use of genetic markers including Diversity Arrays Technology (DArT) and simple sequence repeats (SSRs) are increasingly becoming popular in tracking genes that control quantitatively inherited traits in wheat (Dodig et al., 2010; Bousba et al., 2012; Marmar et al., 2013). The DArTseq protocol is a microarray based platform that employs restriction enzymes to separate low copy sequence repeats from the crop's genome followed by fluorescent labelling of representations and their hybridization to arrays. The technology has been demonstrated to be useful in developing high quality markers useful for high throughput and whole genome profiling in wheat (Akbari et al., 2006). Several putative genes located on quantitative trait loci (QTL) are being mapped on the wheat genome. Dissection of drought tolerance at gene and nucleotide sequence levels in wheat is likely to be enhanced through the use of next generation sequencing (NGS) techniques such as genotyping by sequencing, which is relatively cheap and provides a wide genomic coverage for genome-wide association studies (GWAS) (Elshire et al., 2011; Poland et al., 2012). This has been difficult with traditional marker techniques considering the fact that the wheat genome is huge and complex (Edwards et al., 2013). The use of new technologies should therefore be embraced in wheat breeding programs.

Testing the combining ability and gene action among drought tolerant germplasm introductions will assist in identifying potential parents for hybridization and trait improvement. In this case, good general combiners for particular traits would be deployed into local breeding programs to contribute additive genes that confer adaptability to target growing conditions. If good specific

combiners are identified among targeted crosses, they can be targeted during selection to identify superior transgressive segregates. In this view, breeding for drought tolerance in wheat can be achieved through a strategic interconnection of methodologies and strategies.

Rationale of this study

The increasing negative impacts of drought on wheat productivity is causing South African rain-fed wheat growers to reduce their scales of wheat production. Others are shifting their investments towards the production of alternative crops that are better adapted to drought. However, no alternative crop can satisfy the need for wheat in the bread and pasta industries. Drought is negatively impacting the livelihoods of smallholder and emerging rain-fed wheat farmers, as well as commercial growers who do not have adequate irrigation facilities. Therefore, there is need to develop wheat cultivars that can thrive under limited water or supplementary irrigation. Despite the increasing need for drought tolerance in wheat, the country does not have sufficient genetic resources and breeding programs specifically focused on enhancing the productivity of drought stressed wheat. This challenge emanates from the fact that drought tolerance is difficult to breed. Drought tolerance is a complex trait that is influenced by numerous minor genes with additive and non-additive effects, and is not static. The use of controlled water application and rain-out shelters facilitates intensive drought tolerance screening of large pools of genotypes. Drought tolerance improvement in wheat is further complicated by the large size of the crop's genome which needs efficient high throughput genotyping tools to dissect the genetic bases of complex traits. To achieve this, candidate germplasm that has been pre-bred for drought tolerance by leading international wheat breeding organizations like CIMMYT should be screened and evaluated using various technologies. Among the technologies, advanced sequencing facilities such as the Diversity Arrays Technology sequencing could offer opportunities for providing wide genomic coverage that can be exploited through GWAS to discover markers and genes that can be used for marker-assisted selection and gene introgression into cultivated germplasm through designed crosses. Lastly, the combining ability of superior parents should be evaluated to determine their capacity to improve local genotypes and to produce superior specific crosses.

Overal Objective

The study aimed to improve wheat productivity under water-limited environments through drought tolerance improvement.

Specific Objectives

i. To screen bread wheat genotypes for drought tolerance using phenotypic and proline analyses to select promising lines for use in breeding for drought tolerance.
ii. To estimate variance components and heritability of yield and yield components of wheat under drought-stressed and non-stressed conditions to find traits that can give high genetic gain from selection.
iii. To quantify genome-wide marker-trait association of agronomic traits in wheat under drought-stressed and non-stressed conditions and to identify potential markers for marker-assisted selection.
iv. To determine the combining ability and gene action controlling yield and yield components in wheat under drought stressed and well-watered conditions and select best combiners for effective breeding.

Hypotheses

i. Phenotypic traits and proline contents significantly vary among bread genotypes under drought-stressed and non-stressed conditions.
ii. Yield and yield components of bread wheat genotypes are highly heritable.
iii. Agronomic traits and DArTseq markers are significantly associated in bread wheat genotypes.
iv. The selected parents and their crosses show good combining ability for yield and yield components when evaluated under drought stressed and well-watered conditions.

Outline of thesis

This thesis consists of five chapters in accordance with the number of objectives (see Table 0.1). Chapter 1 is written as a separate review paper, while chapters 2 to 5 are written as discrete research papers, each following the format of a stand-alone research paper followed by a general overview and implications of findings from the study. The literature review and four experimental chapters of the study made the thesis chapters that were condensed into discrete but inter-dependant papers according to the University of KwaZulu-Natal's dominant thesis format. There are some overlaps and unavoidable repetitions of references and some introductory information between chapters. The Crop Science Journal referencing system was used in all chapters of this thesis. Chapter 1 was published in the Journal of Integrative Agriculture (2016, 15(5): 935–943, doi: 10.1016/S2095-3119(15)61102-9). Chapter 2 was published in Frontiers in Plant Science (2016, 7:1276, doi: 10.3389/fpls.2016.01276). Chapter 4 was published in PLoS ONE (2017, 12(2): e0171692. doi:10.1371/journal.pone.0171692).

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CHAPTER 1. A REVIEW OF THE LITERATURE

Abstract

Recurrent drought associated with climate change is among the principal constraints to global productivity of wheat [*Triticum aestivum* (L.) and *T. turgidum* (L.)]. Numerous efforts to mitigate the effects of drought through breeding resilient varieties are underway across the world. Progress is, however, hampered because drought tolerance is a complex trait that is controlled by many genes, and its full expression is affected by the environment. Furthermore, wheat has a structurally intricate and large genome. Consequently, breeding for drought tolerance requires the integration of various knowledge systems and methodologies from multiple disciplines in plant sciences. This chapter summarizes the progress made in rain-fed wheat improvement, germplasm resources for drought tolerance breeding, advances in knowledge, complementary methodologies, and perspectives towards breeding for drought tolerance in the crop to create a coherent overview. Phenotypic, biochemical and genomics-assisted selection methodologies are discussed as leading research components used to exploit genetic variation. Advances in phenomic and genomic technologies are highlighted as options to circumvent existing bottlenecks in phenotypic and genomic selection, and gene transfer. The prospects of further integration of these technologies with proteomics, transcriptomics and metabolomics is also provided. Further, combining ability and gene action controlling drought tolerance in wheat is discussed. Integrating the above components may hasten the breeding of drought tolerant genotypes with adaptation to marginal rainfall conditions.

Keywords: drought tolerance, genomic selection, genotyping, phenotyping, wheat

1

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1.1 Introduction

Wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD) is an important staple food crop that is cultivated on millions of hectares for various domestic and industrial purposes across the globe, offering numerous health benefits (Alexandratos and Bruinsma, 2012; Šramková et al., 2009; Mason et al., 2012). However; global wheat production in the major production regions is being threatened by recurrent drought that is predicted to increase with climate change (Li et al., 2009). Drought tolerant wheat varieties are the ultimate means of safeguarding the crop against adverse effects of drought. However, drought tolerance is a complex trait that is controlled by numerous genes, each with minor effects. Some of the genes are located as quantitative trait loci (QTL) exhibiting additive and non-additive gene effects (Bernardo, 2008). Due to its polygenic inheritance and genotype by environment interaction, drought tolerance typically has low heritability (Blum, 2010; Khakwani et al., 2012). Despite these challenges, determination of the genetic diversity existing within and between wheat populations remains the basis for elucidation of the genetic structure and for improvement of quantitative traits, including drought tolerance. In wheat, greater genetic variability can be explored on germplasm from its centres of origin and diversity (Dvorak et al., 2011). Besides cultivated wheat varieties and breeding stocks, extensive variability for drought tolerance remains within wild relatives and landraces (Nevo and Chen, 2010; Dodig et al., 2012). Manipulation of this diversity to improve drought tolerance among cultivars may be achieved through genetic modification or selection for adaptive mechanisms; including drought escape, dehydration avoidance and dehydration tolerance (Blum, 2010). If promising heterotic parents and improved F1 or F2 progenies are obtained, quick fixation of such genotypes into complete homozygous lines is needed to preserve them from undesirable segregation. This could be achieved through production of double-haploids, to attain complete homozygosity in one generation which could otherwise take more than seven generations of selfing.

Genomics-assisted selection has not yet contributed much to the improvement of drought tolerance in wheat. This may be attributed to the polygenic nature of the trait, and the structural complexity and large size of the crop's genome, which is approximately 17 Gigabase base pairs (Gbp) (Paux et al., 2006; Berkman et al., 2012). Also, lack of standardized phenotyping techniques could be limiting the application of genomic tools in drought tolerance improvement.

Therefore, advanced phenotyping and genotyping technologies may offer prospects towards precise genomic characterization, genomic selection, molecular marker discovery, QTL mapping, and candidate genes discovery. Thus far, much progress in drought tolerance improvement in wheat, has been made through conventional breeding, which involves cycles of phenotypic selection and crossing. Apart from genomic selection, several studies by physiologists suggest that biochemical analyses could also help in the selection for drought tolerant cultivars. This can be achieved through quantifying biochemical indicators of drought tolerance such as proline under stressed conditions. Biochemical and genomic techniques could enhance understanding of the genetic and physiological bases of drought tolerance which is useful for selection and improvement of the polygenic trait (Fleury et al., 2010). Therefore, a multi-disciplinary approach involving application of phenotypic, biochemical, and genomic techniques is required to improve the trait (Tuberosa and Salvi, 2006; Fleury et al., 2010).

1.2 Breeding Progress for Water Limited Environments

CIMMYT has contributed to the worldwide adoption of modern wheat varieties that are adapted to marginal environments through multi-environmental testing and collaboration with national breeding programmes (Manes et al., 2012). The wheat yield progress under marginal conditions, obtained from CIMMYT's international yield trial data for overlapping periods between 1964 and 2010 is presented by Mwadzingeni et al. (2016). The rates of yield increase are still too low to catch up with the projected 70% rise in wheat demand by 2050 (CIMMYT, 2014). However, increasing rain-fed wheat productivity is a potential option of meeting this growing demand, since yields under optimum conditions may be approaching a ceiling. Much of the yield progress reported under low yielding environments has been based on evaluations under several biotic and abiotic constraints including drought. Moreover, much of the documented yield increase was partly a result of spillover benefits from selection for yield improvement under optimum conditions. Development of candidate genotypes at target growing environments and drought conditions, and minimizing confounding effects of other stresses in the breeding programs, will enhance selection for drought tolerance. Though CIMMYT data presented by Mwadzingeni et al. (2016) represent international yield trends, there is still a need to compile a comprehensive documentary of the progress observed by national breeding programs to provide a clear map of where to acquire new innovations and germplasm.

1.3 Germplasm Resources for Improving Drought Tolerance in Wheat

Exploration and exploitation of the diversity existing within and between wheat populations remain central to the improvement of quantitative traits including drought tolerance. The extent of variability however changes over space and time owing to species evolution, natural selection, mutations, genetic drift, gene flow and artificial selection (Ren et al., 2013). In wheat, greater variability can be explored from centres of origin and diversity which lay in the Mediterranean region and Southwest Asia (Alvarez and Guzmán, 2013). Other recently explored regions with substantial diversity include Southeast Europe, where Jaccard's genetic distance ranging from 5.3% to 88,9% was reported among winter wheat cultivars, as well as Ethiopia, North America and Western Europe, where considerable diversity was found within durum wheat collections (Karsai et al., 2012; Ren et al., 2013). Genetic variability is instrumental in the introgression and maintenance of wheat adaptation and exists within cultivated species, landraces, progenitor species like *Aegilopes tauschii* L.*,* wild relatives like *Triticum dicoccoides* L., related grasses and unrelated key sources of trans-genes (Nevo and Chen, 2010; Dodig et al., 2012). Among these sources, cultivated drought tolerant species and landraces are of immediate usefulness since they are cross compatible with elite cultivated materials.

1.3.1 Exploiting Drought Tolerant Germplasm

Yield benefits from the use of wild relatives and landraces in hybridization programs is often realized after a long period of time due to linkage drag associated with the co-inheritance of undesirable genes and rare alleles with desired genes. Considerable genetic diversity for drought tolerance improvement still exist in cultivated wheat including lines from the CIMMYT that could serve as primary sources of variability. Well characterized and released drought tolerant wheat varieties from different countries that have been useful in either development of mapping and breeding populations, drought tolerance studies or in variety improvement are available in literature (Sadras and Lawson, 2011; Alexander et al., 2012; Bennett et al., 2012). Breeders targeting particular drought scenarios can actually make use of such materials through appropriate combination of genes for specific traits into local elite genotypes. National breeding institutions can develop local gene banks of well characterized elite drought tolerant germplasm from different parts of the world for utilization in improving local cultivars. This ideology came from the realization that (1) drought tolerant cultivars grown in different parts of the world

possess different adaptability mechanisms that, if properly combined, will enhance genotypes that are being developed for various target environments (Sadras and Lawson, 2011; Alexander et al., 2012; Bennett et al., 2012; Karsai et al., 2012; Ren et al., 2013). (2) Breeding for target drought scenario will be much progressive if the program is initiated with useful genetic variation based on germplasm that were already well characterized and confirmed to harbor QTLs and genes for adaptive and constitutive traits contributing to drought tolerance, without compromising the yield potential (Mir et al., 2012; Mwadzingeni et al., 2016). This idea can be supported by the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), which has a mandate to promote open access by all states to diverse germplasm from other countries, in order to enhance global food security through crop improvement and productivity. Such provisions allow easy access to much of the accumulated diversity for drought tolerance for breeding purposes. Apart from elite genotypes, additional useful variation for drought tolerance can be tapped from landraces and wild relatives of the hexaploid spps.

1.3.2 Landraces and Synthetic Hexaploid Wheats

Following cultivated species, landraces offer great opportunities for breeding for drought tolerance since they had evolved through natural and partly artificial selection for adaptability. Landraces are also cultivated types that show less drought sensitivity and higher stability when compared to cultivated species (Dodig et al., 2012; Nevo and Chen, 2010). However, most of the landraces are out-yielded by modern cultivars, though less stable. This suggests that elite germplasm can further be improved by some genes with additive and non-additive effects on drought tolerance and hence yield stability from local landraces (Denčić et al., 2000; Reynolds et al., 2007). Conservation and collection of landraces should be prioritized to allow continuous breeding for specific adaptation as a strategy to minimize the devastating effects of climate change such as the resurgence and re-emergence of different pathogens, pests, and weeds, on top of abiotic stresses, particularly drought.

Genes within diploid progenitor and wild relatives can be exploited through production of synthetic hexaploid wheats (SHWs) that are genetically compatible with cultivated lines. A recent study by Ali et al. (2015) revealed better adaptability of synthetic wheat derivatives to drought stress as compared to conventional wheat lines as exhibited by higher root fresh weight,

root length, sugar and protein content under induced drought stress. With increased backcrossing of SHWs to elite cultivated germplasm as the recurrent parent, yield advantages will eventually be realized as unfavorable alleles from the wild relative will be eliminated. CIMMYT produced SHW germplasm harboring genes that are useful for drought tolerance breeding through interspecific hybridization of *Ae. Tauschii*, the D genome donor, with durum wheat (*T. Turgidum* L. subsp. *durum*) (Lage and Trethowan, 2008). This should be emulated by national breeding programs. However, most national wheat breeding institutions lack systematic pre-breeding programs, due to lack of appropriate skills, limited access by local gene bank curators to the germplasm diversity required, or reluctance by governments and policy makers to fund programs that do not offer immediate benefits. In any case, communication between breeders and policy makers should aim to expose the value of pre-breeding programs as primary sources of novel genes for variety development.

1.4 Selection Methods and Technologies for Drought Tolerance

1.4.1 Phenotyping Wheat for Drought Tolerance

Knowledge of phenotypic traits contributing to improved yields under stress is fundamental to the understanding of the complex physiological and genetic mechanisms of wheat adaptability (Reynolds et al., 2005). Important target traits include; reduced plant height, which is associated with high harvest index (Slafer et al., 2005); reduced number of days to anthesis and maturity, which enable the crop to evade terminal drought stress (Blum, 2010); and root architectural traits such as root distribution and root length density, which enable effective water uptake (Ehdaie et al., 2012; Manschadi et al., 2006). Also, seedling traits associated with vigorous seedling establishment, such as coleoptiles length, can increase adaptation to drought through early ground cover, which reduces evaporative losses (Spielmeyer et al., 2007). Wheat traits associated with reduced evaporative losses and photo-assimilate production such as leaf rolling, flag leaf persistence, stomatal conductance, and canopy temperature should be selected, based on their positive correlation with yield under stress (Dodig et al., 2012). For instance, high stomatal conductance was reported to be positively correlated with water stressed yield ($r = 0.94$) (Fischer et al., 1998) due to increased transpiration which is associated with optimum water uptake from a depth, low canopy temperature and high photo-assimilate production (Blum, 2009; Kumar et al., 2012; Lopes and Reynolds, 2010). The ultimate criteria for genotype selection should, however,

be guided by how well the variety integrates its adaptive mechanisms to optimize yields, other than being based on a single trait. Selection based on yield should be supported by proper calculation, utilization, and interpretation of various drought indices which evaluate genotypic yield response to water stress (Fernandez, 1992). Recently, Khakwani et al. (2012) noted that an adapted genotype, Hashim-8, had the highest mean productivity (2.13), geometric mean productivity (1.69), and stress tolerance index (0.34) but with the lowest stress susceptibility index (0.93) and stress tolerance (1.79) when stressed to 25 to 35% of the field capacity. Their study identified significant positive correlations of the first three indices with yield and recognized stress tolerance index as the best yield predictor under stressed conditions, results which were also supported by Fernandez (1992).

1.4.1.1 Use of rain-out shelter and controlled water application

Artificial simulation of drought through controlled water application and utilization of rain-out shelters plays key roles in reducing experimental error in field experiments through improving homogeneity in moisture levels and eliminating confounding effects of untimely rainfall. Several designs of fixed-location and automated moveable rain-out shelters have been documented for utilization in drought tolerance research in major field crops including wheat (Dodig et al., 2012). Movable rain-out shelters which only cover the plot when it is raining cause minimum alteration of non-target variables such as temperature, which may have confounding effects. Timing of drought induction and water regimes should be guided by the typical drought patterns in the targeted environments. Also, the whole system should be monitored with standard and well serviced soil moisture sensors which suit the researcher's particular requirements (SU et al., 2014). In the past various researchers employed different water regimes to simulate drought. Table 1.1 summarizes different water regimes previously adopted on wheat, which may serve as important guidelines for drought tolerance studies.

Table 1.1. Previously used water regimes for drought tolerance evaluation in wheat

Water regime (treatments)	Reference
Control: Water to 60% field capacity (FC)	Majer et al. (2008)
Stress: Water to 20% FC	
Control: Irrigate at 60% FC	Omar et al. (2010)
Stress: Irrigate at 40% FC	
Control: Irrigate after 70 mm Eo	Golabadi et al. (2011)
Stress: Irrigate after 140 mm Eo	
Control: Moisture content kept at 100% FC	Khakwani et al. (2011)
Stress level 1: Watering done at 35% FC back to 100%	
Stress level 2: Maintain moisture between 25 and 35% FC	
Control: Moisture content kept at 100% FC	Khakwani et al. (2012)
Stress: Withhold water for 20 days at booting and after anthesis	
Control: Provide normal irrigation	Mohamed et al. (2013)
Stress: Withhold water from tillering to anthesis then stressing up to maturity	

Eo, evaporation from a class A pan

1.4.1.2 Need for high-throughput and automated phenotyping techniques

The slow pace, high costs, and inconsistencies associated with trait quantification and data management using traditional phenotyping techniques still limits the progress of drought tolerance improvement. This could, also, have been contributing to the complexities of understanding the genetic and physiological basis of drought tolerance both at the phenotypic and genomic level (Xu and Crouch, 2008). The utilization of sophisticated, non-destructive, high-throughput phenotyping technologies with automated systems for capturing, storage, and statistical analysis of large volumes of data, allows for fast and precise large scale quantification and monitoring of various phenotypic traits (Araus and Cairns, 2014). Ground and remote sensing techniques based on near or far-infrared reflectance digital sensors, thermometers, and cameras are reported to precisely measure various phenotypic traits (Zhu et al., 2011; Araus and Cairns, 2014; Honsdorf et al., 2014). These include automated camera systems which are connected to computers for monitoring complex root architectural traits through periodic image

capturing (Iyer-Pascuzzi et al., 2010). Some of these tools are graced with image processing and analysis software (Schneider et al., 2012). These advanced phenotyping technologies may create local databases for easy management of the vast amounts of data that will be generated.

1.4.2 Applications of Biochemical Markers to Improve Drought Tolerance

Drought stress triggers the expression of many genes influencing the metabolism of several bio-chemicals including key enzymes, transcription factors, hormones, amino acids, and carbohydrates (Yang et al., 2010). Notable among these include the phytohormone abscisic acid (ABA), proline, tryptophan, late embryogenesis abundant (LEA) proteins, trehalose, raffinose, mannitol, glycine-betaine and superoxide dismutase (Sivamani et al., 2000; Hameed et al., 2011; Nio et al., 2011). These bio-molecules are involved, among other functions in dehydration avoidance or dehydration tolerance events such as osmotic adjustment, membrane stabilization, anti-oxidation, scavenging of reactive oxygen species (ROS), and gene regulation (Ashraf, 2010; Yang et al., 2010). However, the specific drought responsive mechanisms and functions of the majority of these metabolites are still ambiguous. This necessitates further studies to reveal their roles to allow for informed manipulation of the genetic diversity existing in the expression of their respective genes under stress. Yield benefits from biochemical accumulations should be considered in breeding programs because osmotic adjustment seems to use energy to accumulate photo-assimilates in other plant organs to ensure survival at the expense of grain yield.

Application of knowledge gained on signalling and metabolism of these drought-related bio-chemicals has mainly been mediated through transgenic plants derived from other crop species beyond the *Triticum* genome. For instance, improved water use efficiency, biomass accumulation, and root weight occurred among water stressed transgenic wheat lines expressing the barley [*Hordeum vulgare* (L.)] gene, *HVA1*, encoding for some late embryogenesis abundant proteins that work as osmo-protectants (Sivamani et al., 2000). On the other hand, a proline inducing gene (*P5CS*) boosted drought tolerance of transgenic lines in a response that was possibly due to proline's antioxidant protection of cells from oxidative damage by oxygen free radicals (Vendruscolo et al., 2007). Also, a mannitol biosynthesis (*mtlD)* gene from *Escherichia coli* increased drought tolerance in wheat by acting as an osmo-protectant (Abebe et al., 2003). The potential contribution of the various genes to drought tolerance improvement may be

overstated because most evaluations are carried out on seedling plants under artificial conditions. However, this may not represent the performances of the trans-genes through all growth stages of wheat under field conditions. Intensive screening of the diverse wheat germplasm based on biochemical accumulation could enhance the introgression of the genes involved using conventional breeding techniques.

1.4.3 Quantitative Trait Loci (QTLs)/Genes Controlling Drought Tolerance

Application of marker technologies eliminates confounding effects of the environment during selection, especially when considering polygenic traits like drought tolerance and allows for indirect selection of traits independent of the stage of plant development. Several molecular markers have been used, of which, sequence-based DNA markers, notably SNPs, are gaining popularity and are expected to advance the dissection of complex traits on complex genomes due to their high linkage with heritable variation (Gupta et al., 2011; Berkman et al., 2012). Thus far, several phenotypic drought-responsive traits on wheat have been correlated with molecular markers allowing precise mapping of their respective QTLs on chromosomes (Ibrahim et al., 2012; Ahmad et al., 2014). However, QTL identification for tracing drought tolerance remains a challenge due to the large number of genes influencing the trait, instability of some QTLs, the large size of the wheat genome, and epistatic QTL interactions, among other constraints (Ashraf, 2010; Kumar et al., 2010; Sharma et al., 2011). Further, the full benefits of molecular markers in selecting for quantitative traits will remain a challenge as most marker techniques are just qualitative measures indicating the presence of a gene with no further information on; whether the gene is expressed or not, the levels of its expression and its impact on the complex trait, and the presence and expression of other genes influencing it. There is, therefore, a need to integrate molecular tools with precise high-throughput phenotyping and biochemical analysis to confirm the consistency of molecular markers.

Detection of QTLs containing the genes conferring quantitative traits including drought tolerance has revolutionized the selection process towards marker assisted and genomic selection (Mir et al., 2012). To date, several putative QTLs for drought tolerance related traits have been mapped in wheat, particularly on the A and B genomes where most of relevant QTLs seem to be localized on chromosomes 2B, 3A, 4A, 4B, 7A, and 7B (Table 1.2). However, there are no QTLs

detected for grain quality under drought stress in wheat, yet drought stress and high temperature cause dough quality deterioration. The utilization of the abundant QTLs identified so far through marker assisted selection (MAS), candidate gene detection, and QTLs introgression or pyramiding for drought tolerance improvement in wheat has not been reported as utilized in practice. Also, the utilization of above mentioned markers seem to be reliable for detecting QTLs with major phenotypic effects (Kumar et al., 2012), yet drought tolerance is a function of many QTLs of major and minor effects. Further, cloning of these QTLs is necessary for the determination of their molecular mode of action so that effective selection can be carried out based on their breeding values. Statistical analysis is also a requisite to determine epistatic QTL interactions and complex QTL by environment interactions to account for the error variances due to the environment (Kumar et al., 2010). These limitations may be resolved through the use of advanced sequence-based techniques to improve the consistency of detecting QTLs, including those with minor effects as outlined in Table 1.2.

Table 1.2. Putative QTL regions identified for drought tolerance related traits in wheat either under stressed conditions only or on both stressed and optimal conditions

Chromosome	Traits associated with the putative QTL	Mapping populations	Reference
2A	Relative water content, awn length, grain weight, coleoptiles length, shoot length, and extrusion length	Core collection	Ahmad et al. (2014)
1B, 4A, 4B, 7A, 7D	Thousand-grain weight	Core collection	Nezhad et al. (2012)
1A, 1D, 2B, 3A, 3B, 4B, 4D, 5B, 6A	Potential quantum efficiency of photosystem (PS) II, chlorophyll content, flag leaf temperature, and grain yield	Recombinant inbred lines (RILs) derived from a cross between cultivars 'C306' and 'HUW206'	Kumar et al. (2012)
1D, 2A, 2B, 2D, 3A, 4A, 4B, 5B, 5D, 6D, 7A, 7D	Root diameter, volume, surface area, crossings, forks, and tips	Advanced backcross population derived from a spring wheat cultivar 'Devon' and a synthetic hexaploid accession 'Syn084'	Ibrahim et al. (2012)
1D, 2B, 3A, 3B, 4A, 4B, 5A, 5B, 6A, 6D, 7A, 7B	Grain yield and yield related traits	RILs from a cross between common wheat cultivars 'Dharwar Dry' and 'Sitta'	Alexander et al. (2012)
3BL	Grain yield	Doubled haploid (DH) population from a cross between Line 'RAC875' and variety 'Kukri'	Bennett et al. (2012)
All except 1D and 6A	Grain yield, number of grains per ear, and chlorophyll a fluorescence	DH lines derived from genotypes 'Chinese Spring' and 'SQ1'	Czyczyło-Mysza et al. (2011)

Table 1.2. (Continued)

Chromosome	Traits associated with the putative QTL	Mapping populations	Reference
1B, 2B, 3B, 5B, 7B, 7A	Grain weight, grain weight/spike, grain number/spike, spikes/m^2, spike weight, spike harvest index, and harvest index	RILs obtained from a cross between genotypes 'Oste-Gata' and 'Massara-1'	Golabadi et al. (2011)
All except 2A, 2D, 3D, 5D, 6D, and 7D	Agronomic, phenological, and physiological traits	RILs derived from a cross between variety 'Seri M82' and a fixed line 'Babax'	Pinto et al. (2010)
1A, 3D, 7B	Stay green	RILs derived from a crosses between a stay green cultivar 'Chirya 3' and a non-stay green synthetic 'Sonalika'	Kumar et al. (2010)
2B, 4A, 5A, 7B	Crop productivity, morpho-physiological, and phenological traits	RILs derived from a cross between durum wheat cultivar 'Langdon' and a wild emmer accession 'G18-16'	Peleg et al. (2009)
1B, 1D, 2B, 3A, 4A, 4B, 4D, 5A, 5B, 6A, 6B, 7A, 7B	Yield, anthesis, and height	RILs derived from a cross between elite spring bread wheats, 'Seri M 82' and 'Babax'	Mathews et al. (2008)
6A	Coleoptiles, seedling vigour, and plant height	RILs derived from a cross between a Chinese semi-dwarf wheat 'Chuan-Mai18' and a tall breeding line 'Vigour18'	Spielmeyer et al. (2007)

1.4.4 Applications of Next Generation Sequencing (NGS) and Genome Engineering Technologies

Most traditional marker techniques do not detect some sequences including those within low-copy genomic regions, non-coding regions, transposable elements, and less prolific repeats that may, however, play crucial roles in regulating some key phenotypic traits (Elshire et al., 2011; Edwards et al., 2013). These problems can be mitigated through the employment of next generation sequencing (NGS) techniques including the Illumina and Roche/454 technologies to achieve a wider *de novo* genome sequencing and gene expression analysis under stress (Berkman et al., 2012). The advent of NGS techniques has given birth to robust, as well as, cost, labour, and time effective genotyping by sequencing (GBS) approaches that are expected to aid the analysis of the structurally complex wheat genome through elimination of ascertainment biases and the need for prior genome sequence information associated with traditional techniques (Elshire et al., 2011; Poland et al., 2012). Therefore, NGS technologies, including GBS and some transcriptomic approaches, should be considered because they can contribute to the elucidation of gene expression, variety screening, single nucleotide polymorphism (SNP) marker detection, exposition of QTLs, and the discovery of candidate genes controlling drought tolerance in wheat (Berkman et al., 2012; Edwards et al., 2013). These technologies generate vast amounts of genomic data in real time. However, this requires investments and expertise in bioinformatics for management of the data. Given reduction in costs of NGS, genes involved in drought tolerance will soon be cloned to generate gene–derived markers and to enable their effective usage in breeding for drought tolerance in wheat. Among the advanced sequencing technologies, the Diversity Arrays Technology sequencing (DArTseq) platform could help to speed up the understanding and dissection of the genetic basis of complex traits including drought tolerance through provision of large marker data sets for use in genome-wide-association studies (Jaccoud et al., 2001; Akbari et al., 2006; Crossa et al., 2007; Neumann et al., 2011; Tadesse et al., 2015).

Rapid genetic gains could be realized through the use of genome engineering technique, as a promising option for improving drought tolerance through gene pyramiding, gene stacking, and gene transfer of cloned genes. Genes involved in drought stress tolerance within other species that are cross-incompatible with wheat means that a transgenic approach is the only option

available to utilize such genes (Valliyodan and Nguyen, 2006). This approach manipulates signalling molecules including transcription factors such as the dehydration-responsive element binding factors (DREB1 and DREB2) that bind to the dehydration-responsive element (DRE); ABA-responsive element binding factor (AREB) that binds to the ABA-responsive element (ABRE); and several protein kinases involved in the expression of several genes under stress (Shinozaki and Yamaguchi-Shinozaki, 2007). Important regulatory genes, those encoding proteins involved in the biosynthesis and accumulation of stress related bio-chemicals, and genes involved in post transcriptional modification of ribonucleic acid (RNA) and proteins in response to water stress have been widely reported in the literature (Umezawa et al., 2006; Valliyodan and Nguyen, 2006; Shinozaki and Yamaguchi-Shinozaki, 2007; Yang et al., 2010; Deikman et al., 2012). Yield benefits should however, be considered since some drought regulators trigger several genetic responses to drought stress, including some which cause yield reductions (Blum, 2010; Rong et al., 2014). Despite the existence of numerous potentially useful genes, the technology has not contributed to the release of drought tolerant wheat cultivars. This requires knowledge on the genetic and molecular bases of trans-genes and favourable environments for multi-location field testing of transgenic plants.

1.4.5 Integration of Transcriptomic, Proteomic, Metabolomic and Phenomic Approaches in Drought Tolerance Improvement in Wheat

Future progress in breeding for drought tolerance in wheat could be enhanced by integrating transcriptomic, proteomic, metabolomic and phenomic approaches to further unfold drought-responsive genes and signalling pathways. Lack of a genome sequence, poor genomic resources (Fleury et al., 2010), and failure to integrate such approaches may hinder further understanding of the flow of genetic information influencing drought tolerance in wheat. Advances in sequence based gene expression analysis through the use of NGS techniques could shade more light on the regulatory mechanisms and networks of this polygenic trait (Poland et al., 2012; Edwards et al., 2013). Gene expression analysis and genome-wide transcript profiling under managed stress could increase knowledge on the functions and levels of expression of thousands of drought-responsive genes. To date, several classes of genes have been confirmed to be up or down-

regulated by drought stress to enable dehydration avoidance or tolerance in various plant species including wheat (Hu and Xiong, 2014; Langridge and Reynolds, 2015).

Proteomic, metabolomic and phenomic approaches can now quantify the levels of expression of the entire set of proteins, metabolites or phenotypes under stress. Recent studies combining both transcriptomics and proteomics on wheat, showed genotypic differences in the expression of defence genes, dehydration induced transcripts associated with metabolism of carbohydrate and phyto-hormones, coupled with a rise in bio-chemicals like abscisic acid (ABA) under stress (Reddy et al., 2014; Yin et al., 2014). This envisions the application of genome wide association mapping analysis using the vast amounts of data from various OMICs analyses. Consequently, researchers can model drought co-expression networks using all gene nodes co-influencing the same biological process to further characterize the multiple signalling pathways influencing the performance of the crop under drought stress (Yin et al., 2014). Additionally, this could improve further understanding of the genetic and morpho-physiological bases of drought tolerance in wheat, enabling identification of putative QTL/gene sequences influencing drought tolerance, and ultimately allowing the realisation of significant genetic gains from selection.

1.5 Analyses of Combining Ability and Heterosis for Drought Tolerance in Wheat

Successful production of hybrids and crop genetic improvement can be achieved by determining and exploiting heterotic and combining ability effects of selected parental lines and their crosses (Akinci, 2009). Exploitation of heterosis through hybrid production has been proved to offer yield advantages and has resulted in a rapid advance in the development of high yielding and widely adapted hybrid varieties in crops such as maize (Duvick, 2005). The existence of broad genetic variability in bread wheat genotypes developed around the world offers future prospects of taking advantages of a combination of partial dominance, complete dominance, over-dominance and non-allelic gene interactions after hybridization to improve wheat adaptability (Jordaan, 1996). Nevertheless, commercial production of hybrid wheat varieties remain restricted due to fixed inter-genomic heterosis, lack of heterotic parents and difficulties in crossing due to the cleistogamous nature of its flowering system (Sharma, 2013). Several technological strategies are being improvised to overcome some of these challenges and ensure enhanced hybrid wheat production. Use of both genetic and cytoplasmic male sterility system is

increasingly being utilized to prevent selfing in promising female parents with good general combining ability effects, thus enforcing cross pollination and reducing the labor expenses associated with manual emasculation (Dong et al., 2012; Whitford et al., 2013; Ru et al., 2015). Efforts are underway to modify the floral architecture of heterotic parents to allow plants to open their flowers before pollen release to allow easy pollen transfer during hybridization (Whitford et al., 2013).

Wheat genotypes showing high and significant general combining ability effects as well as superior crosses with high and significant specific combining ability effects for important agronomic traits are widely reported in the literature (Analizleri, 2008). For complex traits like drought tolerance, heterosis can be exploited through the component approach where parents with varying traits contributing to drought tolerance are selected and crossed (Hassan et al., 2007; Farshadfar et al., 2014; Jatoi et al., 2014). Target traits that have been widely studied in combining ability studies include adaptive and constitutive morpho-physiological traits, yield and yield components (Farshadfar et al., 2014). Combining ability and gene action can be analyzed using different mating designs such as North Carolina and diallel; depending on breeding objectives, parental lines available, their mating type, and availability of skilled labor (Hill et al., 1998; Dabholkar, 1999; Omar et al., 2010;). Among these designs, diallel crosses according to Griffing (1956) are commonly used in wheat breeding to evaluate either the F1, F2, or both F1 and F2 crosses together with or without parents and reciprocals (Hill et al., 1998; Dabholkar, 1999; Omar et al., 2010; Acquaah, 2009; Akinci, 2009; Omar et al., 2010; Farshadfar et al., 2014).

1.6 Conclusions and Future Prospects

Recurrent drought associated with climate change limits global wheat production and supply. Achievements made in drought tolerance improvement are minimal, relative to investments and breeding efforts put in by various crop science disciplines working in isolation. Thus, significant progress will be achieved if breeders and other interdisciplinary experts work together with a common goal of timely production of drought tolerant and high yielding wheat cultivars. Recent technologies such as high-throughput phenotyping, next generation sequencing (NGS), and genetic engineering should be utilized for drought tolerance improvement in wheat. It should

also be noted that drought does not occur independent from other abiotic stresses and is normally associated with heat stress. Therefore, future studies should target improving prevailing stresses concurrently, to achieve improved grain yield and quality of wheat under water limited conditions.

1.7 References

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[2]CHAPTER 2. SCREENING OF BREAD WHEAT GENOTYPES FOR DROUGHT TOLERANCE USING PHENOTYPIC AND PROLINE ANALYSES

Abstract

Drought stress is one of the leading constraints to wheat (*Triticum aestivum* L.) production globally. Breeding for drought tolerance using novel genetic resources is an important mitigation strategy. This study aimed to determine the level of drought tolerance among bread wheat genotypes using agronomic traits and proline analyses and to establish correlation of proline content and agronomic traits under drought-stress conditions in order to select promising wheat lines for breeding. Ninety-six genotypes including 88 lines from the International Maize and Wheat Improvement Center (CIMMYT)'s heat and drought nurseries, and eight local checks were evaluated under greenhouse and field conditions during 2014/15 and 2015/16 making four testing environments. The following phenotypic traits were collected after stress imposed during the heading to anthesis period: the number of days to heading (DTH), days to maturity (DTM), productive tiller number (TN), plant height (PH), spike length (SL), spikelet per spike (SPS), kernels per spike (KPS), thousand seed weight (TSW), grain yield (GY), and proline content (PC). Analysis of variance, Pearson's correlation coefficient, principal component analysis and stress tolerance index were calculated. Genotypes with high yield performance under stressed and optimum conditions maintained high values for yield components. Proline content significantly increased under stress, but weakly correlated with agronomic traits under both optimal and water limited conditions. The positive correlation observed between grain yield and proline content under-drought stress conditions provides evidence that proline accumulation might ultimately be considered as a tool for effective selection of drought tolerant genotypes. The study selected 12 genotypes with high grain yields under drought stressed conditions and favorable adaptive traits useful for breeding.

Keywords: agronomic traits, drought tolerance, proline accumulation, water stress, wheat

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2.1 Introduction

Breeding drought tolerant wheat genotypes with relevant agronomic and adaptive traits is key to enhance productivity and food security among wheat growing communities. Adoption of drought tolerant genotypes is one of the most sustainable ways to reduce the impacts of marginal rainfall and prolonged dry spells on wheat production and productivity. The International Maize and Wheat Improvement Center (CIMMYT), and other national and international breeding programs are developing drought tolerant and agronomically superior wheat lines for evaluation and utilization in breeding programs (Lantican et al., 2001; Manes et al., 2012).

Phenotyping remains a key criterion for screening breeding materials based on drought adaptive and constitutive morpho-physiological characteristics including yield and its components (Monneveux et al., 2012; Passioura, 2012). Selection for such traits through the conventional plant breeding technique has significantly improved wheat productivity under both optimum and marginal rainfall conditions. Among important agronomic traits, reduced plant height (PH) is strongly related to harvest index in rain-fed cereal crops especially in water limited environments (Blum, 2010). Yield components of wheat that are relevant for drought screening include the following: number of spikelet per spike (SPS), kennels per spike (KPS), productive tiller number (TN) and thousand seed weight (TSW). Reduced number of days to heading (DTH) and days to maturity (DTM) are also important when breeding for terminal drought stress tolerance since they allow for drought escape (Lopes et al., 2012). Typically, selection should target genotypes with relatively high yields under both stressed and optimum conditions for their improved adaption to changing climatic conditions, hence there is a need to determine stress tolerance index (STI) of test genotypes. Thus, there is a need to select genotypes with a good combination of agronomically important traits, cumulatively contributing to improved yields under target drought conditions (Tardieu, 2012). Selection using controlled water application with the aid of various drought indices offers effective yield based germplasm screening, allowing for selection of high yielding genotypes under both stressed and optimum conditions.

Biochemical analyses including mannitol, glycine betaine, trehalose and proline contents, have long been proposed to be useful as a complementary strategy for selection of drought tolerant genotypes in plant breeding (Abebe et al., 2003; Bowne et al., 2012; Mwadzingeni et al., 2016).

However, this approach still requires validation for its usefulness in screening germplasm for improved yield under stressed conditions. Previous studies indicated that proline is among key biochemicals that accumulate in significant proportions in plants that are exposed to various kinds of stress, including dehydration (Hong-Boa et al., 2006; Khamssi, 2014). Proline, which is an α-amino acid, has been associated with several osmo-protection roles, including; osmotic adjustment (Marek et al., 2009; Zadehbagheri et al., 2014), membrane stabilization (Hayat et al., 2012), and gene signaling to activate anti-oxidizing enzymes that scavenge reactive oxygen species (ROS) (de Carvalho et al., 2013). Other studies have reported the regulation mechanisms of proline biosynthesis and degradation by enzymes such as Δ^1- pyrroline-5-carboxylate synthetase (P5CS) and proline dehydrogenase (PDH) respectively (Kishor et al., 2005; Szabados and Arnould, 2009). Saeedipour (2013) reported that proline content accumlated faster and in higher proportions in drought tolerant genotypes than sensitive counterparts under drought-stress conditions suggesting its value in breeding for drought tolerance. Proline content has been reported to be controlled by genes with additive effects by Maleki et al. (2010).

Information on the correlation between proline accumulations at critical growth stages of wheat with drought stressed yield and other agronomic traits is limited. Most previous studies quantified proline at the seedling stages without considering the ultimate grain yield. Also, some of the studies used too few genotypes to make conclusions that are relevant to plant breeding. Exploration of proline content under severe stress in a pool of diverse genotypes at critical growth stages and description of its correlation with the yield and its component traits will provide useful information for rapid germplasm screening when breeding for drought tolerance. There is therefore a need to intensively screen a large pool of wheat breeding lines for drought tolerance using yield, yield related traits and proline analyses. The objectives of the study were to determine the genotypic variation for drought tolerance among diverse bread wheat genotypes based on agronomic traits and proline analysis, and to identify promising lines for breeding. The study was conducted with the hypotheses that proline content at a critical drought-stress stage tends to be highly correlated with agronomic traits, particularly with grain yield, hence it can be considered as a useful and complementary selection marker. Further, it was hypothesized that candidate CIMMYT wheat lines evaluated will have higher yield potential under drought-stressed and non-stressed conditions than the local checks for drought tolerance breeding.

2.2 Materials and Methods

2.2.1 Plant Materials and Study Site

The study evaluated 96 bread wheat genotypes consisting of 88 lines from CIMMYT's heat and drought nurseries; and 8 local checks. The CIMMYT lines were selected based on their differential pedigrees. Table 2.1 lists the details of the germplasm used in the study. The lines were evaluated under greenhouse and field conditions during 2014/15 and 2015/16 making four testing environments, hereafter referred to as E1 (greenhouse 2014/15), E2 (field 2014/15), E3 (greenhouse 2015/16) and E4 (field 205/16) at the University of KwaZulu-Natal (UKZN). The greenhouse's day/night temperatures were 30ºC/20ºC, while the humidity ranged between 45% and 55%. The field experiment was conducted using soil covered with a custom-made plastic mulch to exclude rainfall and soil water evaporation at UKZN's Ukulinga Research Farm (29° 40' S, 30° 24' E; 806 m above sea level) from mid-December to May during the 2014/15 and 2015/16 growing seasons. Based on annual averages of long term climatic data, Ukulinga has a mean annual temperature and rainfall of 18°C and 738 mm, respectively. Weather data for the periods of the field trials is presented in Table 2.2.

Table 2.1. List of wheat genotypes used in the study

Entry code	Pedigree/Name Genotypes from CIMMYT's heat nursery
LM01	ACHTAR*3//KANZ/KS85-8-5/4/MILAN/KAUZ//PRINIA/3/BAV92/5/MILAN/KAUZ//PRINIA/3/BAV92
LM02	MILAN/KAUZ//PRINIA/3/BAV92/5/TRAP#1/BOW//VEE#5/SARA/3/ZHE JIANG 4/4/DUCULA
LM03	FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/ONIX
LM04	ONIX/4/MILAN/KAUZ//PRINIA/3/BAV92
LM05	ACHTAR/4/MILAN/KAUZ//PRINIA/3/BAV92
LM06	CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/KUKUNA//FRET2/6/MILAN/KAUZ//PRINIA/3/BAV92
LM07	CMSA04M00297S-040ZTP0Y-040ZTM-040SY-23ZTM-03Y-0B
LM08	SOKOLL*2/TROST
LM09	SOKOLL*2/ROLF07
LM10	GK ARON/AG SECO 7846//2180/4/2*MILAN/KAUZ//PRINIA/3/BAV92
LM11	SW89-5124*2/FASAN/3/ALTAR 84/AE.SQ//2*OPATA
LM12	SOKOLL/ROLF07
LM13	ROLF07/3/T.DICOCCON PI94625/AE.SQUARROSA (372)//3*PASTOR
LM14	MILAN/KAUZ//PRINIA/3/BAV92/4/WBLL1*2/KUKUNA
LM15	RL6043/4*NAC//PASTOR/3/BAV92/4/ATTILA/BAV92//PASTOR
LM16	PASTOR*2/BAV92/3/FRET2/KUKUNA//FRET2
LM17	ESDA/KKTS
LM18	GOUBARA-1/2*SOKOLL
LM19	SOKOLL*2/4/CHEN/AEGILOPS SQUARROSA (TAUS)//FCT/3/STAR
LM20	PBW343
LM21	PRL/2*PASTOR
LM22	MUNAL #1
LM23	QUAIU
LM24	WBLL1*2/BRAMBLING
LM25	WHEAR//2*PRL/2*PASTOR
LM26	ATTILA*2/PBW65//TAM200/TUI
LM27	YUNMAI 48//2*WBLL1*2/KURUKU
LM28	ATTILA/3*BCN//BAV92/3/TILHI/4/SHA7/VEE#5//ARIV92
LM29	PRL/2*PASTOR*2//SKAUZ/BAV92
LM30	C80.1/3*BATAVIA//2*WBLL1/3/ATTILA/3*BCN*2//BAV92/4/WBLL1*2/KURUKU
LM31	ATTILA*2/HUITES//FINSI/3/ATTILA*2/PBW65
LM32	ATTILA*2//CHIL/BUC*2/3/KUKUNA
LM33	ATTILA*2/PBW65//KACHU
LM34	WBLL1/KUKUNA//TACUPETO F2001/5/WAXWING/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
LM35	WBLL1//UP2338*2/VIVITSI
LM36	WBLL1*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/KACHU
LM37	KACHU/SAUAL
LM38	SAUAL/3/MILAN/S87230//BAV92
LM39	ATTILA/3*BCN//BAV92/3/TILHI/5/BAV92/3/PRL/SARA//TSI/VEE#5/4/CROC_1/AE.SQUARROSA (224)//2*OPATA
LM40	WBLL1*2/VIVITSI/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*JANZ
LM41	C80.1/3*BATAVIA//2*WBLL1/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES
LM42	TRCH/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES
LM43	ROLF07*2/6/PVN//CAR422/ANA/5/BOW/CROW//BUC/PVN/3/YR/4/TRAP#1
LM44	ROLF07/TUKURU/5/WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ
LM45	ROLF07/YANAC//TACUPETO F2001/BRAMBLING
LM46	FRET2/KUKUNA//FRET2/3/PARUS/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
LM47	FRET2/KUKUNA//FRET2/3/YANAC/4/FRET2/KIRITATI
LM48	FRET2/KUKUNA//FRET2/3/PASTOR//HXL7573/2*BAU/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
LM49	TRCH/SRTU//KACHU
LM50	HUW234+LR34/PRINIA*2//SNLG
LM51	HUW234+LR34/PRINIA*2//YANAC
LM52	HUW234+LR34/PRINIA*2//WHEAR

Table 2.1. (Continued)

Entry	Pedigree/name
	Genotypes from CIMMYT's heat nursery
LM53	HUW234+LR34/PRINIA*2//KIRITATI
LM54	PBW343*2/KUKUNA*2//KITE
LM55	PBW343*2/KUKUNA//PARUS/3/PBW343*2/KUKUNA
LM56	PBW343*2/KUKUNA*2//YANAC
LM57	PBW343*2/KUKUNA//SRTU/3/PBW343*2/KHVAKI
LM58	ATTILA*2/PBW65/6/PVN//CAR422/ANA/5/BOW/CROW//BUC/PVN/3/YR/4/TRAP#1/7/ATTILA/2*PASTOR
LM59	FRET2/KUKUNA//FRET2/3/WHEAR/4/FRET2/TUKURU//FRET2
LM60	ALD/CEP75630//CEP75234/PT7219/3/BUC/BJY/4/CBRD/5/TNMU/PF85487/6/PBW343*2/KUKUNA/7/CNO79//PF70354/MUS/3/PASTOR/4/BAV92
	Local checks
LM61	Check
LM62	Check
LM64	Check
LM65	Check
LM66	Check
LM67	Check
LM68	Check
LM70	Check
	Genotypes from CIMMYT's drought nursery
LM71	BABAX/3/PRL/SARA//TSI/VEE#5/4/CROC_1/AE.SQUARROSA (224)//2*OPATA
LM72	BABAX/3/PRL/SARA//TSI/VEE#5/4/WBLL1
LM73	BAU/KAUZ//PASTOR
LM75	BUC/MN72253//PASTOR
LM76	MILAN/KAUZ//PRINIA/3/BABAX
LM77	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*FRAME
LM78	SW89.5277/BORL95//SKAUZ
LM79	CROC_1/AE.SQUARROSA (205)//BORL95/3/KENNEDY
LM80	CROC_1/AE.SQUARROSA (205)//KAUZ/3/SLVS
LM81	CROC_1/AE.SQUARROSA (224)//2*OPATA/3/2*RAC655
LM82	HD30/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI
LM83	PASTOR/3/VEE#5//DOVE/BUC
LM84	SRN/AE.SQUARROSA (358)//MILAN/SHA7
LM85	SW94.60002/4/KAUZ*2//DOVE/BUC/3/KAUZ/5/SW91-12331
LM86	CHAM 6
LM87	KLEIN CHAMACO
LM88	HIDHAB
LM89	DHARWAR DRY
LM90	CROC_1/AE.SQUARROSA (205)//BORL95/3/KENNEDY-2
LM91	FRTL/CMH83.2517
LM93	PASTOR/FLORKWA.1//PASTOR
LM94	CROC_1/AE.SQUARROSA (224)//OPATA/3/PASTOR/4/PASTOR*2/OPATA
LM95	D67.2/P66.270//AE.SQUARROSA (320)/3/CUNNINGHAM
LM96	ALTAR 84/AE.SQ//2*OPATA/3/PIFED
LM97	KRICHAUFF/2*PASTOR
LM98	KABY//2*ALUBUC/BAYA
LM99	ALTAR 84/AEGILOPS SQUARROSA (TAUS)//OCI/3/VEE/MJI//2*TUI
LM100	SW89.5277/BORL95//SKAUZ

LM1-LM60, genotypes sourced from CIMMYT's heat nursery; LM61-LM70, local checks; M71-LM100, genotypes sourced from CIMMYT's drought nursery.

Table 2.2. Monthly weather data during the field trial at Ukulinga, Pietermaritzburg (2014 /15 and 2015/2016)

Year	Month	Tmax (°C)	Tmin (°C)	RHmax (%)	RHmin (%)	Rs (MJ/m^2)	ET0 (mm)
2014/15	December	26.04	15.96	99.63	53.74	17.63	109.81
	January	27.76	17.1	98.3	52.28	19.69	123.21
	February	26.22	16.55	99.87	55.42	19.44	105.66
	March	27.08	16.76	96.18	48.65	17.83	108.9
	April	23.86	13.51	97.21	46.88	14.58	81.15
2015/16	December	29.29	17.42	60.36	41.76	19.57	140.68
	January	28.38	17.41	99.85	63.85	17.47	109.47
	February	29.40	17.16	99.33	62.68	18.84	108.76
	March	28.95	17.00	98.92	61.17	16.29	102.44
	April	27.47	14.72	95.96	54.08	13.22	80.70

Tmax, average maximum temperature; Tmin, average minimum temperature; RHmax, average maximum relative humidity; RHmin, average minimum relative humidity; Rs, average total radiation; ET0, average total relative evapo-transpiration.

2.2.2 Experimental Design and Crop Establishment

The 96 genotypes were evaluated using a lattice design with two replications containing six incomplete blocks with sixteen genotypes each and two water regimes (under stressed and non-stressed conditions). The stressed treatment involved withholding irrigation to 35% field capacity (FC) before re-watering. Stressed treatment was induced from 50% heading to physiological maturity in order to simulate terminal drought stress. The field plots were 1.5 m long rows with inter-row and in-row spacing of 45 cm and 15 cm respectively. Concurrent drought tolerance studies were conducted in an environmentally controlled greenhouse using pots as experimental units. Plastic pots of 5L capacity filled with composted pine bark growing media were used, with seven plants of one genotype established in each pot. Other agronomic practices were carried out following standard guidelines for wheat production in South Africa (DAFF, 2010).

2.2.3 Data Collection

Data was collected on the following phenotypic traits: days to heading (DTH) were calculated as the number of days between the sowing date and the date when 50% of all the shoots in a plot had fully emerged spikes. The number of productive tillers (TN) was recorded at physiological maturity and plant height (PH) was measured in centimeters (cm) from the ground to the tip of the spike from five randomly sampled and tagged plants in each plot before harvesting. Days to maturity (DTM) were calculated from sowing date to 50% senescence of the spikes. The spike length (SL) [measured in cm], the number of spikelets per spike (SPS) and the numbers of kennels per spike (KPS) were recorded after harvesting from the main tillers of five randomly selected plants. Thousand seed weight (TSW) was determined using a sensitive balance measured from randomly sampled 1000 seeds after harvest and expressed in g/1000 seed. Finally grain yield per plot (GY) was determined as the weight (grams) of the grain from a plot; where the plot sizes were 1.5 meter rows with 30 plants, and seven plants per pot for the field and the greenhouse experiments respectively. From the pot experiment grain yield was extrapolated based on 30 plants to agree with field data.

2.2.4 Determination of Proline Content

Proline analysis was carried out at the University of KwaZulu Natal's Crop Science laboratories. Samples of the second top leaves from the flag leaf were harvested from the stressed and non-stressed plots of the two greenhouse experiments. The leaf samples were temporarily stored at ultra-low temperature (-74ºC) then freeze dried. The dry leaf tissue was ground and 0.1g samples were homogenized in 10 mls of 3% aqueous sulfosalicylic acid. Proline extraction was done following the acid-ninhydrin method according to Bates et al. (1973). This was followed by UV-visible spectrophotometer analysis of the absorbance of the proline extract in toluene at a wavelength of 520 nm, using a model UV-1800 spectrophotometer, Shimadzu Corporation, Kyoto, Japan. The proline concentration was calculated using the following formula:

Proline content (µg per gram of dry leaf tissue) = [(µg proline/ml) x ml toluene)/115.5 µg/µmole]/ [(g sample)/5]. Where, 115.5 is the molecular weight of proline (Bates et al., 1973).

2.2.5 Data Analysis

Phenotypic and proline data were analyzed separately following the lattice procedure of SAS 9.3 (SAS, 2011) and GenStat® version 17, VSN, International (Payne, 2014). Combined analysis of variance was performed following a test of homogeneity of variances. Pearson's correlation coefficients (*r*) were calculated separately for the stress and control treatments using the SPSS version 23 (Spss, 2012). Principal component analysis (PCA) based on the correlation matrix was performed using SPSS to identify influential traits for selection. PCA biplots were plotted separately for the stressed and optimum conditions using GenStat to show the relationships among studied genotypes based on recorded traits. Stress tolerance index (STI) was calculated using the following formula according to (Fernandez, 1992):

STI = (Yp*Ys) / $(Xp)^2$; where Ys = grain yield of a test genotype under drought-stressed condition; Yp = grain yield of a test genotype under non-stressed condition, and Xp = mean yield of test genotypes under non-stressed condition.

2.3 Results

2.3.1 Effect of Genotypes, Water Regimes and Testing Environments on Agronomic Performance and Proline Content

Separate analysis of variance showed significant ($P < 0.001$) effects of the genotype, water regime, environments and their interactions for the studied traits, hence, combined analysis of variance was carried out. Table 2.3 summarizes the results from the combined analysis of variance for agronomic traits and proline content. Highly significant differences were observed among the main effects of genotypes, water regimes, environments, and their interactions for most traits. DTH, DTM, SL and SPS were not significantly affected by the interaction of the genotype by water regime and environment by genotype by water regime, while TN showed non-significant effects of the genotype by water regime by environment interaction only.

Table 2.3. Mean squares and significant tests after combined analysis of variance for nine phenotypic traits and proline content of 96 wheat genotypes evaluated across the four test environments and two water regimes

	Agronomic traits										Proline content	
Sources of variation	DF	DTH	DTM	TN	PH	SL	SPS	KPS	TSW	GY	DF	PC
Gen	95	346.83**	199.35**	3.15**	729.28**	18.76**	31.33**	340.26**	193.22**	9229.26**	95	10392.18**
WR	1	47.83*	6651.85**	455.43**	7791.86**	7.01**	83.45**	5128.34**	6804.68**	1978219**	1	3330364**
Env	3	6324.15**	44781.15**	118.90**	7888.87**	289.99**	2215.79**	30244.35**	2664.44*	1252898**	1	985417.73**
Gen.WR	95	9.201 ns	18.27 ns	1.64**	40.47**	0.25 ns	1.64 ns	43.79**	27.91*	4287.05**	95	10395.28**
Gen.Env	285	43.72**	53.12**	0.86**	54.85**	0.58**	3.06**	34.09*	44.19**	2666.05**	95	8014.72**
Env.WR	3	45.07*	450.59**	2.19*	1064.72**	4.28**	44.10**	387.08**	264.56**	22525.67**	1	1730641.89**
Env.Gen.WR	285	9.47 ns	18.77 ns	0.65 ns	29.14*	0.29 ns	1.74 ns	30.94*	23.17*	2171.13*	95	7710.46**
Residual	765	9.14	21.43	0.64	24.77	0.28	1.68	26.11	19.65	1736.83	382	45.67

DF, degrees of freedom; DTH, days to 50% heading; Env, testing environment; Gen, genotype; PH, plant height; TN, number of productive tillers; DTM, days to maturity; SL, spike length; SPS, number of spikelets per spike; KPS, number of kennels per plant; TSW, thousand seed weight; GY, grain yield per plot; PC, proline content; WR, water regime; *, $P < 0.05$; **, $P < 0.01$; ns, non-significant difference.

Table 2.4 summarizes the mean values; standard error of differences (SED), least significant differences (LSD) at 5% significant levels, and coefficients of variation (CVs) obtained for all traits recorded under the two water regimes. The table shows the best fifteen and bottom five genotypes in terms of grain yield under stressed conditions. The pooled mean values obtained for all traits recorded across all testing environments for all genotypes and their respective stress tolerance index (STI) values are presented in Appendix 2.1. Significant differences were noted in the overall means of the different variables recorded. Significant differences were noted in the overall means of the different variables recorded. The mean DTH was 53.62 days with the earliest genotypes being the local checks LM66 and LM67 which took 43 and 43.63 days to heading respectively, and the latest genotype was LM100 from the heat nursery which took 61.88 days. The mean plant heights under stressed and optimum conditions were 73.52 cm and 78.03 cm, respectively. Under stressed conditions, the shortest genotype was the local cultivar LM67 (58.51 cm), while the tallest was LM77 (89.88 cm) from the drought nursery. The lines LM90, LM84 and LM100 were the tallest under optimum conditions with average height of 90.68, 90.53 and 90.06 cm respectively, while genotype LM53 was the shortest (61.18 cm).

A reduction in average tiller numbers was observed from 4.45 to 3.36 due to severe drought stress. Genotypes LM64 and LM84 developed the highest number of productive tillers, 4.74 and 6.33 under stressed and optimum conditions, respectively; while LM62 and LM95 had the least number of tillers, 1.99 and 2.81, under stressed and optimum conditions, respectively. A slight decrease in average spike length from 8.79 cm under optimum growing conditions to 8.65 cm under stress was observed. Average DTM were slightly lower under stress (98.97 days) than under optimum conditions (103.13 days). Lines LM89 which took 106 days to mature was the latest under stress while lines LM84 (109.62 days) and LM49 (109.87 days) were among the latest genotypes under optimum conditions. LM03 which took 91 days to maturity and LM08 which matured after 94.37 days were the earliest under stressed and optimum conditions, respectively. Means of SPS, KPS and TSW under stress were slightly lower than the values under optimum conditions (Table 2.4). The average grain yield per plot was reduced by 40.64% under stress as compared to the control. The minimum and maximum stress tolerance index were 0.12 and 1.0 observed on the genotypes LM61 and LM23, respectively. Mean STI was 0.60 with 75% of the genotypes having above average STI.

Proline content varied significantly among genotypes, water regimes and the genotype by water regime interactions. Water regime accounted for much of the variation observed, explaining 54.75% of the variation in proline content. The genotype explained only 0.17% while testing environments, genotype by water regime, genotype by environment and genotype by water regime by environment interactions accounted for 16.2%, 0.17%, 0.13 and 0.13% respectively (Table 2.3). The mean PC was 24.5 µg and 156.2 µg per gram of dry leaf tissue under optimum and stressed conditions, respectively. The highest PC contents were 381.18 and 46.72 µg/gram of dry leaf sample, obtained from lines LM41 and LM29 under stressed and optimum conditions, respectively (Table 2.4).

Table 2.4. Means for nine agronomic traits and proline content of 96 wheat genotypes and the top 15 best and five bottom performing genotypes when evaluated under stressed and non-stressed across the test environments, ranked according to their performance under stressed conditions

Top fifteen genotypes																				
	DTH		DTM		TN		PH		SL		SPS		KPS		TSW		GY1		PC	
Entry	WR1	WR2	WR1	WR2	WR1	WR2	WR1	WR2	WR1	WR2	WR1	WR2	WR1	WR2	WR1	WR2	WR1	WR2	WR1	WR2
LM29	55.63	58.13	103.12	107.25	3.80	4.66	76.28	79.20	8.39	8.77	15.03	15.53	37.37	38.85	34.70	37.20	149.70	205.00	67.89	46.72
LM22	56.24	57.50	101.12	104.12	4.09	4.73	74.61	79.06	8.59	8.72	14.68	15.35	32.20	38.72	32.49	36.37	143.30	201.10	214.89	26.00
LM04	56.25	56.88	101.87	105.00	3.48	4.19	76.93	77.68	10.41	10.04	16.85	16.33	39.40	41.05	33.43	36.51	138.90	192.20	93.77	13.08
LM77	55.00	55.00	99.75	103.00	3.70	4.14	87.90	89.71	9.10	9.10	14.45	14.43	31.72	33.50	36.79	45.43	136.20	194.00	86.52	24.12
LM15	57.50	56.88	103.12	107.75	3.59	4.61	75.95	85.81	9.61	9.92	15.58	16.98	37.62	45.90	30.33	36.47	135.00	231.70	307.70	24.92
LM71	53.88	53.88	94.87	101.87	4.05	4.86	75.31	80.01	9.29	9.46	14.43	15.05	34.10	36.87	29.44	32.86	131.50	181.90	97.37	42.91
LM23	56.25	57.50	98.87	103.50	3.95	6.08	80.68	88.64	9.54	10.15	14.78	16.23	34.52	36.12	32.16	38.50	128.80	258.40	217.09	19.58
LM100	61.88	61.88	106.00	108.00	3.48	4.03	79.49	90.06	9.77	10.04	14.73	15.80	33.57	41.07	36.50	38.77	127.20	198.10	204.71	23.08
LM27	54.38	56.25	95.50	98.25	3.41	3.97	76.60	75.03	8.37	8.20	15.78	15.60	37.75	39.27	31.96	33.56	126.90	162.30	82.92	25.84
LM85	56.88	56.25	100.25	104.00	3.74	4.86	72.83	79.82	8.60	8.92	15.03	15.67	35.47	35.59	30.75	35.09	126.50	225.50	78.56	28.96
LM96	55.63	55.63	102.75	106.25	4.08	4.65	83.28	88.27	7.89	7.99	15.23	15.13	35.47	36.05	27.99	35.69	126.30	179.20	322.91	23.36
LM03	51.50	51.88	91.75	100.00	3.79	4.53	83.16	86.58	9.50	9.54	15.88	15.88	36.07	38.15	28.07	39.98	126.00	210.30	159.30	20.22
LM31	52.00	54.38	96.50	101.87	3.52	4.24	78.32	78.34	9.67	9.24	14.13	13.30	32.72	30.79	33.11	42.82	125.50	175.00	192.59	28.92
LM35	52.00	50.38	97.87	101.62	3.52	5.30	76.85	82.63	8.35	8.71	15.30	15.85	37.45	39.67	30.96	34.31	125.30	226.20	176.90	20.44
LM44	52.63	54.38	94.75	98.12	3.43	3.95	83.02	85.77	9.82	10.00	15.73	16.00	42.02	45.47	26.87	28.93	123.80	162.50	97.96	19.56
Bottom five genotypes																				
LM20	61.25	60.00	103.72	106.00	2.86	4.32	69.93	72.73	9.14	8.95	15.75	15.68	22.47	29.82	37.10	38.34	69.90	151.30	190.68	25.39
LM95	45.50	48.00	94.50	97.37	2.57	2.81	60.90	62.54	7.08	6.97	12.33	12.60	29.30	30.35	28.68	31.45	64.30	79.90	75.44	16.02
LM68	48.13	50.63	96.25	101.75	3.39	4.28	59.05	64.64	6.67	7.06	13.07	14.68	30.23	36.42	21.05	24.89	60.90	130.80	234.88	15.06
LM62	60.63	58.13	104.87	106.87	1.99	3.33	64.06	65.64	9.78	10.21	15.78	17.14	32.20	39.75	27.15	30.71	58.70	124.40	165.07	26.65
LM61	45.38	44.75	93.12	96.62	3.32	3.52	59.92	62.26	5.97	5.96	10.98	10.63	19.72	20.80	29.09	30.18	50.30	75.00	85.99	16.53
Mean	53.45	53.80	98.97	103.13	3.36	4.45	73.52	78.03	8.65	8.79	14.71	15.18	32.87	36.52	30.72	34.93	104.83	176.60	156.20	24.50
SED	1.07	1.07	1.64	1.64	0.28	0.28	1.76	1.76	0.19	0.19	0.94	0.94	1.81	1.81	3.20	3.20	29.79	29.79	3.38	3.38
LSD (5%)	4.41	4.41	6.77	6.77	0.55	0.55	7.08	7.08	0.74	0.74	0.90	0.90	3.55	3.55	6.28	6.28	28.93	28.93	6.64	6.64
CV (%)	5.60	5.60	4.60	4.60	20.50	20.50	6.60	6.60	6.00	6.00	8.70	8.70	14.70	14.70	13.50	13.50	29.60	29.60	7.50	7.50

DTH, days to 50% heading; CV, coefficient of variation; DTM, days to maturity; E1, test environment 1 (greenhouse 2014/15); E2, test environment 2 (field 2014/15); E3, test environment 3 (greenhouse 2015/16); E4, test environment 4 (field 2015/16); GY, grain yield per plot; LSD, list significant difference; KPS, number of kennels per plant; PH, plant height; SED; standard error of differences; SPS, number of spikelets per spike; SL, spike length; TN, number of productive tillers; TSW, thousand seed weight; WR 1, water regime 1 (water-stressed), WR2, water regime 2 (control).

2.3.2 Correlations of Phenotypic Traits and Proline Content

Table 2.5 summarizes correlation coefficients (r) describing the degree of correlations among measured agronomic traits and proline content. Under well-watered conditions, the number of days to heading showed a significant and positive correlation ($r > 0.5$, $P < 0.05$) with most of the variables recorded except for TSW and PC. Under stress, the number of days to heading was highly and significantly correlated with DTM, PH, SL and SPS, but a weakly negative correlation with TN. Plant height significantly correlated with all traits except proline content under both stressed and well-watered conditions, as well as with the number of days to maturity under stressed conditions. Notably, productive tiller numbers showed a positive correlations with GY under both stressed and optimum conditions. Days to maturity had a positive correlations with DTH under both stressed and optimum conditions, but with weak negative and insignificant correlations with TN and PC. Further, spike length had a positive and significant correlations with DTH, PH, SPS, and KPS under both stressed and optimum conditions, as well as with grain yield under well-watered conditions. Grain yield under stress was highly correlated with TN, with moderately high correlations with PH, KPS and TSW under stress. On the other hand, under optimum conditions, grain yield was highly and significantly correlated with all yield components except TSW which showed moderate correlation. Proline content had weak positive and non-significant correlations ($r < 0.3$, $P > 0.05$) with all traits under both stressed and optimum conditions, except for DTM and TSW which were weak and negatively correlated with PC under stress.

Table 2.5. Pearson's correlation coefficients (*r)* describing association of nine phenotypic traits and proline content of 96 wheat genotypes evaluated under two greenhouse and two field experiments of stressed (lower diagonal) and optimal (upper diagonal) conditions

		Optimum conditions									
		DTH	DTM	TN	PH	SL	SPS	KPS	TSW	GY	PC
Stressed conditions	DTH	1	0.776**	0.178 ns	0.689**	0.648**	0.749**	0.491**	0.269**	0.498**	0.178 ns
	DTM	0.723**	1	0.092 ns	0.472**	0.473**	0.581**	0.365**	0.216*	0.349**	0.126 ns
	TN	-0.299**	-0.135 ns	1	0.251*	0.047 ns	0.059 ns	0.033 ns	0.04	0.653**	0.199 ns
	PH	0.557**	0.191 ns	-0.106	1	0.687**	0.611**	0.461**	0.473**	0.634**	0.161 ns
	SL	0.630**	0.300**	-0.370**	0.619**	1	0.727**	0.603**	0.292**	0.510**	0.069 ns
	SPS	0.709**	0.386**	-0.394**	0.618**	0.725**	1	0.773**	0.141 ns	0.547**	0.122 ns
	KPS	0.297**	0.034 ns	-0.238*	0.500**	0.530**	0.668**	1	-0.104	0.593**	0.081 ns
	TSW	0.308**	0.398**	-0.062	0.254*	0.215*	0.136 ns	-0.209*	1	0.414**	0.078 ns
	GY	0.141 ns	0.115 ns	0.543**	0.443**	0.244*	0.270**	0.466**	0.336**	1	0.197 ns
	PC	0.002 ns	-0.043 ns	0.118 ns	0.030 ns	0.170 ns	0.057 ns	0.138 ns	-0.218*	0.080 ns	1

DTH, days to 50% heading; DTM, days to maturity; GY, grain yield per plot; KPS, number of kennels per plant; PC, proline content; PH, plant height; TN, number of productive tillers; SL, spike length; SPS, number of spikelets per spike; TSW, thousand seed weight; *, $P < 0.05$ (2-tailed); **, $P < 0.01$ level (2-tailed); ns, non-significant.

2.3.3 Principal Component Analysis (PCA)

The rotated component matrix (Table 2.6) shows the proportion of total variance explained by different principal components and their correlations with variable traits. From the stress treatment, three principal components were important, contributing 72.44% of the total variation observed. The first two principal components were the most influential with a cumulative contribution to the total variation of 56.44%. Variables SPS, SL, KPS, PH and DTH had high positive loading into the first principal component while DTH, TSW and DTM had high positive loading into the second principal component. These were followed by GY and PC which had high positive loading into the third principal components respectively. Similarly, three principal components were important under optimum conditions, accounting for 73.38% of the total variation of which 61.92% was accounted for by the first two components. All traits except TN, PC and TSW had high positive loading into the first principal component while TN had high positive loading into the second principal component.

Table 2.6. Rotated component matrix of nine phenotypic traits and proline content of 98 wheat genotypes evaluated in four test environments under stressed and optimum conditions

Stressed conditions				Optimum conditions			
Trait	PC-1	PC-2	PC-3	Trait	PC-1	PC-2	PC-3
SPS	0.908	0.097	-0.097	SPS	0.864	-0.315	0.145
SL	0.854	0.095	-0.071	DTH	0.862	-0.127	-0.100
KPS	0.791	-0.397	0.119	PH	0.835	0.130	-0.222
PH	0.764	0.119	0.251	SL	0.817	-0.222	-0.092
DTH	0.725	0.513	-0.13	GY	0.778	0.470	0.181
TSW	0.104	0.802	0.246	KPS	0.715	-0.337	0.455
DTM	0.361	0.712	-0.058	DTM	0.701	-0.184	-0.150
PC	0.196	-0.442	0.141	TN	0.296	0.782	0.388
GY	0.375	0.058	0.89	PC	0.224	0.383	0.188
TN	-0.372	-0.078	0.838	TSW	0.378	0.376	-0.780
Explained variance (eigenvalue)	3.934	1.71	1.6		4.743	1.449	1.146
Proportion of total variance (%)	39.34	17.1	16.004		47.432	14.487	11.458
Cumulative variance (%)	39.34	56.44	72.444		47.432	61.919	73.377

DTH, days to 50% heading; DTM, days to maturity; GY, grain yield per plot; KPS, number of kennels per plant; PC, proline content; PC-1, principal component 1; PC-2, principal component 2; PC-3, principal component 3; PH, plant height; TN, number of productive tillers; SL, spike length; SPS, number of spikelets per spike; TSW, thousand seed weight.

2.3.4 Principal Component Biplot Analysis

The relationships between the different variables and genotypes with respective principal components are further illustrated by the principal component biplots in Figure 2.1 and Figure 2.2 for the stressed and optimum conditions respectively. Smaller angles between dimension vectors in the same direction indicated high correlation of the variable traits in terms of discriminating genotypes. Genotypes excelling in a particular trait were plotted closer to the vector line and further in the direction of that particular vector, often on the vertices of the convex hull. Under stress, most of the genotypes were scattered in the positive side of the first principal component, with genotypes LM22, LM96, LM02 and LM15 excelling in yield which

was contributed mostly by high tiller numbers and KPS, as well as optimum values for other yield components (Figure 2.1). Under optimum conditions, the genotypes were also more concentrated on the positive side of the first principal component with genotype LM09, LM17, LM80, LM84 and LM23 being more inclined in the direction of GY, PC, PH, TSW and TN (Figure 2.2). The local checks LM61, LM64, LM66 and LM67, and line LM95 clustered together in the direction of early heading and short stem height.

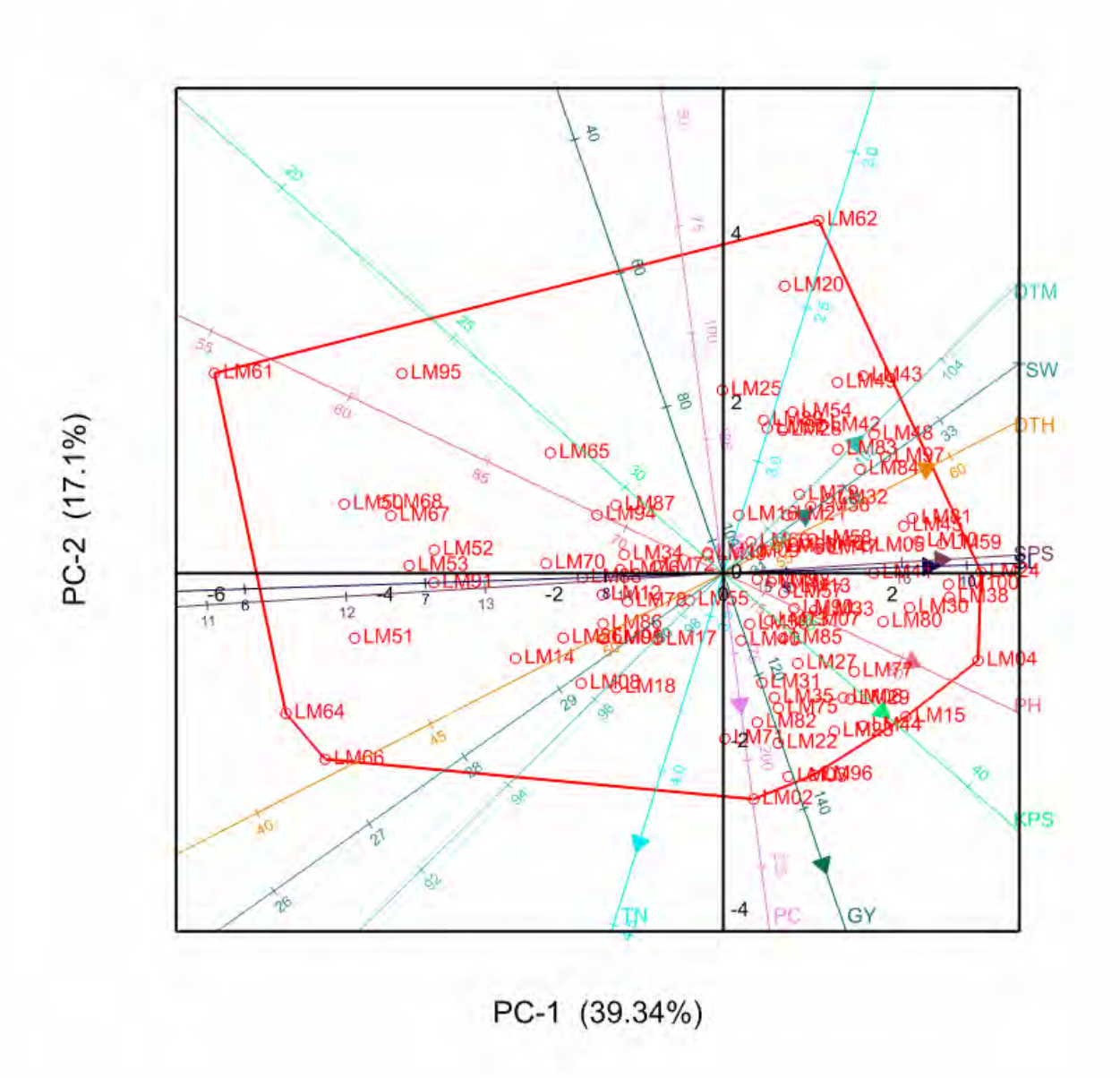

Figure 2.1 Principal component biplot showing genotypic grouping under stress. DTH, days to 50% heading; DTM, days to maturity; GY, grain yield per plot; SL, spike length; KPS, number of kennels per plant; PC, proline content; PH, plant height; TN, number of productive tillers; SPS, number of spikelets per spike; TSW, thousand seed weight.

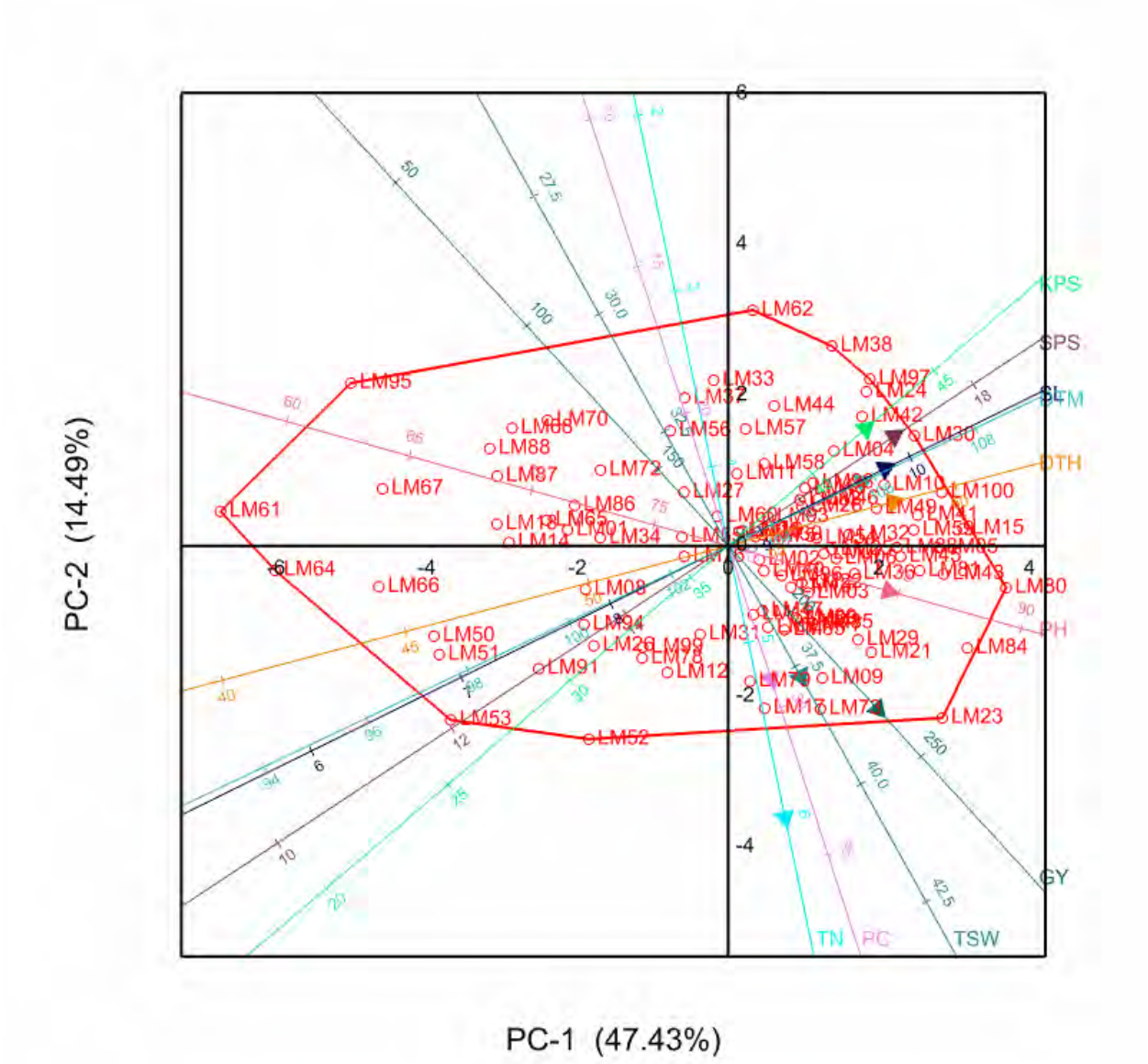

Figure 2.2 Principal component biplot showing genotypic grouping under optimum conditions. DTH, days to 50% heading; DTM, days to maturity; GY, grain yield per plot; SL, spike length; KPS, number of kennels per plant; PC, proline content; PH, plant height; TN, number of productive tillers; SPS, number of spikelets per spike; TSW, thousand seed weight.

2.4 Discussion

Effective germplasm screening for drought tolerance particularly under managed drought conditions is an effective way of selecting materials for advanced breeding programs. The highly significant genotype differences observed among all the traits recorded indicate that the germplasm pool used in this study is a rich source of genetic diversity for breeding purposes (Table 2.3). Thus, the germplasm pool can be used to identify genotypes with high levels of tolerance to water stress, as indicated by differential genotype responses to the two water regimes.

2.4.1 Effect Of Genotypes and Water Regime on Grain Yield

Selecting for improved grain yield under both stressed and optimum conditions allow genotypes to maintain ranks for high yields since the same genotypes will be expected to perform well in either situation. The observed maintenance of high yields under stressed and optimum conditions in some genotypes such as LM03, LM23 and LM85 supports the findings of Foulkes et al. (2007) that genotypes performing well under optimum conditions retain high yield under stress. However, the high cross-over interactions observed in this study was due to severe stress imposed on the genotypes resulting in average yield losses of about 41% compared to 26% observed under mild stress imposed by Foulkes et al. (2007). Interestingly, twenty-two genotypes from the heat and drought nurseries including LM15, LM22, LM29, LM27, LM77 and LM96 yielded better than all local checks under stress. Generally, most of the materials from the heat and drought nurseries were better adapted to the summer planting than the local checks because they were prebred for heat and drought tolerance. Therefore, can provide useful diversity for spring cultivation.

2.4.2 Association of Agronomic Traits under Different Water Regimes and Testing Environments

The positive and significant correlations ($r > 0.3$) of GY with TN, KPS and TSW under both stressed and optimum conditions, imply the direct contribution of these yield components to yield and should be considered as important target traits during selection, as is supported by the findings of Dodig et al. (2012) and Sareen et al. (2014). This resulted in high stressed GY in

lines such as LM22, LM29, LM77, LM15, LM24 and LM100. From Figure 2.1, it can be confirmed that maintenance of a large number of productive tillers and kernels per spike contributes more to the grain yield when compared to the other yield components under stress because the number of grains produced per plant will compensate better for the reduction in seed weight (Slafer et al., 2014). However, under optimum conditions (Figure 2.2), all the yield components have considerable contribution to grain yield implying that selection for any of the yield components could significantly improve the yields. Late maturing and tall genotypes have enough time and capacity to accumulate photo-assimilates resulting in higher grain yields, which explains the positive correlation of DTH, DTM, PH and SL with GY under optimum conditions. However, under stress, genotypes excelling in the former traits succumbed to drought stress due to high evapo-transpiration losses and ultimately suffered much yield losses. This resulted in the moderate to low correlations of DTH, DTM, PH and SL with GY under water stress. This could be the reason for the decline in ranks under stress of most genotypes including LM23 and LM80 which excelled under optimum conditions. However, plant height could also be associated with deeper and extensive rooting systems since some tall genotypes such as LM23 and LM03 maintained high yield under both stressed and well-watered conditions. Genotypes with high yield under both stressed and non-stressed conditions exhibited high STI which further confirm the reliability of this index in selecting for high productivity under either condition (Fernandez, 1992).

Early heading and maturity have an advantage of allowing drought escape, enabling the genotype to efficiently utilize irrigation or rainfall during critical growth stages (Blum, 2010). However, the plant cycle should not be too short and the plant size should not be too small since such traits will compromise yields in either situation as evidenced by a yield penalty in earliest and shortest genotypes like LM70, LM95 and the local checks LM61 and 68. This is in agreement with the findings of Butler et al. (2005) where short wheat genotypes with two alleles for dwarfness; *Rht-B1b* and *Rht-D1b,* yielded lower than those with one or none of the dwarfing alleles, under both stressed and optimum conditions. The findings may be attributed to low capacity to accumulate sufficient stem reserves for subsequent partitioning to the grain (Borrell et al., 1993). The local check LM66 was among early and short genotypes and excelled in stressed yield by its ability to maintain a high number of productive tillers and a relatively high TSW. This could have resulted

from a lengthy grain filling period (Dodig et al., 2012). However, the small plant stature compromises other yield components of such genotypes, thereby reducing the rank in yield potential under optimum conditions.

The principal component analysis indicated that under stress SPS, SL, DTH, PH and KPS have much influence during selection and can be selected together followed by TSW and DTM respectively (Table 2.6). This further emphasizes the importance of selecting genotypes based on yield components which could result in simultaneous selection for complementary genes adding up to yield. Putting much emphasis on few major genes may result in increased survival rate at the expense of grain yield (Passioura, 2012). Under optimum conditions, high positive loading of SPS, DTH, PH, SL, GY, KPS and DTM into the first principal component indicate that they have much influence and can be simultaneously selected for because of their direct influence on each other (Table 2.6). This could be explained by the fact that genotypes with longer life cycles and increased plant height have more time for photo-assimilate production and have the capacity to accumulate more biomass. Hence they will have high grain yield.

2.4.3 Effect of Water Regime on Proline Accumulation

The variation in proline content observed among the different genotypes under both stressed and well-watered conditions and its accumulation under stress was in accordance with previous findings. Rampino et al. (2006), Vendruscolo et al. (2007), Bowne et al. (2012) and Qayyum et al. (2013) reported genotypic differences in proline concentration, and in proline accumulation in wheat genotypes exposed to water stress. Nio et al. (2011) reported of increased proline content in wheat exposed to stress, implying some levels of osmotic adjustment. Similar effects of water stress and increased PC were observed in other crops, including sugar beet (*Beta vulgaris* L.) (Gzik, 1996), alfalfa (*Medicago sativa* L.) (Irigoyen et al., 1992), and pea (*Pisum sativum* L.) (Sánchez et al., 1998).

The non-significant correlation observed between the proline content and stressed yield under controlled environment suggests that, although proline plays an important role of osmo-protection, it may not be a good reflection of stressed yield levels. These findings are in agreement with by Tardieu (2005) who argued that genes encoding desiccation tolerance may

not enhance yield under agricultural drought. The findings from this study are also reported by Marek et al. (2009) who observed low and non-significant correlations of proline content and grain yield under drought stress. The results do not support the hypothesis that proline can serve as an important biochemical marker or selection index for indirect selection for stressed yield, which is of breeders' interests. However, the presence of positive correlation between proline content and grain yield suggests that PC remains an important trait in enhancing the capacity of genotypes to optimize grain yields under drought-stress. However, further research under multiple environments is required to confirm if this is not only true under the specific experimental design applied in this study. Despite the poor correlation of proline content with stressed yield suggesting that some genotypes take advantage of the capacity to accumulate more proline under stress as was noted in the drought tolerant wheat cultivar, Chinese Spring, when compared to the susceptible cultivar, SQ1 (Marcińska et al., 2013). There is therefore a need to take advantage of such genotypes. These results provide a good practical insight and add on to previous studies that used external osmotica such as polyethylene glycol (PEG), which may need to be confirmed by actual soil water deficit since hydroponic conditions cannot provide an accurate mimic of the soil environment. Some of the studies evaluated a small number of genotypes which needed to be increased to make meaningful conclusions and recommendations for breeding. Others determined the proline accumulation at seedling stage which needed to be confirmed by genotypic responses when exposed to water stress at critical growth stages. The positive correlation of grain yield with proline content under drought stressed conditions observed in the present study supported these previous studies in that proline accumulation is a good indicator of drought tolerance in wheat which could be useful during genotype selection.

2.5 Conclusions

Proline accumulates under stress, but proline, when measured at a single time point, may not serve as a good predictor or marker for indirect selection for yield under agricultural conditions. However, the positive correlation between grain yield and proline content under-drought stress conditions provides already evidence that proline accumulation might ultimately be considered as a tool for effective selection. Further studies are required to quantify proline content at different stress levels to explore the rate of proline accumulation in different genotypes during time of stress exposure and yield potential of genotypes. The current study also deduced that the

material evaluated contain useful genetic diversity for drought tolerance. Promising lines such as LM02, LM04, LM05, LM09, LM13, LM17, LM21, LM22, LM23, LM29, LM45 and LM85 with high yield under stressed conditions have been selected for use in breeding for drought tolerance based on their diverse and complementary agronomic traits recorded in this study that could further enhance grain yield. The currently selected lines showed higher mean grain yields under drought-stress and higher stress tolerance indices than the local checks (LM61 to LM70). The lines are part of CIMMYT's nursery distributed worldwide. In South Africa, they will add to the germplasm pool identified by Dube et al. (2015).

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Appendix 2.1. Means of agronomic traits, proline content and stress tolerance index (STI) of 96 wheat genotypes when evaluated under stressed and non-stressed conditions

	DTH		DTM		TN		PH		HL		SPS		KPS		TSW		GY			PC1	
Entry	WR1	WR2	WR1	WR2	WR1	WR2	WR1	WR2	WR1	WR2	WR1	WR2	WR1	WR2	WR1	WR2	WR1	WR2	STI	WR1	WR2
LM01	44.75	44.25	96.25	97.37	3.34	4.20	68.76	73.54	8.42	8.47	14.10	14.03	34.00	37.87	30.62	32.94	112.30	170.10	0.61	153.69	18.07
LM02	50.38	49.00	92.75	100.62	3.51	3.89	79.61	81.98	9.28	9.29	14.73	14.88	37.92	43.30	28.23	36.88	122.00	195.00	0.76	350.65	36.51
LM03	51.50	51.88	91.75	100.00	3.79	4.53	83.16	86.58	9.50	9.54	15.88	15.88	36.07	38.15	28.07	39.98	126.00	210.30	0.85	159.30	20.22
LM04	56.25	56.88	101.87	105.00	3.48	4.19	76.93	77.68	10.41	10.04	16.85	16.33	39.40	41.05	33.43	36.51	138.90	192.20	0.86	93.77	13.08
LM05	58.88	60.00	106.62	108.87	3.42	5.06	71.78	80.88	8.91	8.95	15.43	16.40	35.32	46.32	32.50	34.34	115.30	239.80	0.89	173.52	20.82
LM06	52.63	53.75	98.75	104.75	3.35	4.47	77.66	82.54	9.85	9.90	15.15	15.50	36.92	39.80	32.29	37.98	121.50	201.30	0.78	309.59	25.75
LM07	56.88	58.13	103.25	106.00	3.69	4.85	79.38	82.53	7.97	8.07	15.30	16.14	33.60	40.69	29.84	32.06	113.30	196.60	0.71	184.32	27.05
LM08	50.25	49.63	93.62	94.37	3.71	4.34	68.47	74.13	8.02	8.13	13.99	14.35	33.15	35.80	27.69	33.87	109.60	165.00	0.58	201.19	29.42
LM09	59.38	56.88	101.62	106.87	3.84	5.83	69.35	83.55	8.86	8.73	14.63	14.40	29.27	33.82	32.23	37.69	111.30	234.80	0.84	114.76	9.82
LM10	57.50	58.75	102.50	106.62	3.09	4.41	72.59	81.22	10.26	10.42	15.88	16.13	36.37	40.37	35.24	35.57	114.80	198.00	0.73	180.66	19.78
LM11	52.50	51.38	97.75	97.87	3.21	4.25	73.84	79.93	9.08	9.50	14.83	16.50	31.37	42.42	28.91	31.03	95.40	171.10	0.52	196.93	24.05
LM12	52.00	51.50	94.87	96.37	3.80	4.67	75.18	79.93	8.25	8.52	14.35	14.70	27.47	31.45	28.62	39.82	95.20	179.20	0.55	122.78	31.27
LM13	55.63	53.13	98.87	99.87	3.51	5.05	80.07	83.98	8.53	8.94	15.38	15.93	35.20	41.07	29.36	32.69	108.10	212.70	0.74	48.62	21.77
LM14	44.25	44.25	98.75	98.87	3.84	3.83	68.22	72.00	7.74	7.92	12.80	13.29	29.17	33.81	32.07	33.84	117.20	138.50	0.52	145.79	28.61
LM15	57.50	56.88	103.12	107.75	3.59	4.61	75.95	85.81	9.61	9.92	15.58	16.98	37.62	45.90	30.33	36.47	135.00	231.70	1.00	307.70	24.92
LM16	53.25	53.75	96.62	98.37	2.93	4.44	77.07	82.72	9.17	9.81	13.95	15.35	33.30	37.37	33.28	34.06	96.40	174.50	0.54	127.82	21.97
LM17	50.25	50.25	95.12	100.25	3.29	5.32	71.87	78.11	8.78	9.20	14.00	14.68	35.32	38.52	27.92	35.28	103.60	226.90	0.75	222.73	38.11
LM18	47.88	47.38	97.25	100.37	3.80	3.70	69.90	68.37	8.12	8.04	14.10	13.59	33.50	29.27	30.07	33.48	120.80	116.00	0.45	135.57	32.72
LM19	55.63	56.25	104.25	108.87	4.10	4.52	76.88	81.59	8.37	8.30	15.08	14.78	24.90	28.75	38.25	40.03	116.50	164.00	0.61	149.11	30.41
LM20	61.25	60.00	103.72	106.00	2.86	4.32	69.93	72.73	9.14	8.95	15.75	15.68	22.47	29.82	37.10	38.34	69.90	151.30	0.34	190.68	25.39
LM21	56.25	56.88	102.00	107.75	3.15	4.73	71.33	76.85	8.34	8.62	15.18	15.73	35.25	39.77	32.56	39.95	109.50	233.00	0.82	90.11	38.92
LM22	56.24	57.50	101.12	104.12	4.09	4.73	74.61	79.06	8.59	8.72	14.68	15.35	32.20	38.72	32.49	36.37	143.30	201.10	0.92	214.89	26.00
LM23	56.25	57.50	98.87	103.50	3.95	6.08	80.68	88.64	9.54	10.15	14.78	16.23	34.52	36.12	32.16	38.50	128.80	258.40	1.07	217.09	19.58
LM24	58.75	58.13	101.00	104.87	3.30	3.68	76.97	80.64	10.56	10.38	17.48	17.65	36.85	42.02	32.35	34.61	118.60	159.60	0.61	101.16	25.75
LM25	56.25	56.88	100.12	105.37	2.95	4.69	69.96	76.22	8.49	8.50	15.45	16.10	31.80	44.10	29.59	34.60	79.00	213.40	0.54	36.37	27.16
LM26	45.50	43.63	92.87	98.25	3.43	4.87	68.74	68.92	8.36	8.44	14.43	14.58	34.65	36.02	25.78	32.81	99.60	185.60	0.59	120.63	35.90
LM27	54.38	56.25	95.50	98.25	3.41	3.97	76.60	75.03	8.37	8.20	15.78	15.60	37.75	39.27	31.96	33.56	126.90	162.30	0.66	82.92	25.84
LM28	56.88	55.63	100.37	105.12	3.01	4.20	73.01	78.82	9.25	9.25	14.70	16.03	30.20	38.27	37.11	38.13	93.50	188.80	0.57	103.11	19.16
LM29	55.63	58.13	103.12	107.25	3.80	4.66	76.28	79.20	8.39	8.77	15.03	15.53	37.37	38.85	34.70	37.20	149.70	205.00	0.98	67.89	46.72
LM30	55.63	56.25	98.87	106.12	2.91	3.90	73.87	82.06	9.83	9.71	16.78	17.60	40.50	46.52	29.68	37.85	112.70	211.70	0.77	239.78	17.56
LM31	52.00	54.38	96.50	101.87	3.52	4.24	78.32	78.34	9.67	9.24	14.13	13.30	32.72	30.79	33.11	42.82	125.50	175.00	0.70	192.59	28.92
LM32	54.50	54.38	99.12	106.50	2.71	4.62	80.02	87.17	8.74	9.06	15.25	15.68	37.32	42.37	31.40	34.64	98.50	200.90	0.63	87.17	22.02
LM33	53.75	55.63	102.37	108.25	3.43	3.40	73.97	76.86	8.40	8.39	16.80	16.10	34.15	37.00	29.21	31.47	119.50	126.00	0.48	167.88	26.24
LM34	48.25	49.50	94.75	98.50	3.17	3.98	73.15	77.58	8.49	8.32	14.65	15.15	32.20	30.79	28.42	34.82	89.00	142.00	0.41	142.14	26.76
LM35	52.00	50.38	97.87	101.62	3.52	5.30	76.85	82.63	8.35	8.71	15.30	15.85	37.45	39.67	30.96	34.31	125.30	226.20	0.91	176.90	20.44
LM36	55.63	57.50	101.37	106.12	3.02	4.80	76.56	82.93	9.05	9.42	14.48	15.50	34.75	38.62	33.37	36.85	106.70	207.70	0.71	85.64	22.83
LM37	53.25	52.50	99.50	105.25	2.44	3.42	69.98	72.72	8.79	8.80	15.30	15.98	33.97	36.29	34.45	37.89	89.20	144.70	0.41	191.28	12.18
LM38	55.00	53.13	100.00	107.75	2.78	3.08	76.32	78.90	8.94	9.57	17.28	17.90	44.25	43.62	31.69	36.56	120.60	158.60	0.61	129.15	23.28
LM39	50.75	50.88	98.00	103.00	3.11	4.58	74.65	81.41	8.32	9.08	14.68	16.13	35.85	39.30	29.62	33.21	101.70	180.00	0.59	83.67	25.81
LM40	50.75	51.38	93.12	95.37	3.30	4.68	75.32	78.58	9.41	9.51	15.95	16.80	36.25	40.02	27.13	35.66	101.90	218.30	0.71	166.19	20.01
LM41	54.38	55.75	100.12	107.25	2.67	4.45	75.09	80.53	9.89	10.26	16.18	17.23	38.42	43.05	27.57	35.12	94.50	209.30	0.63	381.18	32.27
LM42	57.50	59.39	102.00	107.65	3.02	4.24	77.63	79.09	8.87	9.32	16.88	17.98	31.90	41.15	25.86	30.41	77.70	168.40	0.42	105.16	25.56
LM43	60.63	59.38	103.62	108.87	2.99	5.00	77.02	87.65	10.15	10.47	15.20	16.23	26.77	35.65	35.47	40.27	86.80	216.30	0.60	127.35	15.38
LM44	52.63	54.38	94.75	98.12	3.43	3.95	83.02	85.77	9.82	10.00	15.73	16.00	42.02	45.47	26.87	28.93	123.80	162.50	0.65	97.96	19.56
LM45	59.38	56.25	103.62	107.75	3.31	4.56	73.86	81.48	10.14	10.24	15.45	16.23	33.72	38.00	35.00	41.77	115.50	218.60	0.81	129.15	18.27
LM46	53.25	55.63	100.00	105.12	3.21	4.47	72.02	79.04	9.63	9.71	15.70	16.35	31.42	38.00	35.98	36.88	113.30	192.00	0.70	155.05	14.94
LM47	55.00	56.25	100.12	105.00	3.18	4.44	75.97	78.06	9.74	9.42	15.00	14.90	32.05	31.50	34.23	38.26	109.00	165.10	0.58	180.78	39.72
LM48	55.63	58.13	97.87	103.62	2.59	4.20	79.30	84.30	10.42	10.24	15.60	15.40	33.62	35.07	34.01	36.42	91.80	167.30	0.49	75.89	17.76
LM49	57.50	60.63	103.25	109.87	2.76	4.19	77.72	86.30	9.28	9.78	15.15	16.23	30.65	33.77	33.94	36.71	89.80	158.20	0.46	61.73	32.18
LM50	45.50	44.88	94.25	98.12	3.30	4.54	63.59	65.10	5.88	6.16	11.65	12.50	27.15	32.72	30.28	32.43	85.60	153.60	0.42	79.57	29.84
LM51	44.75	45.25	93.25	97.25	3.86	4.52	60.62	65.37	6.61	6.83	11.95	12.45	27.80	29.32	30.02	35.87	105.60	148.50	0.50	127.93	28.76

Appendix 2.1. (continued)

Entry	DTH		DTM		TN		PH		HL		SPS		KPS		TSW		GY			PC1	
	WR1	WR2	WR1	WR2	WR1	WR2	WR1	WR2	WR1	WR2	WR1	WR2	WR1	WR2	WR1	WR2	WR1	WR2	STI	WR1	WR2
LM52	50.75	50.63	97.00	99.12	3.73	5.37	65.30	69.57	6.42	6.99	12.33	13.44	25.75	31.02	29.97	38.14	94.10	198.10	0.60	143.08	27.75
LM53	44.88	44.25	96.25	96.25	3.68	5.86	60.42	61.18	7.10	7.24	12.68	12.73	27.14	29.57	30.90	32.61	100.00	180.50	0.58	95.53	18.07
LM54	58.75	58.75	102.25	105.62	2.72	4.40	73.52	76.40	7.02	7.44	15.88	18.10	32.30	37.45	35.71	38.26	95.20	188.80	0.58	133.63	26.93
LM55	52.13	50.75	97.00	101.37	3.27	4.64	71.65	73.55	7.61	7.90	14.65	15.70	35.45	40.70	32.21	31.00	116.80	184.60	0.69	92.53	24.22
LM56	53.75	53.75	99.00	102.25	3.52	3.72	72.46	77.41	7.92	7.89	15.85	16.35	36.37	37.15	29.37	31.57	116.50	139.60	0.52	106.63	23.12
LM57	52.50	55.63	96.87	100.87	3.16	4.13	73.20	75.58	8.84	8.80	16.05	16.40	37.67	43.35	30.32	32.39	109.30	177.60	0.62	114.89	13.45
LM58	55.00	57.50	101.87	106.62	3.04	4.03	76.19	82.82	8.86	8.63	15.48	15.25	36.55	38.00	27.75	32.14	97.30	155.40	0.48	186.52	24.54
LM59	60.00	61.25	104.25	107.62	3.49	4.84	74.60	80.34	11.28	11.33	16.15	16.90	31.97	34.37	32.33	34.18	108.20	172.40	0.60	219.63	34.25
LM60	54.38	56.25	101.12	104.12	3.16	3.93	78.03	80.11	8.67	8.61	13.65	14.40	33.37	35.60	31.50	36.29	105.70	156.30	0.53	130.30	26.81
LM61	45.38	44.75	93.12	96.62	3.32	3.52	59.92	62.26	5.97	5.96	10.98	10.63	19.72	20.80	29.09	30.18	50.30	75.00	0.12	85.99	16.53
LM62	60.63	58.13	104.87	106.87	1.99	3.33	64.06	65.64	9.78	10.21	15.78	17.14	32.20	39.75	27.15	30.71	58.70	124.40	0.23	165.07	26.65
LM64	44.63	43.63	99.37	98.75	4.74	4.00	60.20	62.80	6.33	6.46	10.21	10.50	23.42	21.57	30.65	31.75	110.90	97.00	0.34	222.79	18.56
LM65	55.00	53.13	105.12	105.25	3.54	4.67	62.27	63.80	7.73	7.80	12.25	13.10	29.25	32.42	26.17	28.19	88.10	135.10	0.38	96.12	23.57
LM66	42.38	43.63	97.62	100.62	4.73	4.58	62.36	63.23	6.15	6.29	11.78	12.08	24.75	28.17	31.20	31.66	118.80	134.30	0.51	211.07	16.00
LM67	43.63	43.63	96.62	100.12	3.28	3.64	58.51	62.89	6.69	7.01	12.58	12.05	27.75	30.70	31.33	32.69	95.60	123.10	0.38	107.60	14.44
LM68	48.13	50.63	96.25	101.75	3.39	4.28	59.05	64.64	6.67	7.06	13.07	14.68	30.23	36.42	21.05	24.89	60.90	130.80	0.26	234.88	15.06
LM70	51.25	49.38	94.60	99.62	3.29	3.69	65.27	71.03	8.39	8.51	13.38	13.83	32.47	37.72	24.64	28.06	82.20	128.10	0.34	234.36	20.41
LM71	53.88	53.88	94.87	101.87	4.05	4.86	75.31	80.01	9.29	9.46	14.43	15.05	34.10	36.87	29.44	32.86	131.50	181.90	0.77	97.37	42.91
LM72	49.25	49.13	94.00	102.75	3.10	3.43	77.74	80.85	8.90	8.62	14.60	13.80	31.95	32.95	27.28	33.22	82.30	119.90	0.32	216.15	28.49
LM73	52.13	55.75	98.87	103.87	3.18	5.59	80.18	83.00	8.49	8.68	14.50	14.88	37.40	36.88	28.94	35.68	111.80	220.70	0.79	137.39	33.95
LM75	55.25	56.38	97.25	103.62	3.83	4.14	77.65	80.25	8.95	8.95	15.23	15.20	34.55	35.85	29.49	36.57	119.30	165.80	0.63	218.87	29.74
LM76	48.63	49.25	98.00	101.50	3.29	4.49	70.78	74.16	8.67	9.00	13.42	14.45	29.44	37.57	34.32	38.05	95.60	197.00	0.60	275.33	12.81
LM77	55.00	55.00	99.75	103.00	3.70	4.14	87.90	89.71	9.10	9.10	14.45	14.43	31.72	33.50	36.79	45.43	136.20	194.00	0.85	86.52	24.12
LM78	53.88	55.13	100.25	102.62	3.98	5.07	72.46	80.24	6.77	7.17	14.73	14.65	26.17	25.62	34.47	38.15	114.40	156.20	0.57	142.86	21.47
LM79	54.50	53.25	99.37	100.87	3.13	4.73	85.91	86.33	9.03	9.07	14.63	14.48	28.87	30.50	35.77	42.24	95.40	185.70	0.57	111.76	29.65
LM80	59.38	63.99	100.78	106.75	3.34	4.57	85.98	87.19	8.33	8.83	15.28	16.80	37.02	46.85	29.54	37.31	114.40	251.30	0.92	165.84	35.84
LM81	58.75	58.75	102.25	106.50	3.02	4.73	75.30	89.07	9.79	9.87	16.08	16.38	32.87	37.77	36.24	38.59	111.40	208.20	0.74	218.88	23.42
LM82	56.38	56.88	97.25	106.37	3.77	4.74	71.16	75.72	8.85	8.71	15.63	16.05	38.30	43.40	23.37	29.16	108.80	182.50	0.64	317.40	29.32
LM83	60.00	58.75	102.12	107.62	3.03	4.76	74.15	80.15	9.06	9.29	16.05	16.40	32.77	42.35	29.59	34.99	86.50	216.60	0.60	173.38	27.48
LM84	60.00	62.50	105.00	109.62	3.22	6.33	79.10	90.55	9.17	9.44	15.40	16.70	33.70	38.35	27.46	28.81	90.70	206.10	0.60	118.50	32.40
LM85	56.88	56.25	100.25	104.00	3.74	4.86	72.83	79.82	8.60	8.92	15.03	15.67	35.47	35.59	30.75	35.09	126.50	225.50	0.91	78.56	28.96
LM86	50.25	50.63	99.12	101.62	3.77	4.82	66.61	64.33	8.55	8.59	14.13	14.60	33.35	34.32	24.66	28.46	96.80	145.40	0.45	197.67	18.90
LM87	48.00	47.25	94.62	102.62	2.93	4.03	71.85	69.59	8.39	7.98	14.53	12.92	32.42	30.95	29.40	32.55	85.20	132.80	0.36	105.22	8.79
LM88	49.25	49.75	93.75	100.87	3.08	3.68	71.44	70.85	8.30	7.92	14.15	13.28	33.05	30.40	24.06	29.90	77.70	112.50	0.28	286.30	18.17
LM89	57.50	55.63	106.25	105.37	3.16	5.72	76.84	81.84	8.46	8.69	14.60	15.40	31.25	39.60	26.70	26.12	79.00	189.00	0.48	125.25	35.57
LM90	53.38	55.00	96.75	101.75	3.48	4.11	85.21	90.68	9.33	9.34	14.30	14.68	30.42	32.17	36.61	45.00	120.70	189.00	0.73	56.46	23.92
LM91	46.13	46.75	98.12	100.50	3.85	5.05	61.81	69.15	7.07	7.41	12.55	13.48	27.97	29.75	30.95	35.11	98.70	166.40	0.53	131.50	26.88
LM93	55.13	55.00	95.50	102.87	2.93	4.34	79.39	84.15	9.76	9.85	14.48	14.95	30.20	35.30	33.46	37.70	93.90	170.00	0.51	294.94	14.90
LM94	55.00	53.75	98.25	103.87	3.60	4.44	72.07	79.27	6.83	7.14	14.23	14.02	26.40	21.80	32.75	40.42	94.30	125.10	0.38	127.50	23.21
LM95	45.50	48.00	94.50	97.37	2.57	2.81	60.90	62.54	7.08	6.97	12.33	12.60	29.30	30.35	28.68	31.45	64.30	79.90	0.16	75.44	16.02
LM96	55.63	55.63	102.75	106.25	4.08	4.65	83.28	88.27	7.89	7.99	15.23	15.13	35.47	36.05	27.99	35.69	126.30	179.20	0.73	322.91	23.36
LM97	56.25	59.38	103.87	105.87	2.94	3.80	74.95	82.92	9.83	10.26	16.03	16.98	35.72	42.77	31.40	33.55	98.80	163.10	0.52	68.60	18.77
LM98	55.63	56.25	96.00	101.25	3.25	4.43	76.50	84.93	9.92	9.95	15.13	15.73	35.00	42.00	24.33	31.59	93.10	183.50	0.55	176.99	20.51
LM99	52.13	53.25	93.75	99.37	3.75	5.63	74.89	76.85	8.34	8.15	13.88	13.95	32.85	33.62	24.16	28.69	98.10	180.10	0.57	95.21	24.15
LM100	61.88	61.88	106.00	108.00	3.48	4.03	79.49	90.06	9.77	10.04	14.73	15.80	33.57	41.07	36.50	38.77	127.20	198.10	0.81	204.71	23.08
mean	53.45	53.80	98.97	103.13	3.36	4.45	73.52	78.03	8.65	8.79	14.71	15.18	32.87	36.52	30.72	34.93	104.83	176.60	0.60	156.20	24.50

DTH, days to 50% heading; DTM, days to maturity; GY, grain yield per plot; SL, spike length; KPS, number of kennels per plant; PC, proline content; PH, plant height; TN, number of productive tillers; SPS, number of spikelets per spike; TSW, thousand seed weight; WR1, water regime 1 (water stress); WR2, water regime 2 (control); STI, stress tolerance index.

CHAPTER 3. VARIANCE COMPONENTS AND HERITABILITY OF YIELD AND YIELD COMPONENTS OF WHEAT UNDER DROUGHT-STRESSED AND NON-STRESSED CONDITIONS

Abstract

Grain yield of wheat is a complex polygenic trait that is highly influenced by genotype by environment interaction. The objective of this study was to determine variance components and heritability of yield and yield related traits of a population of 96 bread wheat genotypes under drought-stressed and non-stressed conditions. Genotypes were evaluated across eight testing environments during the 2014/2015 and 2015/2016 growing seasons using an alpha lattice design with two replications. The results indicated the presence of significant effects of genotypes, seasons, sites, and water regimes and their interactions. High levels of genotypic variance (σ^2_g) were found for spike length (73%), number of spikelets per spike (44.19%), plant height (51.26%), number of kennels per spike (32.98%), number of days to heading (44.24%) and thousand seed weight (22.98%), resulting in high broad-sense heritability estimates of > 0.50. Conversely, genotypic variation was relatively moderate for the number of days to maturity, grain yield and number of productive tillers per plant, accounting for 15.03%, 8.46% and 6.13% of the total variation, respectively. The heritability estimates of the latter traits were $20\% \leq H^2 < 50\%$ which may limit their selection gains under drought-stressed environments. Further, quantitative trait loci analysis and progeny testing are required to discern the number of genes and associated genetic effect and to pinpoint genomic regions in the tested wheat genetic resources for effective breeding for drought tolerance.

Keywords: drought stress, heritability, variance components, wheat, yield components

3.1 Introduction

Grain yield of wheat is a complex trait consisting of various components such as the number of productive tillers per plant, number of spikelets per spike, number of kernels per spike and grain weight. Other complementary traits affecting yield response include the number of days to heading and maturity, plant height and spike length (Lopes et al., 2012; Slafer et al., 2014). Partitioning of the effect of genotype (g), environment (e) and g x e interaction (GEI) provides reasonable estimates of their relative contribution to phenotypic variation during selection. Genotype x environment interaction leads to differential response of genotypes due to polygenic effect and the influence of the growing environment (Hall, 2000; Sanchez-Garcia et al., 2012; Rad et al., 2013). This requires evaluation of diverse candidate genotypes across representative testing environments to select promising lines for further breeding or for cultivar recommendation. Selection response for grain yield can be achieved through direct or indirect selection of yield components that have complementary effects, contributing to enhanced crop productivity.

The magnitudes of genetic variance components and heritability affect selection response of a trait (Falconer and Mackay, 1996). Heritability is the proportion of genetic variation to the total phenotypic variation, which is one of the useful parameters to estimate the likelihood of genetic gain after selection in a given population and environment. Heritability values estimate the likelihood of tracing genes affecting particular traits. Several studies have attempted to estimate the heritability of important economic traits that directly affect yield response in wheat, particularly under drought-stressed and non-stressed conditions (Aycicek and Yildirim, 2006; Eid, 2009; Abdolshahi et al., 2015). Aycicek and Yildirim (2006) reported heritability estimates as low as 2.07%, 1.01% and 0.1% for days to heading, plant height and grain yield, respectively, due to high genotype by environment interaction. Heritability estimates are specific to the test population or individuals evaluated under the prevailing environments. Therefore, heritability values should be determined in a given breeding population to estimate the response to selection.

In an attempt to select drought tolerant wheat genotypes, diverse germplasm that are tolerant to drought and heat stresses were acquired from CIMMYT. The lines were screened for drought tolerance based on phenotypic and proline analyses (Mwadzingeni et al., 2016; Chapter 2). The

germplasm needs to be further evaluated for their selection response towards agronomic traits. These will allow selection of lines with high breeding values under the prevailing growth conditions to maximize their genetic potential for drought tolerance breeding or for direct production. Therefore, the objective of this study was to determine variance components and heritability of yield and yield related traits of 96 bread wheat genotypes under drought-stressed and non-stressed conditions.

3.2 Materials and Methods

3.2.1 Plant Materials, Study Site and Data Collection

Ninety-six bread wheat genotypes comprising 88 lines from CIMMYT's heat and drought tolerance nurseries and eight locally grown drought-susceptible cultivars were evaluated (Chapter 2, Table 2.1). Data on the number of days to heading (DTH), number of productive tillers per plant (TN), plant height (PH), days to maturity (DTM), spike length (SL), number of spikelets per spike (SPS), number of kennels per spike (KPS), thousand seed weight (TSW) and grain yield per plot (GY), collected in chapter 2, were considered for this analysis. Descriptions on data collection are summarized in Chapter 2, Section 2.2.3.

3.2.2 Data Analysis

Combined analysis of variance was conducted and variance components were estimated following the General Linear Model (GLM) procedure of Agrobase (Agrobase, 2005) by considering the seasons and water regimes as fixed factors. The genotypes and sites were treated as random factors. Negative variances were adjusted to zero (Robinson et al., 1955; Borojevic, 1990). Expected mean of squares (EMS) were calculated following Gordon et al. (1972), Borojevic (1990) and Shimelis and Shiringani (2010) as presented in Table 3.1. The broad sense heritability (H^2) estimates were calculated from the phenotypic variance (σ^2_p) and the genotypic variance (σ^2_g) according to Allard (1999) as;

$H^2 = \sigma^2_g / (\sigma^2_g + \sigma^2_{gwls} / wls + \sigma^2_{gls} / ls + \sigma^2_{glw} / lw + \sigma^2_{gsw} / sw + \sigma^2_{gs} / s + \sigma^2_{gw} / w + \sigma^2_{gl} / l + \sigma^2_e / rlsw) = \sigma^2_g / (\sigma^2_g + \sigma^2_{gxe} / e + \sigma^2_e / re) = \sigma^2_g / \sigma^2_p$.

Where σ^2_e = environmental variance, σ^2_g = genotypic variance, σ^2_{gl} = genotype by site interaction variance, σ^2_{gs}, = genotype by season interaction variance, σ^2_{gw} = genotype by water regime

interaction variance, σ^2_{gls} = genotype by site by season interaction variance, σ^2_{glw} = genotype by site by water regime interaction; σ^2_{gws} = genotype by water regime by site interaction variance, σ^2_{gws} = genotype by site by water regime by season interaction, r = replication.

Table 3.1. Details of computation of expected mean squares for the wheat genotypes evaluated on two sites (l), seasons (s) and water regimes

Source of variation	Degrees of freedom	Expected mean square
Genotype (g)	g – 1	$\sigma^2_e + r\sigma^2_{gwls} + rw\sigma^2_{gls} + rs\sigma^2_{glw} + rl\sigma^2_{gsw} + rwl\sigma^2_{gs} + rls\sigma^2_{gw} + rsw\sigma^2_{gl} + \sigma^2_g$
Site (l)	l – 1	–
Season (s)	s – 1	–
Water regime (w)	w – 1	–
gl	(g – 1)(l – 1)	$\sigma^2_e + r\sigma^2_{gwls} + rw\sigma^2_{gls} + rs\sigma^2_{glw} + rsw\sigma^2_{gl}$
Gs	(g – 1)(s – 1)	$\sigma^2_e + r\sigma^2_{gwls} + rl\sigma^2_{gws} + rw\sigma^2_{gls} + rwl\sigma^2_{gs}$
Gw	(g – 1)(w – 1)	$\sigma^2_e + r\sigma^2_{gwls} + rl\sigma^2_{gws} + rs\sigma^2_{glw} + rsl\sigma^2_{gw}$
gls	(g – 1)(l – 1)(s – 1)	$\sigma^2_e + r\sigma^2_{gwls} + rw\sigma^2_{gls}$
glw	(g – 1)(l – 1)(w – 1)	$\sigma^2_e + r\sigma^2_{gwls} + rs\sigma^2_{glw}$
gws	(g – 1)(w – 1)(s – 1)	$\sigma_e^2 + r\sigma^2_{gwls} + rl\sigma^2_{gws}$
glws	(g – 1)(l – 1)(w - 1)(s - 1)	$\sigma^2_e + r\sigma^2_{gwls}$
Replication within water regime, seasons and sites	lws (r – 1)	–
ME_e	lws (g – 1)(r - 1)	σ^2_e

σ^2_e, environmental variance; σ^2_g, genotypic variance; σ^2_{gl} genotype by site interaction variance; σ^2_{gs}, genotype by season interaction variance; σ^2_{gw}, genotype by water regime interaction variance; $\sigma^2_{gls,}$ genotype by site by season interaction variance; σ^2_{glw}, genotype by site by water regime interaction; $\sigma^2_{gws,}$ genotype by water regime by site interaction variance; $\sigma^2_{gws,}$ genotype by site by water regime by season interaction; r, replication.

3.3 Results

3.3.1 Influence of Genotypes, Water Regimes, Seasons and Testing Environments on Trait Variability

Highly significant differences ($P < 0.01$) were observed among the main effects of the genotype, site, season and water regime for most of the studied traits (Table 3.2). The number of days to heading and number of productive tillers per plant were not significantly affected by the water regime. Similarly; thousand seed weight was not significantly affected by seasonal variability. Most of the interaction effects of grain yield with site, season and water regime were significant ($P < 0.05$).

Table 3.2. Mean squares and significant tests after combined analysis of variance for nine phenotypic traits of 96 wheat genotypes evaluated in two localities over two seasons, under two water regimes and two replications

	Traits									
Source	DF	DTH	DTM	PH	TN	SL	SPS	KPS	TSW	GY
Genotype (Gen)	95	344.606***	199.43***	728.957***	3.115***	18.549***	30.677***	331.134***	186.037***	8631.239***
Gen*Site	95	37.272***	47.964***	49.566***	0.465ns	0.451***	2.597***	25.188ns	55.38***	2045.785ns
Gen * Season	95	67.301***	80.449***	81.983***	1.582***	0.756***	3.604***	55.57***	33.155***	3345.954***
Gen * Water Regime (WR)	95	8.825ns	18.368ns	40.419***	1.634***	0.243ns	1.572ns	43.257***	26.791**	4066.003***
Gen*Site * Season	95	25.616***	30.912*	32.934*	0.498ns	0.521***	2.993***	21.728ns	40.848***	2262.287*
Gen*Site * WR	95	7.54ns	14.1ns	32.462*	0.362ns	0.308ns	1.919ns	23.443ns	19.081ns	1425.247ns
Gen * Season * WR	95	10.547ns	21.376ns	30.266ns	1.148***	0.196ns	1.565ns	39.592***	26.725**	2726.462***
Gen*Site * Season * WR	95	10.113ns	20.717ns	24.678ns	0.444ns	0.343ns	1.66ns	26.342ns	20.469ns	2010.68ns
Site	1	16318.39***	128214.9***	6164.719***	341.128***	604.201***	3546.493***	86899.79***	775.639ns	3342901***
Season	1	2405.143***	5077.068***	6955.221***	0.048ns	214.505***	3018.38***	419.758***	932.91***	26429.13***
WR	1	42.684ns	6628.792***	7762.736***	455.257***	7.053***	82.78***	4992.579***	6887.799***	1922719***
Error	760	9.584	22.542	25.384	0.648	0.279	1.717	26.483	20.056	1755.159

DTH, days to 50% heading; DF, degrees of freedom; PH, plant height; TN, number of productive tillers; DTM, days to maturity; SL, spike length; SPS, number of spikelets per spike; KPS, number of kennels per plant; TSW thousand seed weight; GY, gain yield; *, $P < 0.05$ (2-tailed); **, $P < 0.01$ level (2-tailed); ***, $P < 0.001$ level (2-tailed); ns, non-significant.

3.3.2 Variance Components and Heritability Estimates

The variance component estimates for the nine phenotypic traits of the 96 wheat genotypes evaluated across the two test sites in two seasons under two water regimes are presented in Table 3.3. Generally marked genotypic variation existed among the studied traits, except for the number of days to maturity, number of productive tillers and grain yield that were considerably influenced by the environment. The mean values of traits, the least significant differences (LSD) and the coefficient of variation (CV) were presented in Chapter 2. Spike length, number of spikelets per spike, plant height, number of kennels per spike, number of days to heading and thousand seed weight had moderate to high genotypic variances (σ^2_g) of 73%, 44%, 51%, 32.98%, 44.24% and 22.98%, respectively, largely due to genotypic differences, hence, had high heritability estimates above 50% (Table 3.3). Moderate heritability values ($20\% \geq H^2 < 50\%$) were observed for the number of days to maturity (47.29%), grain yield (38.93%) and number of productive tillers per plant (28.83%). For the latter traits, much of the variation was explained by the residual component (σ^2_e) as compared to the interactions of the genotype by other components. Variation in grain yield (σ^2_g =8.46) was considerably influenced by the genotype by environment interaction.

Table 3.3. Variance components for nine phenotypic traits of 96 wheat genotypes evaluated in two sites over two seasons, under two water regimes and two replications

	Traits																	
	DTH		DTM		PH		TN		SL		SPS		KPS		TWS		GY	
Component	Var	%	var	%	var	%	var	%	var	%	var	%	var	%	var	%	var	%
Genotype (Gen)	16.58	44.24	6.16	15.03	39.27	51.26	0.06	6.13	1.12	73.00	1.75	44.19	16.77	32.98	8.74	22.98	229.09	8.46
Gen*Site	1.78	4.75	2.97	7.24	1.11	1.44	0.01	0.58	0.00[1]	0.00[1]	0.00[1]	0.00[1]	0.80	1.58	2.00	5.26	46.97	1.73
Gen*Season	5.17	13.79	6.13	14.95	5.44	7.10	0.05	4.67	0.05	3.12	0.09	2.25	2.60	5.11	0.00[1]	0.00[1]	45.48	1.68
Gen*Water regime (WR)	0.11	0.28	0.45	1.11	0.00	0.00[1]	0.07	6.91	0.01	0.65	0.00[1]	0.00[1]	0.83	1.63	0.18	0.47	248.40	9.17
Gen*Site*season	3.89	10.37	2.56	6.25	2.07	2.70	0.01	1.36	0.05	2.93	0.34	8.54	0.00[1]	0.00[1]	5.24	13.77	63.59	2.35
Gen*Site*WR	0.00[1]	0.00[1]	0.00[1]	0.00[1]	1.95	2.54	0.00[1]	0.00[1]	0.00[1]	0.00[1]	0.07	1.67	0.00[1]	0.00[1]	0.00[1]	0.00[1]	0.00[1]	0.00[1]
Gen*Season*WR	0.11	0.29	0.17	0.41	1.40	1.83	0.18	17.23	0.00[1]	0.00[1]	0.00[1]	0.00[1]	3.37	6.63	1.62	4.25	186.21	6.87
Gen*Site*Season*WR	0.27	0.71	0.00[1]	0.00[1]	0.00	0.00[1]	0.00[1]	0.00[1]	0.03	2.15	0.00[1]	0.00[1]	0.00[1]	0.00[1]	0.21	0.56	133.86	4.94
Residual	9.58	25.57	22.54	55.02	25.38	33.13	0.65	63.10	0.28	18.15	1.72	43.36	26.49	52.08	20.06	52.72	1755.16	64.80
Total variance	37.48	100.00	40.97	100.00	76.61	100.00	1.03	100.00	1.54	100.00	3.96	100.00	50.85	100	38.04	100.00	2708.76	100.00
Phenotypic variance (V*p*)	21.74		13.03		45.49		0.21		1.18		2.01		21.38		12.83		588.40	
Heritability (H^2)	0.76		0.47		0.86		0.29		0.95		0.87		0.78		0.68		0.39	
Heritability (%)	76.26		47.29		86.33		28.83		94.61		87.28		78.43		68.15		38.93	

DTH, days to 50% heading; PH, plant height; TN, number of productive tillers; DTM, days to maturity; SL, spike length; SPS, number of spikelets per spike; KPS, number of kennels per plant; TSW, thousand seed weight; GY, gain yield; Var, variance; [1], the value was negative.

3.4 Discussion

The significant genotypic differences ($P < 0.001$) observed for the studied traits reflects that the germplasm pool from which the sampled lines were selected contains a broad genetic base (Table 3.2). Some of these genetic resources could, therefore, be useful in breeding of locally cultivated varieties to marginal growing conditions. Significant differences due to the water regime that was observed on all traits except days to heading is expected since drought stress is known to negatively affect yield and its components (Fischer and Maurer, 1978). Water stress is reported to influence tissue elongation, resulting in reduced plant height and spike length (Moayedi et al., 2010; Sanjari Pireivatlou and Yazdansepas, 2010). The non-significant effect of water regime on the number of days to heading was expected since the genotypes were stressed at 50% heading. Drought stress shortens the grain filling period, resulting in a significant reduction of the number of days to maturity, which could explain the significant differences observed among genotypes due to water regimes (Kilic and Yagbasanlar, 2010). Shortening of the effective grain filling period results in shriveled kernels and hence reduced seed weight with a subsequent yield penalty. It is also worth noting that genotype by water regime interaction had non-significant effects on the number of days to maturity, as well as on spike length and number of spikelets per spike, which could explain the capacity of respective genotypes to maintain their rankings under different water regimes. The high influence of the environment on the phenotypic variation for the number days to maturity, grain yield and number of productive tillers indicate the existence of considerable variation due to sites, seasons and water regimes singly or their combinations. Low heritability estimates are reported for polygenic traits studied under varying conditions, particularly involving drought stress (Eid, 2009).

The high heritability estimates of spike length (94.61%), number of spikelets per spike (87.28%), plant height (86.33%), number of kennels per spike (78.43%), number of days to heading (76.26%) and thousand seed weight (68.15%) are indicative of the effect of some major genes on these traits under both water-stressed and non-stressed conditions. Previous studies identified some of the major QTL encoding for functional genes that control most agronomic traits in wheat under drought-stressed conditions (Spielmeyer et al., 2007 ; Mathews et al., 2008; Li et al., 2015). Some of the genetic components affecting plant height could also influence spike length since the two traits are highly correlated and both have high heritability estimates

(Mwadzingeni et al., 2016). Owing to the high heritability estimates observed in this study, association mapping based on the studied set of germplasm and traits could probably identify genetic determinants influencing these traits under contrasting environments. The moderate heritability values (20% < H2 < 50%) observed for the number of productive tillers (28.83%), number of days to maturity (47.29%) and grain yield (38.93%) could also reflect the presence of major or minor genes controlling these traits (Table 3.3). The results from this study concurs with the findings of Abdolshahi et al. (2015) who reported high heritability estimates for several morphological traits of wheat evaluated under drought-stressed and non-stressed conditions, including plant height (79%), thousand seed weight (85%) and days to flowering (85%), and moderate heritability for grain yield (45%).

Grain yield is a polygenic trait that is highly influenced by the environment under drought-stressed condition; hence, the moderate heritability estimate of this trait was expected. Genetic gains in grain yield is achieved through selection of component traits. Selection for highly heritable traits that positively correlate with other quantitative traits enhances the efficiency of selection (Shimelis and Shiringani, 2010). Heritability for grain yield was estimated at 38.93% which is in agreement with the moderate value of 45% reported by Abdolshahi et al. (2015). However, the heritability estimates obtained in this study are higher than moderate and low values (H^2 < 50%) reported by Yagdi and Sozen (2009) from a set of durum wheat genotypes tested under different environmental conditions. This confirms that heritability values are subject to the particular set of genotypes being evaluated and the target testing environments. Such differences in a set of populations and test environments could explain variable heritability estimates for similar key traits obtained in various studies (Eid, 2009; Mohsin et al., 2009).

3.5 Conclusions

The tested germplasm pool is a vital source of genetic variation for drought tolerance breeding. The lines exhibited high levels of genotypic and phenotypic variability for the studied traits. Under the test environments, selection based on the studied traits can result in significant genetic advances for drought tolerance owing to high heritability values. The number of days to maturity, number of productive tillers per plant and grain yield showed moderate heritability values. The tested wheat germplasm constitutes a useful resource which could be used by wheat

breeding programs in sub-Saharan Africa to exploit their genetic variation and potential for drought adaptation across marginal rainfall growing environments. It is recommended to explore the variability existing within the germplasm through molecular markers, QTL analysis or progeny testing to pinpoint the number of genes and their gene action for effective breeding for drought tolerance through population structure analysis and marker trait association studies.

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CHAPTER 4. GENOME-WIDE ASSOCIATION ANALYSIS OF AGRONOMIC TRAITS IN WHEAT UNDER DROUGHT-STRESSED AND NON-STRESSED CONDITIONS

Abstract

The objective of this study was to determine the population structure and genome-wide marker-trait association of key agronomic traits of wheat for drought tolerance breeding. A population of 93 bread wheat genotypes was genotyped using the Diversity Arrays Technology sequencing (DArTseq) protocol. The following agronomic traits, assessed under drought-stressed and non-stressed conditions, were considered for the study: the number of days to heading (DTH), number of days to maturity (DTM), plant height (PH), spike length (SL), number of kernels per spike (KPS), thousand seed weight (TSW) and grain yield (GY). Population structure analysis and genome-wide association mapping were undertaken based on 16,383 silico DArTs with < 10% missing data. The population evaluated was grouped into nine distinct genetic structures. Inter-chromosomal linkage disequilibrium showed the existence of linkage decay as physical distance increased. A total of 62 significant ($P < 0.001$) marker-trait associations (MTAs) were detected explaining more than 20% of the phenotypic variation observed under both drought-stressed and non-stressed conditions. Significant ($P < 0.001$) MTA event(s) were observed for DTH, PH, SL, SPS, and KPS under both stressed and non-stressed conditions, while additional significant ($P < 0.05$) associations were also considered for TSW, DTM and GY under non-stressed condition. The MTAs reported in this population could be useful to initiate marker-assisted selection (MAS) and targeted trait introgression of wheat under drought-stressed and non-stressed conditions, and for fine mapping and cloning of the underlying genes.

Keywords: Diversity Arrays Technology, drought tolerance, genome wide association study, linkage disequilibrium, population structure, wheat

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4.1 Introduction

Genome-wide association study (GWAS) facilitates understanding of the genetic bases and dissection of complex genes controlling economic traits such as drought tolerance. GWAS rely on marker-trait association (MTA) involving representative markers and genetically diverse populations such as elite breeding lines and improved cultivars. The goal of GWAS is to discern genomic regions that could either be markers or genes associated with key agro-morphological traits for marker-assisted breeding, gene discovery or gene introgression (Edae et al., 2014). Understanding the population structure and the magnitude of linkage disequilibrium (LD) present in the prevailing genetic resources are important pre-requisites to deduce the genetic makeup, composition and genomic predictions of traits of interest during selection. Linkage disequilibrium *per se* could serves as a predictor of the resolution at which influential genomic regions can be detected through marker-trait-association analysis. Linkage analysis establishes associations among sets of genes, and provides insights on the effect of genetic drift, selection, mutation, recombination, quantitative trait loci, linked genes, or gene-flow in a given population (Baird, 2015; Xu, 2010). Identification of diagnostic genetic markers and candidate genes associated with target traits will facilitate marker-assisted selection, and trait introgression. A considerable number of markers and QTL associated with several polygenic traits has been mapped along the 21 chromosomes of bread wheat (Kuchel et al., 2007; Tsilo et al., 2010; Le Gouis et al., 2012; Edae et al., 2014; Sukumaran et al., 2015). These genomic resources are crucial to understand the genetic mechanism of drought tolerance and other economic traits present in complex polyploid crops including wheat.

Several DNA-based marker systems have been successfully applied in association mapping of complex traits in different crop species. The most widely used marker systems include simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), single nucleotide polymorphism (SNP) and microarray based Diversity Arrays Technology (DArT) markers (Jaccoud et al., 2001; Breseghello and Sorrells, 2006; Wang et al., 2014; Gouy et al., 2015). Advanced and high-throughput genotyping technologies such as genotyping by sequencing (GBS) are effective tools to detect abundant and highly reproducible SNPs and DArT markers (Sonah et al., 2013; Spindel et al., 2013). These marker systems are used in population genetics, GWAS, marker assisted selection (MAS), genomic selection, haplotype mapping, genetic

diversity analyses or linkage map construction (Raman et al., 2011). The Diversity Arrays Technology has been successfully used in wheat, though it was initially developed for crops with less complex genomes such as rice (Jaccoud et al., 2001; Akbari et al., 2006; Crossa et al., 2007; Neumann et al., 2011; Tadesse et al., 2015). The DArT sequencing platform provides a database of sequences which are useful resources to advance marker-trait association analyses.

A diverse population of drought and heat tolerant lines were acquired from CIMMYT for selection and drought tolerance breeding in South Africa. The population comprising of 87 introductions and six local drought-susceptible released varieties were screened using key agronomic traits and proline analyses under drought-stressed and non-stressed conditions as described in Chapter 2. Evaluated traits showed moderate to high heritability estimates (Chapter 3). This provided a comprehensive database of agronomic traits useful for further selection and to undertake marker-trait association analysis. Further, these genetic resources should be systematically genotyped using a fairly large marker density representing the 21 chromosomes to identify additional candidate genes controlling key traits to initiate marker-assisted selection of drought tolerance in wheat. Therefore, the objective of this study was to determine the population structure and genome-wide marker-trait association of key agronomic traits of wheat for drought-tolerance breeding, using representative DArTseq markers.

4.2 Materials and Methods

4.2.1 Plant Materials and Phenotyping

The study used a population of 93 bread wheat genotypes presented in Chapter 2 (Table 2.1). Genotypes LM23, LM62 and LM65 were excluded during this study because their DNA was not sent for sequencing. The 93 genotypes were rigorously phenotyped for key agronomic traits as described in Chapter 2, section 2.2. The following eight phenotypic traits were considered for this study; number of days to heading (DTH), plant height (PH), number of days to maturity (DTM), spike length (SL), number of spikelets per spike (SPS), numbers of kennels per spike (KPS), thousand seed weight (TSW) and grain yield per plot (GY), and the data were analyzed as described in section 2.2.5. Data for the number of productive tillers and proline content were not used in this study since they had low heritabilities (Chapter 3).

4.2.2 DNA Extraction and DArT Sequencing

Genomic DNA of the 93 genotypes was extracted from fresh leaf tissue of 2 week old seedlings following the plant DNA extraction protocol for DArT (DArT, 2014). The quality of DNA was checked for nucleic acid concentration and purity using a NanoDrop 2000 spectrophotometer (ND-2000 V3.5, NanoDrop Technologies, Inc.). The DNA samples were sent to Diversity Arrays Technology Pty Ltd, Canberra, Australia in a 96 well microtiter plate for destructive DNA analysis. Samples were genotyped using the DArTseq protocol using 38,611 silico DArTs. After eliminating the DArT loci with unknown chromosome positions and filtering markers with more than 10% missing data, a total of 16,383 markers distributed across the 21 chromosomes were maintained for analysis. The number of markers used from each chromosome were 681; 1,068; 289; 1,114; 1,887; 455; 754; 1,322; 396; 995; 334; 86; 512; 1,242; 150; 868; 1,303; 264; 1,145; 1,231 and 287 in chromosomes 1A, 1B, 1D, 2A, 2B, 2D, 3A, 3B, 3D, 4A, 4B, 4D, 5A, 5B, 5D, 6A, 6B, 6D, 7A, 7B and 7D, in that order. The silico DArTs used had reproducibility values of 1, polymorphic information content (PIC) values ranging from 0.02 to 0.50, a mean call rate of 0.93 with a range from 0.84 to 1, and a read mean depth of 14.92 ranging from 5 to 399.

4.2.3 Population Structure, Linkage Disequilibrium and Marker-Trait Association Analyses

Population structure was determined using the software STRUCTURE v2.3.4 (Pritchard et al., 2010). The parameters of the project were set at 10,000 burn-in periods, with 10,000 Markov chain–Monte Carlo (MCMC) repetitions after burn-in. Ten iterations were ran for K (clusters) values of 1 to 20 to allow selection of the replication with the highest mean value of ln likelihood. Genotypic data was imputed for missing values using TASSEL v4.3.15 (https://sourceforge.net/p/tassel/tassel4-standalone/ci/master/tree/). Linkage disequilibrium was estimated using the squared allele frequency correlations R^2 value from which the number of significant allele pairs ($P < 0.01$) was determined using 1,000 permutations. Marker trait association analysis was carried out following the GLM procedure in TASSEL v4.3.15.

4.3 Results

4.3.1 Phenotypic Traits Evaluation

Analysis of variance indicated significant differences ($P < 0.05$) due to the genotype, site, water regime and their interaction effects for all the studied traits. High and positive correlations and high heritability estimates were detected for most of the traits considered in the current study. Spike length, number of spikelets per spike, plant height, number of kennels per spike, number of days to heading and thousand seed weight had higher levels of genotypic variance (σ^2_g), hence high heritability values of > 50%. The number of days to maturity and grain yield had moderate heritability estimates ($20\% \leq H^2 < 50\%$).

4.3.2 Population Structure

Population structure was constructed to reveal the genetic relationships and to aid genotype selection. Nine distinct populations were recognised (Figure 4.1) after the LnP (D) kept increasing from -766,307 at K = 1 to -627,026 (with a mean value of ln likelihood of -590,791) at K=9. Figure 4.1 presents the population structure for K = 9 where each colour represents a different genetic cluster. The list of genotypes and the overall representation of membership of the sample in each of the 9 clusters are presented on Table 4.1. The expected heterozygosity of genes among individuals varied from 0.07 to 0.29 with fixation index (Fst) varying from 0.31 to 0.89 among clusters.

In the structure, Cluster 1 consisted of six and four genotypes from the heat and drought tolerance nurseries, respectively (Table 4.1). Cluster 2 consisted of only four genotypes from the heat tolerance nursery. This was followed by the largest group, Cluster 3, which comprised 29 genotypes of which 21 were from the heat tolerance nursery while the remaining eight were from the drought tolerance nursery. Cluster 4 had only genotypes from the heat tolerance nurseries, while Clusters 5, 6 and 7 had mixtures of genotypes. All the local checks (LM61, LM64, LM66, LM67 and LM70) were grouped in Cluster 8, together with ten other genotypes including LM12 from the heat tolerance nursery and nine genotypes from the drought tolerance nursery (Table 4.1). Likewise, the last cluster contained the genotypes LM78 and LM94 from the drought tolerance nursery.

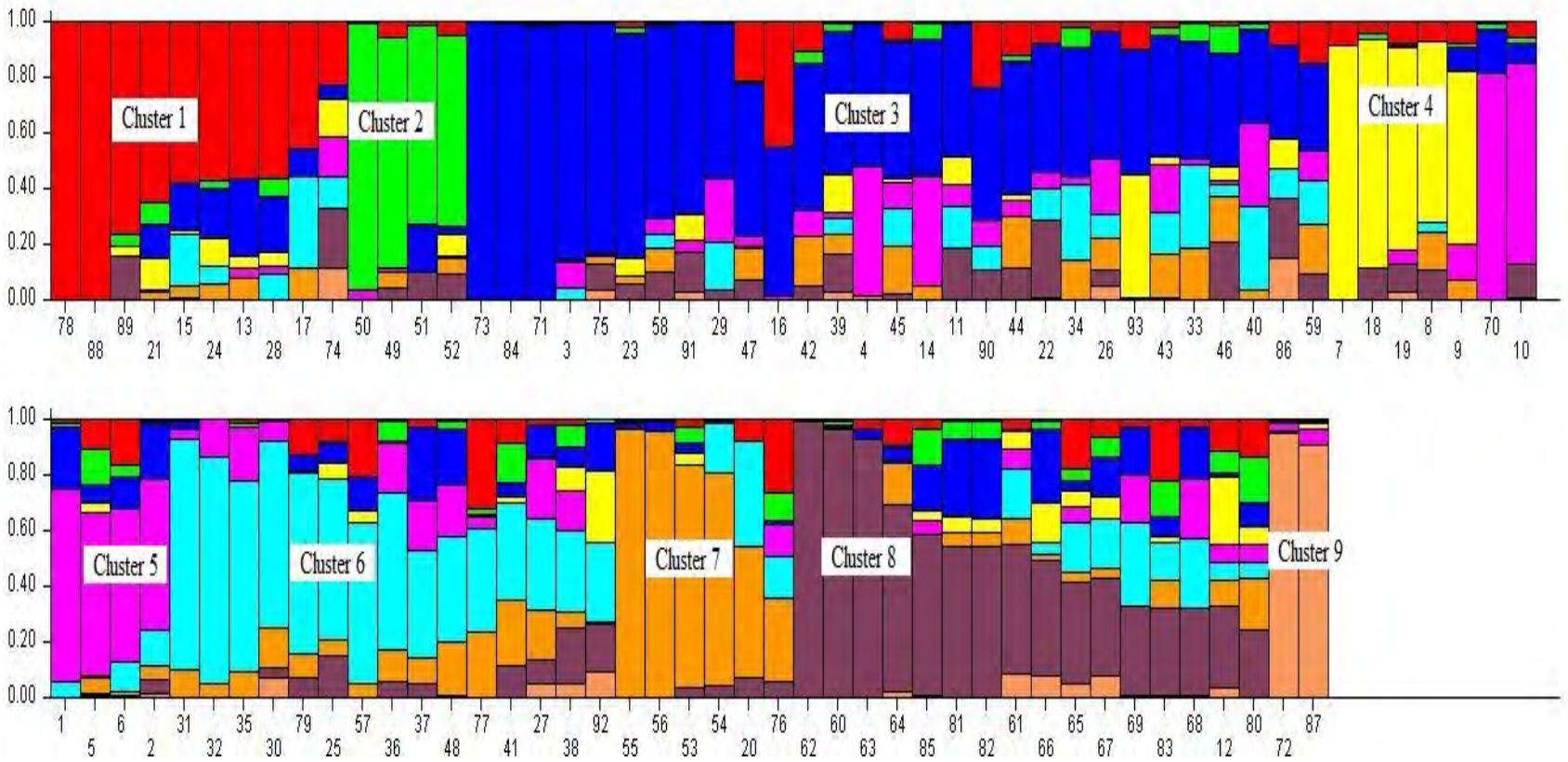

Figure 4.1 Population structure based on 93 genotypes and 16,383 DArTseq markers. Each colored segment per genotype estimates the membership fraction to each of the 9 populations. See Table 4.1 for codes of genotypes.

Table 4.1. Nine genetic clusters with their respective list of wheat genotypes, proportion of membership, expected heterozygosity and the mean values of Fst observed from the study population

Cluster	*Genotypes	% membership	Expected heterozygosity	Mean fixation index (Fst)
1	LM13 (13), LM15 (15), LM17 (17), LM21 (21), LM25 (24), LM29 (28), LM80 (74), LM84 (78), LM95 (88), LM96 (89)	0.118	0.1682	0.6144
2	LM50 (49), LM51 (50), LM52 (51), LM53 (52)	0.06	0.0908	0.8375
3	LM03 (3), LM04 (4), LM11 (11), LM14 (14), LM16 (16), LM22 (22), LM24 (23), LM27 (26), LM30 (29), LM34 (33), LM35 (34), LM40 (39), LM41 (40), LM43 (42), LM44 (43), LM45 (44), LM46 (45), LM47 (46), LM48 (47), LM59 (58), LM60 (59), LM77 (71), LM79 (73), LM81 (75), LM90 (84), LM93 (86), LM97 (90), LM98 (91), LM100 (93)	0.235	0.171	0.561
4	LM07 (7), LM08 (8), LM09 (9), LM18 (18), LM19 (19)	0.073	0.0674	0.892
5	LM01 (1), LM02 (2), LM05 (5), LM06 (6), LM10 (10), LM76 (70)	0.1	0.0863	0.8545
6	LM26 (25), LM28 (27), LM31 (30), LM32 (31), LM33 (32), LM36 (35), LM37 (36), LM38 (37), LM39 (38), LM42 (41), LM49 (48), LM58 (57), LM83 (77), LM85 (79), LM99 (92)	0.142	0.2472	0.441
7	LM20 (20), LM54 (53), LM56 (55), LM57 (56), LM82 (76)	0.102	0.1093	0.7725
8	LM12 (12), LM61 (60), LM64 (61), LM66 (62), LM67 (63), LM68 (64), LM70 (65), LM71 (66), LM72 (67), LM73 (68), LM75 (69), LM86 (80), LM87 (81), LM88 (82), LM89 (83), LM91 (85)	0.136	0.2947	0.3117
9	LM78 (72), LM94 (87)	0.033	0.1027	0.8471

*See Table 2.1 for descriptions of genotypes.

4.2.3 Linkage Disequilibrium

Linkage disequilibrium analysis revealed the presence of 597,871 loci pairs within a physical distance extending up to 16,356 bp. About 45,835 (7.67%) of loci pairs were in significant LD ($P < 0.05$). Further, 5,188 (0.87%) of the pairs were in complete LD ($R^2 = 1$). Marker pairs in LD were observed over long distances, however, a clear and rapid decline in LD with distance was observed. Pearson's correlation coefficients revealed negative correlation ($r = -0.0813$ between the linkage disequilibrium (R^2) and the physical distance (bp) as well as between the P-value and R^2 ($r = -0.59$), revealing the existence of linkage decay. A weak positive correlation ($r = 0.0543$) existed between the genetic distance and the P-value.

4.2.4 Marker-Trait Association

A total of 334 significant ($P < 0.05$) marker-trait associations (MTAs) were observed. Only the MTAs that had P values < 0.001 (Table 4.2) were considered as significant for all traits except for grain yield, thousand seed weight and number of days to maturity where significant ($P < 0.05$) marker-trait associations were considered because the three traits are highly complex, often with moderate to low heritability (Abdolshahi et al., 2015, Chapter 3). Appendix 4.1 presents slightly significant MTAs ($0.05 > P > 0.001$) that were not considered in this study. The MTAs considered in this study (Table 4.2) explained > 20% of the total phenotypic variation observed on all respective traits. Of the MTAs that were considered significant, four loci were identified to be highly associated with the number of days to heading, explaining 24.96% to 37.77% of the total phenotypic variation. Two of these makers were located on chromosome 5A, while the other two were found on chromosomes 5B and 6B (Table 4.2). The number of days to heading were recorded immediately before imposing drought stress but the means from the stressed and non-stressed experiments were used separately for GWAS to check for repeatability.

Table 4.2. DArTseq markers with high association with eight agronomic traits of 93 wheat genotypes evaluated under drought-stressed and non-stressed conditions

	Drought-stressed			Non-stressed		
Trait	Marker	Perm P	Marker R^2	Marker	Perm P	Marker R^2
Days to heading	5A|081.525617550|4542594|4542594	0.001	0.31411	5A|081.525617550|4542594|4542594	0.001	0.29551
	5A|084.411633690|3534155|3534155	0.001	0.37532	5A|084.411633690|3534155|3534155	0.001	0.37773
	5B|000.649324338|1209883|1209883	0.001	0.24964	5B|000.649324338|1209883|1209883	0.001	0.24964
	6B|079.586479380|3949288|3949288	0.001	0.30387	6B|079.586479380|3949288|3949288	0.001	0.30809
Plant height	1B|063.445873190|3937163|3937163	0.001	0.28054	2B|013.546408570|977308|977308	0.001	0.28426
	2D|128.146584600|4021827|4021827	0.001	0.28848	2B|023.182120080|1251215|1251215	0.001	0.26467
				5A|084.411633690|3534155|3534155	0.001	0.2667
				5B|000.649324338|1209883|1209883	0.001	0.23751
				6B|079.586479380|3949288|3949288	0.001	0.25864
				7B|112.004439500|2322338|2322338	0.001	0.26883
Spike length	2B|107.092980900|1029432|1029432	0.001	0.22172	2B|107.092980900|1029432|1029432	0.001	0.21201
	2B|108.086871100|1132117|1132117	0.001	0.22172	2B|108.086871100|1132117|1132117	0.001	0.21201
	2D|128.146584600|4021827|4021827	0.001	0.3196	2D|128.146584600|4021827|4021827	0.001	0.30445
	5B|000.000000000|3023157|3023157	0.001	0.24953	5B|000.000000000|3023157|3023157	0.001	0.24497
	5B|117.097644100|3950938|3950938	0.001	0.22692	5B|117.097644100|3950938|3950938	0.001	0.22025
	6B|079.586479380|3949288|3949288	0.001	0.27346	6B|079.586479380|3949288|3949288	0.001	0.27701
	7A|065.934336980|1118335|1118335	0.001	0.25871	7A|065.934336980|1118335|1118335	0.001	0.24691
	3A|056.634055130|3934533|3934533	0.001	0.23714	6B|031.043100140|1237876|1237876	0.001	0.20889
	1B|063.445873190|3937163|3937163	0.001	0.22512			
	1B|184.429245100|1113389|1113389	0.001	0.22723			
	4B|042.180040830|1081624|1081624	0.001	0.22838			
	4B|042.901192180|1027953|1027953	0.001	0.22838			
	6A|048.638907120|3944784|3944784	0.001	0.23035			
Days to maturity				6B|079.586479380|3949288|3949288	0.012	0.24028
Spikelets per spike	1B|239.642526900|1249348|1249348	0.001	0.33723	1B|239.642526900|1249348|1249348	0.001	0.32517
	2D|128.146584600|4021827|4021827	0.001	0.38306	2D|128.146584600|4021827|4021827	0.001	0.35446
	4B|042.180040830|1081624|1081624	0.001	0.32331	4B|042.180040830|1081624|1081624	0.001	0.33309
	4B|042.901192180|1027953|1027953	0.001	0.32331	4B|042.901192180|1027953|1027953	0.001	0.33309
	5B|000.649324338|1209883|1209883	0.001	0.31848	5B|000.649324338|1209883|1209883	0.001	0.28063
	6B|079.586479380|3949288|3949288	0.001	0.4069	6B|079.586479380|3949288|3949288	0.001	0.36461
	5B|000.000000000|3023157|3023157	0.001	0.34661	5A|084.411633690|3534155|3534155	0.001	0.32471
	2B|107.007987900|1087177|1087177	0.001	0.38011			
Kernels per spike	2D|128.146584600|4021827|4021827	0.001	0.30042	2D|128.146584600|4021827|4021827	0.001	0.30059
	4A|132.059989400|4989948|4989948	0.001	0.30242	2D|148.989565100|374614|wPt-4329	0.001	0.2447
				6B|031.043100140|1237876|1237876	0.001	0.29653
				6B|031.199477690|4990947|4990947	0.001	0.34092
				6B|033.035441000|1300029|1300029	0.001	0.34092
				6B|035.712467660|4989379|4989379	0.001	0.28856
				7A|048.712205470|1129617|1129617	0.001	0.28859
				7A|065.934336980|1118335|1118335	0.001	0.29523
1,000 seed weight				7B|076.034196290|1258792|1258792	0.03	0.23944
Grain yield				5D|138.209637900|7157166|7157166	0.021	0.22568

Perm P = Probability value; R^2 = marker-trait correlation

Two markers located on chromosomes 1A and 2D were associated with plant height under drought-stress. Under non-stressed condition, six markers were associated with plant height, of which two were located on chromosome 2B and the rest were on chromosomes 5A, 5B, 6B, and 7B. These markers explained 23.75% to 28.8% of the variation in plant height. Spike length was associated with 13 markers under drought-stressed condition explaining 22.17% to 31.96% of the total phenotypic variation; and eight markers under non-stressed condition; explaining 21.20% to 30.45% of the variation in spike length. The markers observed for this trait under drought-stress were from chromosomes 1B, 2B, 2D, 3A, 4B, 5B, 6A, 6B and 7A. Eight DArT markers were associated with spike length under non-stressed condition, of which, seven markers were consistent under both drought-stressed and non-stressed conditions from chromosomes 2B, 2D, 5B, and 7A (Table 4.2). Under drought-stress, SPS was highly associated with eight markers located on chromosomes, 1B, 2B, 2D, 4B, 5D and 6B; while under the same stress level, seven significant MTAs were recorded that were located on chromosomes 1B, 2D, 4B, 5A, 5B and 6B. Six of the markers, except for one located on chromosome 2B, one on 5A and one on 5B were consistent with the ones obtained under drought-stressed condition (Table 4.2). The B genome had most of the significant MTAs observed for this trait. The marker 6B|079.586479380|3949288|3949288 explained the highest proportion of the phenotypic variation (R^2 = 41%) under drought-stressed condition, while a marker on chromosome 2B explained the least proportion (R^2 = 28.06%) of the phenotypic variation observed under the non-stressed condition. Under drought-stressed condition, the number of kernels per spike was associated with two markers located on chromosomes 2D and 4A, explaining 30.04% and 30.24% of the observed phenotypic variation, in that order. Eight significant MTAs were detected under non-stressed condition on chromosomes 2D, 6B and 7A explaining 28.06% to 36.46% of the variation observed on the number of spikelets per spike. Three MTAs on chromosomes 6B, 7B and 5D were considered significant ($0.05 > P > 0.001$) for the number of days to maturity, thousand seed weight and grain yield accounting for 24.03%, 23.94% and 22.57% of the phenotypic variation, respectively.

A pleiotropic locus is associated and affects the expression of more than one phenotypic trait. In this study, several pleiotropic loci were identified including the marker 5A|084.411633690|3534155|3534155 that was associated with DTH, PH and SPS under non-

stressed condition (Table 4.2). Days to heading, PH and DTM under non-stressed condition; SL under drought-stressed condition; and SPS under drought-stressed condition were associated with the marker 6B|079.586479380|3949288|3949288 located on chromosome 6B. On chromosome 2D, the locus 2D|128.146584600|4021827|4021827 was associated with PH under drought-stress condition as well as SL, SPS, and KPS under both drought-stressed and non-stressed conditions. Plant height and SL under drought-stressed condition were associated with the marker 1B|063.445873190|3937163|3937163 on chromosome 1B. The marker 7A|065.934336980|1118335|1118335 was associated with SL under both drought-stressed and non-stressed conditions as well as with KPS under non-stressed condition. Additionally, 5B|000.000000000|3023157|3023157 was associated with SL under drought-stressed and non-stressed conditions as well as with SPS under drought-stressed condition only. Spike length and SPS under drought-stressed condition were associated with the marker 2B|108.086871100|1132117|1132117, while the marker 4B|042.180040830|1081624|1081624 was associated with SL under drought-stressed condition only and SPS under both drought-stressed and non-stressed conditions. Further, the locus 5B|000.649324338|1209883|1209883 was associated with DTH and PH under non-stressed condition as well as SPS under drought-stressed condition. Finally, 6B|031.043100140|1237876|1237876 was associated with SL and KPS under drought-stressed condition. Blast searches of the marker 6B|031.043100140|1237876|1237876 on the National Center for Biotechnology Information (NCBI) and GrainGenes databases revealed that this marker has a sequence alignment that is 97% identical to the TaMFT gene that regulates seed dormancy on chromosome 3A (Nakamura et al. (2015); http://www.uniprot.org/uniprot/A0A0K2RW47; http://blast.ncbi.nlm.nih.gov/Blast.cgi; (http://wheat.pw.usda.gov/GG2/blast.shtml).

Out of the 65 significant marker-trait associations observed, 25 trait-specific MTAs were differentiated. Chromosome 2B had four trait specific MTAs, of which one was associated with spike length under drought-stress, two with plant height under non-stressed condition and one with spike length under non-stressed condition. Traits that were represented by at least one significant trait-specific marker-trait association under either of the two water conditions were days to heading, plant height, spike length, number of spikelet per spike, number of kernels per spike, days to maturity, and grain yield (Table 4.2).

4.4 Discussion

Understanding the genetic bases of complex traits in polyploid crops such as wheat, presents an opportunity for drought tolerance breeding. To complement the growing need for such knowledge, the current study explored the population structure and association of genomic regions with yield and yield related traits in a diverse population of drought tolerant and susceptible wheat genotypes. High heritability estimates as well as significant and positive correlations were observed among the studied traits (Mwadzingeni et al. 2016b; Chapter 3) confirming the value of the data in the present marker-trait association analyses. This is supported by Laido et al. (2014) who reported the relevance of traits that had high heritability estimates for QTL detection.

4.4.1 Population Structure and Linkage Disequilibrium

The population evaluated was grouped into nine distinct genetic structures (Figure 4.1). This is expected given that the genetic materials possess diverse pedigrees which were systematically developed by CIMMYT. However, the existence of common origin or parents in the pedigrees of some genotypes often results in some levels of relationship among genotypes. The results obtained from the structure analysis will be useful in tracking potential parents that could be useful for drought tolerance breeding. Thus, future studies could use a sub-sample of the genetically divergent lines from this genetic pool exhibiting farmers-preferred and quality attributes.

Most of the unique groupings identified could be explained by existence of at least one common parent in the pedigree of genotypes within each cluster. For instance, in Cluster 1, five of the six genotypes from the heat nursery; LM13, LM15, LM21, LM25 and LM29; shared the common parent PASTOR in their parentage. Also, the genotypes LM50, LM51, LM52 and LM53 found in Cluster 2 could be related due to the sharing of crosses involving HUW234+LR34/PRINIA*2// in their pedigree. Similarly, the ancestral genotype SOKOLL which was common in most pedigrees of genotypes in Cluster 4 could be causing some similarities observed in that group. Interestingly, all the genotypes in Cluster 5 were descendants from the parents MILAN, KAUZ and PRINIA. Likewise, seven of the genotypes in Cluster 6

had pedigrees containing ATTILA and the parent PBW343 was common in all pedigrees of genotypes in Cluster 7, which could have contributed to formation of these respective clusters.

Existence of marker pairs in LD over long distances and closely linked pairs with non-significant LD observed in the current study has been previously reported in various crop species (Matschegewski et al., 2015; Neumann et al., 2011; Wang and Zhang, 2014). This could reflect that LD is not static as it can be influenced by other factors such as genetic admixtures apart from the genetic or physical distance.

4.4.2 Marker-Trait Association

The present study identified 334 significant ($P < 0.05$) marker-trait associations (Table 4.2). This will add to previously identified genomic regions influencing similar or complimentary traits. Although only those MTAs observed at $P < 0.001$ were considered significant in this study, the rest of these associations observed at $P < 0.05$ (Appendix 4.1) may be useful for drought tolerance breeding. These MTAs could be located on regions that influence the respective traits directly or indirectly. Thus, the proportion of the phenotypic variation ($R^2 > 0.2$) observed for all significant markers suggests their possible influence on respective traits. In this light, the observed MTAs for grain yield, days to maturity and thousand seed weight, were considered significant at $0.05 > P > 0.001$, since the traits are highly complex with low heritability. Drought tolerance is highly influenced by genotype by environment interaction (Blum, 2010; Khakwani et al., 2012) which could explain the low number of significant MTAs observed under drought-stressed than non-stressed condition.

Several research efforts have been directed at locating QTL influencing various agronomic traits to facilitate MAS in wheat improvement in the face of increased droughts along with other key production constraints (Alexander et al., 2012; Czyczyło-Mysza et al., 2011; Pinto et al., 2010). The genes identified in the current population adds to the currently available pool of genetic resources and candidate genes. Some of these loci could be located on regions that were already confirmed to be housekeeping genes for the traits under study. For instance, in the present study, significant MTAs have been identified on chromosomes that had previously been reported to house QTL for respective traits. Plant height was reported to be associated with genomic regions

on chromosomes 1B (Mathews et al., 2008; Pinto et al., 2010), 2B (Alexander et al., 2012; Mathews et al., 2008; Pinto et al., 2010), 5A (Mathews et al., 2008), 5B (Mathews et al., 2008; Pinto et al., 2010) and 6B (Mathews et al., 2008). Chromosome 5D was reported to harbour QTL for grain yield (Quarrie et al., 2005), in agreement with the present study. Further, Peleg et al. (2009) reported loci affecting the number of days to heading on chromosome 5A under varied drought-stress levels. In the present study, an MTA for TSW was recorded on Chromosome 7B, which was previously reported to have significant associations with the same trait using the markers *Xwmc606, Xgwm537, wPt1715* and *wPt2449* in a collection of tetraploid durum wheat genotypes (Laido et al., 2014). Blast search on the NCBI database reviewed that the DArTseq markers associated with DTH on chromosome 5A in the present study seems to be located on a highly conserved region since it has almost 100% sequence similarities with regions in other crop species including *Sorghum bicolor* L. and *Oryza sativa* L. (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Typically, loci or QTL regions that influence a particular trait under stress also control the trait under non-stressed condition (Mathews et al., 2008). This could be the case with loci that influenced spike length under non-stressed condition and were consistently observed under drought-stress in the present study. Similar explanation can be presented for the markers affecting the number of spikelet per spike under non-stressed condition that were consistent under drought-stressed condition, except for the locus at 5A|084.411633690|3534155|3534155. Ideally, the effects of such loci may not be influenced by the change in external environment. Such genomic regions could be useful in MAS or gene introgression when breeding for broad adaptation. On the other hand, some gene loci may influence particular traits differently under different sets of growing environments, resulting in markers or loci becoming inconsistently associated with particular traits when environmental conditions change. This has been witnessed on markers such as 1B|063.445873190|3937163|3937163 and 4B|042.901192180|1027953|1027953 which were associated with plant height and spike length, respectively, only under drought-stress.

High phenotypic trait correlations could be explained in terms of direct or indirect contribution of one trait to another. Looking into the genome, loci controlling such traits could be similar.

This is evidenced by the existence of several multi-trait associations where one gene will have pleotropic effects on highly correlated traits. Dholakia et al. (2003) reported that highly correlated traits are often controlled by a common QTL. For instance, the locus 2D|128.146584600|4021827|4021827 controls several traits such as plant height, spike length, number of spikelets per spike and the number of kernels per spike; which are often highly correlated (Kashif and Khaliq, 2004; Baloch et al., 2013). Such findings support the need to verify if the locus 6B|031.043100140|1237876|1237876 that was associated with spike length and the number of kernels per spike is not also linked to seed dormancy since it shared similar sequence alignment with the region controlling the latter trait in wheat. Interestingly, chromosome 5B which reportedly harbor a region controlling several agronomic traits (Edae et al. (2014) is found to carry genomic regions associated with DTH, PH, SPS and SL in the present study. Some loci, however, influenced only one trait, for instance, 2B|013.546408570|977308|977308 and 2B|023.182120080|1251215|1251215 which only affected plant height.

4.5 Conclusions

Marker trait association is key to identifying genomic regions that are associated with phenotypic traits of breeding significance. The present study identified a total of 65 highly significant marker trait associations under contrasting water regimes. Under drought-stressed condition; 4, 2, 13, 8, and 2 markers were highly associated with days to heading, plant height, spike length, spikelet per spike and kernels per spike, while under non-stressed condition; 4, 6, 8, 7 and 8 highly associated markers were identified, respectively. Only one marker per trait was considered significant at $P = 0.05$ for grain yield, days to maturity and thousand seed weight. The markers identified in this study are useful genomic resources to initiate marker-assisted selection and trait introgression of wheat under drought-stressed and non-stressed conditions, and for fine mapping and cloning of the underlying genes. Further studies are required to validate the significant markers identified in the present study.

4.6 References

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Appendix 4.1. DArTseq markers associated with eight agronomic traits of 93 wheat genotypes evaluated under drought-stressed and non-stressed conditions ($0.05 > P > 0.001$)

Drought-stressed			Non-stressed		
Marker	Perm P	Marker R^2	Marker	Perm P	Marker R^2
		Days to heading			
5B|000.649324338|1209883|1209883	0.003	0.23873	2B|107.007987900|1087177|1087177	0.002	0.29296
5A|086.598960660|1215648|1215648	0.003	0.26821	2D|128.146584600|4021827|4021827	0.003	0.26388
2D|128.146584600|4021827|4021827	0.003	0.27203	5A|086.598960660|1215648|1215648	0.007	0.2608
3A|134.034465000|3945820|3945820	0.003	0.27326	1B|263.548918400|1396299|1396299	0.009	0.28202
2B|107.007987900|1087177|1087177	0.003	0.29771	4A|050.549009130|1118950|1118950	0.014	0.21772
2B|107.092980900|1029432|1029432	0.011	0.21538	6B|078.748041400|3958789|3958789	0.024	0.26966
2B|108.086871100|1132117|1132117	0.011	0.21538	3A|134.034465000|3945820|3945820	0.026	0.24248
6B|078.748041400|3958789|3958789	0.011	0.27278	2B|107.092980900|1029432|1029432	0.034	0.20372
5B|000.000000000|3023157|3023157	0.013	0.24497	2B|108.086871100|1132117|1132117	0.034	0.20372
6A|067.991408960|1237708|1237708	0.023	0.23895			
3B|013.678476040|3958709|3958709	0.027	0.23445			
3A|004.294450061|4990595|4990595	0.028	0.23402			
4A|050.549009130|1118950|1118950	0.029	0.20193			
1B|037.446808880|4991863|4991863	0.035	0.23014			
6A|056.724637920|3945797|3945797	0.043	0.19675			
2B|057.230408490|3064921|3064921	0.043	0.22823			
		Plant height			
5B|000.649324338|1209883|1209883	0.002	0.24165	3A|076.019683050|4394549|4394549	0.002	0.22396
3A|070.942486970|1104502|1104502	0.002	0.24277	1B|063.445873190|3937163|3937163	0.002	0.25054
3B|008.294356567|4261111|4261111	0.002	0.29407	3B|008.294356567|4261111|4261111	0.002	0.27192
3B|012.621927070|3028387|3028387	0.003	0.24898	3B|005.715696741|1125891|1125891	0.003	0.24427
7B|112.004439500|2322338|2322338	0.008	0.24533	3A|070.942486970|1104502|1104502	0.004	0.21477
3A|076.019683050|4394549|4394549	0.01	0.2112	4A|050.549009130|1118950|1118950	0.009	0.20701
3B|011.134281090|3027768|3027768	0.017	0.25656	2D|128.146584600|4021827|4021827	0.013	0.22945
2D|126.901211300|1109826|1109826	0.018	0.20185	3A|134.034465000|3945820|3945820	0.013	0.22981
3B|005.715696741|1125891|1125891	0.018	0.23024	3B|017.067952280|998573|998573	0.015	0.24932
3B|013.678476040|3958709|3958709	0.018	0.23051	2B|107.007987900|1087177|1087177	0.016	0.24799
3B|012.928798990|1165422|1165422	0.02	0.20062	2A|043.555079010|3222362|3222362	0.017	0.19637
3D|147.079710800|1108739|1108739	0.022	0.19969	6A|067.991408960|1237708|1237708	0.024	0.22279
3A|075.704509290|1086515|1086515	0.03	0.22183	6A|028.951443700|1094966|1094966	0.027	0.21995
3B|017.067952280|998573|998573	0.03	0.24508	2B|033.737015920|4733926|4733926	0.027	0.24342
2B|013.546408570|977308|977308	0.032	0.24293	3B|029.893695730|3222379|3222379	0.029	0.18924
2B|107.007987900|1087177|1087177	0.032	0.24322	3B|011.134281090|3027768|3027768	0.029	0.24021
3B|065.420680590|1153055|1153055	0.033	0.2198	2B|012.351178520|2297566|2297566	0.031	0.23973
3A|002.485631701|3952392|3952392	0.041	0.18783	2B|057.230408490|3064921|3064921	0.036	0.21537
3A|004.294450061|4990595|4990595	0.041	0.21675	1B|037.446808880|4991863|4991863	0.036	0.21551
3B|029.893695730|3222379|3222379	0.042	0.18517	2A|104.293924100|1110657|1110657	0.038	0.18584
3B|005.829269954|2256562|2256562	0.042	0.23713	6A|048.226863160|1150993|1150993	0.038	0.21467
2A|047.886253160|5971468|5971468	0.043	0.21221	7B|097.452251360|1126833|1126833	0.041	0.18452
2B|023.182120080|1251215|1251215	0.043	0.21221	7B|100.338066000|1096412|1096412	0.041	0.18452
3B|009.173872085|1083589|1083589	0.044	0.21107	7B|100.338066000|1276616|1276616	0.041	0.18452
			7B|100.475730800|3937689|3937689	0.041	0.18452
			2B|012.351178520|2291877|2291877	0.041	0.21291
			3B|010.530635430|4989647|4989647	0.041	0.23513
			2B|026.939223010|1093835|1093835	0.044	0.20929

Appendix 4.1. (continued)

Drought-stressed			Non-stressed		
Marker	Perm P	Marker R^2	Marker	Perm P	Marker R^2
		Spike length			
3A|070.942486970|1104502|1104502	0.005	0.19608	1B|184.429245100|1113389|1113389	0.002	0.20749
2B|107.007987900|1087177|1087177	0.005	0.23609	3A|056.634055130|3934533|3934533	0.004	0.21891
2B|107.234832300|1159774|1159774	0.006	0.18982	4B|042.180040830|1081624|1081624	0.004	0.22577
5B|118.415533200|2303045|2303045	0.008	0.21189	4B|042.901192180|1027953|1027953	0.004	0.22577
2B|106.869346000|3023780|3023780	0.009	0.18772	2B|107.234832300|1159774|1159774	0.006	0.18905
1B|176.095445600|1259406|1259406	0.01	0.18276	5B|000.649324338|1209883|1209883	0.008	0.17696
7A|065.934336980|4541148|4541148	0.01	0.18356	5D|138.209637900|7157166|7157166	0.008	0.19944
5D|019.203412160|1161544|1161544	0.01	0.18569	1B|063.445873190|3937163|3937163	0.008	0.19985
3B|011.134281090|3027768|3027768	0.011	0.22365	2B|107.007987900|1087177|1087177	0.008	0.22003
2A|060.600016560|2361439|2361439	0.017	0.17704	1B|176.095445600|1259406|1259406	0.01	0.17524
3B|027.228283330|304361|wPt-5432	0.017	0.17801	2A|043.555079010|3222362|3222362	0.011	0.17382
2B|106.857184700|3533741|3533741	0.017	0.20066	2A|060.600016560|2361439|2361439	0.013	0.17187
3A|137.708449300|980006|980006	0.019	0.17557	6A|048.638907120|3944784|3944784	0.013	0.19444
5B|000.649324338|1209883|1209883	0.025	0.17252	1D|079.079510220|1078020|1078020	0.015	0.17008
1B|045.390774230|1294103|1294103	0.03	0.19166	6B|030.982252730|1252863|1252863	0.016	0.19243
6B|031.043100140|1237876|1237876	0.031	0.16808	3A|070.942486970|1104502|1104502	0.022	0.16746
5B|114.361194300|1126378|1126378	0.032	0.20907	5B|120.771719000|2275671|2275671	0.025	0.20819
1B|043.312855080|1162221|1162221	0.034	0.16642	1D|079.958633130|3954634|3954634	0.026	0.16481
1B|044.758067030|1266583|1266583	0.034	0.16642	5B|118.415533200|2303045|2303045	0.026	0.18742
1B|045.177576690|3022879|3022879	0.034	0.16642	1D|082.419690380|1096857|1096857	0.026	0.18855
1B|046.175945760|5325402|5325402	0.034	0.16642	2B|106.869346000|3023780|3023780	0.029	0.16331
1B|047.111474150|7346672|7346672	0.034	0.16642	2A|058.063556250|1062330|1062330	0.029	0.16357
1B|047.741224110|7334370|7334370	0.034	0.16642	6B|031.510249370|4992590|4992590	0.029	0.20528
1B|051.254224490|7346032|7346032	0.034	0.16642	2B|087.475859870|3944716|3944716	0.032	0.16243
1B|051.289701790|3950468|3950468	0.034	0.16642	2B|061.547753160|4542690|4542690	0.033	0.18528
1B|054.040907460|1266945|1266945	0.034	0.16642	6A|067.991408960|1237708|1237708	0.038	0.18309
1B|061.689082610|1122393|1122393	0.034	0.16642	1D|079.061658050|5324336|5324336	0.038	0.18326
1B|061.689082610|4261902|4261902	0.034	0.16642	7B|097.452251360|1126833|1126833	0.04	0.15854
1B|063.445873190|4005038|4005038	0.034	0.16642	7B|100.338066000|1096412|1096412	0.04	0.15854
1B|066.188180470|1164185|1164185	0.034	0.16642	7B|100.338066000|1276616|1276616	0.04	0.15854
1B|070.209793990|7345501|7345501	0.034	0.16642	7B|100.475730800|3937689|3937689	0.04	0.15854
1B|070.891907290|1232724|1232724	0.034	0.16642	5D|019.203412160|1161544|1161544	0.04	0.15888
1B|247.531565800|1699070|1699070	0.034	0.16642	7A|065.934336980|4541148|4541148	0.044	0.15726
1B|263.257205600|1767653|1767653	0.034	0.16642	2B|057.230408490|3064921|3064921	0.044	0.18076
2A|004.227547215|1732419|1732419	0.034	0.16642			
2A|007.484441636|1162329|1162329	0.034	0.16642			
3A|056.634055130|4539513|4539513	0.034	0.16642			
5A|069.124959010|993853|993853	0.034	0.16642			
6A|086.477776400|1238110|1238110	0.034	0.16642			
6B|073.055837020|978839|978839	0.034	0.16642			
7D|013.562439360|1216320|1216320	0.034	0.16642			
7D|082.070949060|1387325|1387325	0.034	0.16642			
6A|067.991408960|1237708|1237708	0.034	0.18993			
2B|087.475859870|3944716|3944716	0.035	0.16606			
4B|042.901192180|3954457|3954457	0.035	0.18907			
5B|001.190695579|1088009|1088009	0.037	0.18818			
5B|120.771719000|2275671|2275671	0.037	0.20658			
3A|134.034465000|3956339|3956339	0.038	0.18774			
6A|048.638907120|4406711|4406711	0.044	0.20467			

Appendix 4.1. (continued)

Drought-stressed			Non-stressed		
Marker	Perm P	Marker R^2	Marker	Perm P	Marker R^2
Spikelet per spike					
6A|028.951443700|1094966|1094966	0.003	0.31618	6B|030.951829030|1094565|1094565	0.004	0.29618
3B|027.228283330|304361|wPt-5432	0.004	0.27662	2B|107.007987900|1087177|1087177	0.004	0.32519
6A|048.638907120|2281875|2281875	0.004	0.3336	6B|031.043100140|1237876|1237876	0.005	0.25697
5D|019.203412160|1161544|1161544	0.006	0.26843	5B|000.000000000|3023157|3023157	0.005	0.28927
2D|126.901211300|1109826|1109826	0.007	0.25933	5A|081.525617550|4542594|4542594	0.006	0.28448
3A|134.034465000|3945820|3945820	0.007	0.28985	2D|144.969480900|1104828|1104828	0.007	0.28341
1B|063.445873190|3937163|3937163	0.007	0.29219	4B|042.901192180|3954457|3954457	0.009	0.28057
1B|176.095445600|1259406|1259406	0.009	0.25508	6B|030.982252730|1252863|1252863	0.012	0.26518
3A|004.294450061|4990595|4990595	0.009	0.28386	6A|028.951443700|1094966|1094966	0.012	0.26633
6A|049.559984320|2280316|2280316	0.009	0.30866	1D|067.245066070|4262641|4262641	0.012	0.2701
3D|147.079710800|1108739|1108739	0.01	0.2527	6B|030.951829030|1765837|1765837	0.012	0.29597
2D|144.969480900|1104828|1104828	0.013	0.27969	6A|049.559984320|2280316|2280316	0.012	0.29643
4B|042.901192180|3954457|3954457	0.017	0.27778	2D|148.989565100|374614|wPt-4329	0.013	0.23457
6B|078.748041400|3958789|3958789	0.018	0.30002	6B|031.199477690|4990947|4990947	0.015	0.26184
3A|056.634055130|3934533|3934533	0.024	0.27215	6B|033.035441000|1300029|1300029	0.015	0.26184
1D|067.245066070|4262641|4262641	0.029	0.26746	3A|004.285602847|3959705|3959705	0.015	0.26207
2A|091.748081010|1117352|1117352	0.037	0.26411	3A|134.034465000|3945820|3945820	0.015	0.26406
3A|004.285602847|3959705|3959705	0.037	0.26454	6B|078.748041400|3958789|3958789	0.017	0.28445
3A|076.019683050|4394549|4394549	0.038	0.23379	6D|000.191508874|5324047|5324047	0.022	0.25655
3A|002.485631701|3952392|3952392	0.04	0.23339	1D|082.419690380|1096857|1096857	0.023	0.25596
5B|117.097644100|3950938|3950938	0.043	0.23173	1D|079.958633130|3954634|3954634	0.028	0.22285
			2D|126.901211300|1109826|1109826	0.028	0.2231
			3A|004.294450061|4990595|4990595	0.028	0.2522
			1D|079.417276640|1094132|1094132	0.029	0.25187
			5D|052.977153290|3532978|3532978	0.03	0.22179
			3A|004.732320657|4989854|4989854	0.031	0.21887
			1D|079.079510220|1078020|1078020	0.031	0.2197
			2B|101.018822200|1113485|1113485	0.031	0.24993
			6B|031.429488930|1131748|1131748	0.031	0.2742
			6B|027.222942130|1090582|1090582	0.032	0.27264
			6B|031.160976350|1724555|1724555	0.037	0.27002
			6B|031.087340250|4398260|4398260	0.039	0.26929
			6A|056.724637920|4539672|4539672	0.04	0.2147
			5B|117.097644100|3950938|3950938	0.042	0.21408
			6B|035.712467660|4989379|4989379	0.042	0.24415
Kernels per spike					
6B|079.586479380|3949288|3949288	0.016	0.23815	6B|030.951829030|1144567|1144567	0.002	0.26852
7D|160.711960400|2358656|2358656	0.017	0.20662	2D|153.055365100|1237263|1237263	0.003	0.23728
2D|153.055365100|1237263|1237263	0.017	0.20691	5B|117.097644100|3950938|3950938	0.003	0.23779
6B|030.951829030|1144567|1144567	0.017	0.23637	6B|030.951829030|1765837|1765837	0.004	0.2811
2D|148.989565100|374614|wPt-4329	0.019	0.20373	6B|030.982252730|1252863|1252863	0.005	0.25133
1D|058.851037050|5411762|5411762	0.021	0.23176	6B|031.197452460|3939783|3939783	0.005	0.27574
6B|029.393612280|1003850|1003850	0.022	0.25482	6B|029.393612280|1003850|1003850	0.006	0.27067
6B|031.822757590|4540541|4540541	0.025	0.25203	6B|031.301254040|2276412|2276412	0.007	0.2463
6B|031.043100140|1237876|1237876	0.029	0.19792	1D|058.851037050|5411762|5411762	0.008	0.23862
5B|000.000000000|3023157|3023157	0.036	0.2251	1B|239.642526900|1249348|1249348	0.008	0.24518
6B|026.564442100|1115276|1115276	0.039	0.24743	6B|031.822757590|4540541|4540541	0.008	0.26855
5B|001.190695579|1088009|1088009	0.044	0.22238	6B|031.993678630|1234486|1234486	0.009	0.26076

Appendix 4.1. (continued)

Drought-stressed			Non-stressed		
Marker	Perm P	Marker R^2	Marker	Perm P	Marker R^2
Kernels per spike					
			2D|148.923115000|1124930|1124930	0.012	0.20103
			2D|149.648499200|1117423|1117423	0.012	0.20103
			2D|149.851806300|1260378|1260378	0.012	0.20103
			2D|150.373883300|1132957|1132957	0.012	0.20103
			6B|079.586479380|3949288|3949288	0.012	0.23068
			4B|042.180040830|1081624|1081624	0.012	0.23374
			4B|042.901192180|1027953|1027953	0.012	0.23374
			1D|079.417276640|1094132|1094132	0.012	0.23376
			6A|049.559984320|2280316|2280316	0.012	0.25411
			6B|021.333495120|1091969|1091969	0.012	0.2551
			6B|026.564442100|1115276|1115276	0.012	0.25656
			1A|080.925753810|3938842|3938842	0.012	0.25695
			2A|127.024087100|3943270|3943270	0.014	0.197
			2A|123.580916200|3949672|3949672	0.014	0.22699
			7A|112.746111900|5331823|5331823	0.014	0.22699
			5B|000.000000000|3023157|3023157	0.014	0.22718
			6B|030.951829030|2322413|2322413	0.023	0.24512
			1D|079.417276640|1039789|1039789	0.024	0.22137
			7B|042.947028100|986776|986776	0.025	0.21966
			6B|023.023153150|1154773|1154773	0.028	0.2423
			2D|152.244494700|1104321|1104321	0.03	0.21798
			2D|155.591825400|1279862|1279862	0.034	0.21645
			6B|077.148647860|2279482|2279482	0.034	0.2399
			6B|030.951829030|1139022|1139022	0.038	0.23815
			6B|029.393612280|1003850|1003850	0.039	0.23789
			2D|148.923115000|1096024|1096024	0.04	0.18498
			2D|150.373883300|1122467|1122467	0.04	0.18498
			6B|041.061995810|2309137|2309137	0.042	0.21383

Perm P = Probability value; R^2 = marker-trait correlation

CHAPTER 5. COMBINING ABILITY AND GENE ACTION CONTROLLING YIELD AND YIELD COMPONENTS IN WHEAT UNDER DROUGHT STRESSED AND WELL-WATERED CONDITIONS

Abstract

This study determined the combining ability and gene action controlling yield and yield related traits under drought-stressed and non-stressed conditions involving 12 wheat parents and their 66 half diallel crosses. The materials were evaluated using a 6 x 13 lattice design with two replications under field and greenhouse conditions during April to October 2016. Plant height (PH), productive tiller number (TN), kernels per spike (KPS), thousand seed weight (TSW) and grain yield (GY) were recorded. Significant effects of genotypes, water regimes and test environments were observed. The effects of general and specific combining abilities were significant for PH, KPS, TSW and GY revealing the influence of both additive and non-additive gene effects in that order. For most traits, the ratios of GCA to SCA variances were less than a unity, indicating the predominance of non-additive gene effects. Parents LM17 and LM21 had consistent negative GCA effects for PH, hence, could be useful in breeding for reduced plant height. Consistently high GCA effects were observed on LM02 for GY; LM02 and LM23 for KPS; and LM04 and LM09 for TSW, suggesting presence of additive genes. LM17 x LM23, LM04 x LM45, LM29 x LM45 and LM09 x LM85 had negative SCA values for plant height, hence could be selected for reduced PH. LM02 x LM45, LM29 x LM85 and LM21 x LM23, LM13 x LM23, and LM09 x LM21 were better specific combiners for drought stressed KPS, TSW and GY, respectively, and are useful for further selection.

Keywords: combining ability, drought tolerance, gene action, wheat, yield components

5.1 Introduction

Combining ability and the nature of gene action controlling the inheritance of grain yield and yield related traits predetermines the usefulness of breeding lines and their resultant progenies under ranges of environmental conditions. Analysis of the general combining ability (GCA) and specific combining ability (SCA) effects allows estimation of the predominant gene actions controlling adaptive traits (Kearsey and Pooni, 1997; Dholariya et al., 2014; Masood et al., 2014). Crossing of parents that combine well to give superior progenies ensure effective transfer of desired traits. Recent studies have demonstrated that several complex agronomic traits in wheat are controlled by both additive and non-additive gene actions (Kumar et al., 2011; Adel and Ali, 2013). In some studies, additive gene action has been reported as the most important (Adel and Ali, 2013), while predominance of non-additive gene interaction has been noted for some agronomic traits (Jadoon et al., 2013; Rad et al., 2013). Considering that yield, is a complex trait that is influenced by several minor genes, its improvement can be achieved through both direct and indirect selection for yield components (Hassan et al., 2007; Farshadfar et al., 2014; Jatoi et al., 2014). Key traits for improvement of grain yield (GY) and drought tolerance include plant height (PH), the number of productive tillers (TN), number of spikelets per spike (SPS), number of kernels per spike (KPS) and thousand seed weight (TSW) (Monneveux et al., 2012). In the case of plant height, parents with low negative GCA effects will be more ideal to obtain relatively short progenies that are better adapted to water-stressed environments. On the other hand, selection for grain yield and yield components should target parents with high positive GCA or crosses with high positive SCA effects (Masood et al., 2014).

Various mating designs and statistical tools are available to determine the nature and magnitude of combining ability, as well as the type of predominant gene action controlling yield and related traits. This is dependent on the ultimate breeding objective and available genetic resources. North Carolina and diallel designs are among the prominently used controlled mating designs. Diallel mating designs have been extensively utilized in wheat breeding following Griffings' Methods and models (Griffing, 1956; Dabholkar, 1999; Omar et al., 2010), or as proposed by Hayman (1954). Where estimation of maternal effects is required, Griffings' full diallel analyses (Method I) that includes parents, crosses and their reciprocals, or Method III that excludes only parents become the designs of choice. Likewise, the rest of the genetic parameters can effectively

be estimated using Method II that exclude reciprocals (Acquaah, 2009). Method II reduces the number of entries from p^2 cross combinations, for a full diallel, to $p/2(p + 1)$. This lowers both labor and cost of the crossing program, particularly for crops with cleistogamous flowering system such as wheat.

Either the F1, F2 or both generations can be used for genetic analysis, depending on the set objectives and availability of enough F1 seeds for evaluation (Kearsey and Pooni, 1997; Joshi et al., 2004; Acquaah, 2009; Al-Naggar et al., 2015). Where hybrid seed production is not a priority, as is the case with most wheat breeding programs, gene action and combining ability can effectively be assessed at the F2 generation to guide hybridization and accumulation of desirable genes. This also allows seed increase for evaluation. Several genetic analyses in wheat and other agronomic crops have been carried out at the F2 generation (Jadoon et al., 2013; Rad et al., 2013). Recent studies using diallel methods at F1 or F2 generations of bread wheat have observed significant ($P < 0.05$) positive heterotic, GCA and SCA effects for yield and yield components, hence, indicating the likelihood of wheat improvement through designed crosses and selection at advanced generations (Akinci, 2009; Omar et al., 2010; Farshadfar et al., 2014; Al-Naggar et al., 2015)

Extensive variability for drought tolerance exists among the nurseries that were received for evaluation from CIMMYT (Mwadzingeni et al., 2016). Such variability, if properly managed could be useful in the development of new cultivars or improvement of the adaptability of existing ones to drought stressed conditions. Acquiring information on the combining ability of parental lines selected from that germplasm pool could determine their potential to improve cultivated wheat. Since the current target in wheat hybridization is not to produce hybrids, due to existing bottlenecks in commercial hybrid production and the challenges in getting sufficient F1 seed for evaluation, combining ability analysis using F2 provides adequate guidelines for selection and trait advancement. There is a need to deduce the importance of the F2 diallel crosses from the selected CIMMYT lines through genetic analysis before deploying them into local drought tolerance breeding programs. This study, therefore, aimed at determing the combining ability and gene action controlling yield and yield related traits under drought-

stressed and non-stressed conditions involving 12 wheat parents and their 66 progenies derived through a half diallel mating design.

5.2 Materials and Methods

5.2.1 Parental Lines, Crosses and Mating Design

Twelve parental bread wheat genotypes of CIMMYT origin; LM02, LM04, LM05, LM09, LM13, LM17, LM21, LM22, LM23, LM29, LM45 and LM85, selected from Chapter 2 were crossed in a 12 x 12 half diallel mating design, Method II. The genotypes were selected based on their divergent agronomic traits and relatively high levels of drought tolerance after extensive phenotyping. Table 5.1 presents the details of the selected parents including their stress tolerance index according to Mwadzingeni et al. (2016). The lines had high STI values, of which line LM23 had the highest stress tolerance index (1.07), while the lowest was LM13 that had a moderate value of 0.55. Crossing was done in the greenhouse where the materials were planted in crossing blocks that were stagger-planted three times at weekly interval to cater for differences in the number of days to maturity and to ensure synchronized flowering and continuous supply of pollen. This was done from June to October 2015. Standard procedures for wheat crossing, involving hand emasculation and subsequent pollination, were followed.

Table 5.1. List of wheat parents used for half diallel analysis

Parent	Name	Pedigree	Stress tolerance index
1	LM02	JIANG 4/4/DUCULA	0.76
2	LM04	ONIX/4/MILAN/KAUZ//PRINIA/3/BAV92	0.86
3	LM05	ACHTAR/4/MILAN/KAUZ//PRINIA/3/BAV92	0.89
4	LM09	SOKOLL*2/ROLF07	0.84
5	LM13	SOKOLL/ROLF07	0.55
6	LM17	ESDA/KKTS	0.75
7	LM21	PRL/2*PASTOR	0.82
8	LM22	MUNAL #1	0.92
9	LM23	QUAIU	1.07
10	LM29	PRL/2*PASTOR*2//SKAUZ/BAV92	0.98
11	LM45	ROLF07/YANAC//TACUPETO F2001/BRAMBLING	0.81
12	LM85	SW94.60002/4/KAUZ*2//DOVE/BUC/3/KAUZ/5/SW91-12331	0.91

5.2.2 Generation of F2 Crosses and Phenotypic Evaluation

The 66 F1 crosses were selfed from November 2015 to March 2016 to produce F2 seeds. During April to October 2016, the 78 genotypes were evaluated under field and greenhouse conditions. Field experiments were carried out at Ukulinga Research Farm (29° 40′ S, 30° 24′ E; 806m above sea level) under two contrasting water regimes involving drought stressed and well-watered conditions (Chapter 2). A custom-made plastic mulch rain-out shelter system was used to eliminate effects of untimely rainfall. Weather conditions at Ukulinga Research Farm during the study period are summarized in Table 5.2. The field temperatures and evapo-transpiration were lower than those experienced during the study by Mwadzingeni et al. (2016). The greenhouse's day/night temperatures were adjusted to 25ºC/15ºC, while the humidity was maintained between 45% and 55%. Field plots were 1.5m long, while in the greenhouse, 7 plants of the same genotype were established using 5 litter capacity plastic pots of composited pine bark media. Standard spacing and agronomic practices were followed during planting and crop establishment. The 78 genotypes comprising of parents and crosses, excluding reciprocal crosses, were laid out in a 6 x 13 lattice design with two replications under stressed and non-stressed conditions in the greenhouse and in the field, providing 4 test conditions. Drought stress was imposed to 35% of the field capacity from 50% heading to maturity. Data on five agronomic traits was recoded. The traits included the number of productive tillers (TN) that was recorded as tillers that successfully set seed at maturity, plant height (PH) measured from ground level to the tip of the head at maturity, numbers of kennels per spike (KPS) recorded after harvesting, thousand seed weight (TSW) and grain yield per plot (GY) recorded at maturity. Grain yield for each plot was standardized to yield per 30 plants for both the greenhouse and field studies to eliminate the plot size differences between the two sites.

Table 5.2. Monthly weather data during the field trial at Ukulinga, Pietermaritzburg, from April to September 2016

Month	Tmax (°C)	Tmin (°C)	RHmax (%)	RHmin (%)	Rs (MJ/m^2)	ET0 (mm)
April	26.743	15.20633	89.406	58.68933	13.22	80.71
May	23.76097	12.00258	91.39194	56.09	11.94774	68.71
June	22.303	11.17533	87.978	55.85133	10.47133	57.24
July	20.0829	9.199032	89.13194	45.08484	10.84516	61.38
August	23.8671	11.1729	78.43355	29.97613	14.31516	103.34
September	22.87433	12.30567	99.20067	50.50933	13.12133	80.1

Tmax, average maximum temperature; Tmin, average minimum temperature; RHmax, average maximum relative humidity; RHmin, average minimum relative humidity; Rs, average total radiation; ET0, average total relative evapo-transpiration.

5.2.3 Data Analysis

Following separate analysis of variance and tests for homogeneity that revealed significant genotypic and water regime differences, as well as homogeneous and comparable variances in the two study sites, combined analysis of variance was performed on Genstat 18 (Payne, 2014) to determine if the genotypes, sites and water regimes were significantly different. The GCA and SCA effects were determined separately for each of the four test conditions according to Griffing (1956)'s Method II, Model I using R statistical software (R_Core_Team, 2013), following the general linear model: $Y_{ijk} = \mu + g_i + g_j + s_{ij} + e_{ijk}$; where Y_{ijk} is the observed measurement for the ij^{th} cross grown in the k^{th} replication; μ is the population mean; g_i and g_j are the GCA effects; s_{ij} the SCA effect; and e_{ijk} the error term associated with the ij^{th} cross evaluated in the k^{th} replication (Griffing, 1956). The relative contribution of GCA to the total sum of squares was estimated by dividing the variances due to GCA by the variances due to SCA (σ^2gca/σ^2sca) according to Baker (1978).

5.3 Results

5.3.1 Mean Performance

Analysis of variance revealed significant ($P < 0.05$) effects of the genotypes, water regimes and test environments. Table 5.3 presents the mean squares and significant tests after combined analysis of variance for the five phenotypic traits of 66 crosses and their 12 parents evaluated across the two test environments and water regimes**.** Most of the interaction effects were significant except the genotype by water regime interaction that did not significantly affect PH and TN; the genotype by test environment interaction that had no significant effects on TN and GY; and the genotype by water regime by site interaction that significantly affected TSW only. Much of the total mean squares were accounted for by the environmental effects except for the number of productive tillers and grain yield that had the highest mean squares due to water regime.

Table 5.3. Mean squares and significant tests after combined analysis of variance for five phenotypic traits of the 66 crosses and their 12 parents evaluated across the two test environments and two water regimes

Source of variation	DF	PH	TN	KPS	TSW	GY
Gen	77	137.92***	1.3712**	146.02***	67.4***	13023***
WR	1	4422.67***	206.7447***	3748.33***	7258.73***	2949294***
Env	1	44143.02***	6.5095**	17003.92***	16137.04***	19835^{ns}
Gen.WR	77	46.66^{ns}	0.9122^{ns}	42.2*	37.48***	8750*
Gen.Env	77	70.51***	0.7453^{ns}	75.98***	43.78***	5425^{ns}
WR.Env	1	474.05***	19.493***	4.37^{ns}	184.98**	60545**
Gen.WR.Env	77	45.66^{ns}	0.6925^{ns}	29.29^{ns}	36.93***	5499^{ns}
Residual	311	35.24	0.8669	30.36	21.06	6569

DF, degrees of freedom; Env, test environment; Gen, genotype; PH, plant height; TN, number of productive tillers; KPS, number of kennels per spike; TSW, thousand seed weight; GY, grain yield per plot; WR, water regime; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, non-significant difference.

Mean values, coefficients of variation (CVs), standard error of differences (SEDs) and list significant differences (LSDs) of the genotypes evaluated under drought stressed and non-stressed conditions are presented on Table 5.4. Mean plant height decreased from 96.82 cm to 89.75 cm; and from 111.90 cm to 108.32 cm in the greenhouse and field experiments, respectively, due to water stress. The number of kernels per spike also decreased from mean values of 41.90 to 37.17; and 52.51 to 47.44 in the greenhouse and field experiments, respectively. Similarly, a decrease in the mean number of productive tilers was observed in both the greenhouse (from 4.53 to 3.07) and the field (from 4.01 to 3.22). A decrease in thousand seed weight due to water stress was also recorded. Grain yield reduction by 48.22% was recorded in the greenhouse, while in the field, yield was reduced by 40%. Some crosses performed better than both parents on some traits, for instance, cross LM09 x LM23 consistently had grain yield performance above the two parents across all test conditions. Others performed consistently better than both parents under particular water regimes, such as the cross LM05 x LM23 with yield performance above both parents under stressed condition. Likewise, among all the traits evaluated, some genotypes performed better or lower than their mid parent values, while some performed lower than both parents. Notably, genotype LM21 maintained the shortest plant height under non-stressed conditions. However, under stress, crosses such as LM05 x LM29 had lower plant height than the latter. Evidently, the values of all traits recorded followed a continuous distribution.

Table 5.4. Means for yield and yield components of 66 F2 crosses and 12 parental genotypes evaluated under stressed and non-stressed conditions in the greenhouse and field

	PH				KPS				TN				TSW				GY			
	Stressed		Non-stressed		Stressed		Non-stressed		Stressed		Non-stressed		Stressed		Non-stressed		Stressed		Non-stressed	
Genotype	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
Parents																				
LM02	101.20	113.75	96.60	107.38	39.13	57.06	45.75	49.02	2.50	3.17	2.70	3.32	46.44	34.75	45.00	42.25	132.80	186.30	173.00	205.60
LM04	99.35	109.19	97.50	113.56	52.50	39.13	51.65	52.63	3.40	3.18	4.50	4.58	40.31	41.94	58.25	54.31	221.40	159.20	402.70	394.30
LM05	76.90	109.19	90.20	105.09	31.38	62.69	48.52	63.31	1.95	2.03	4.80	3.53	45.50	33.81	58.88	41.19	86.30	132.30	410.30	276.40
LM09	88.05	106.56	90.50	110.38	33.69	37.94	33.19	47.94	3.80	3.03	5.50	4.79	42.56	44.81	55.44	52.69	164.10	154.10	299.90	361.10
LM13	104.35	106.75	103.80	109.91	51.44	45.22	58.29	55.75	3.60	2.91	4.00	3.40	31.56	34.81	49.81	51.31	175.50	134.70	348.30	291.40
LM17	88.20	102.03	95.45	105.38	32.94	49.69	34.56	55.75	3.50	4.04	5.30	4.90	36.88	37.19	49.88	45.94	127.90	225.80	273.90	382.00
LM21	82.90	99.00	98.20	97.47	37.69	45.50	32.86	47.81	3.00	3.70	5.20	5.00	54.50	37.69	61.81	47.94	187.60	191.50	317.50	335.30
LM22	92.05	104.62	101.30	110.38	44.75	53.63	35.25	56.96	4.30	3.02	4.10	4.22	54.75	36.44	51.31	42.69	315.20	172.50	221.40	307.10
LM23	97.35	118.22	93.40	116.66	45.38	53.50	43.44	50.75	3.10	3.40	3.50	3.47	43.88	40.25	54.44	45.44	183.50	216.90	249.30	239.20
LM29	93.25	102.31	91.90	105.44	36.00	42.19	48.56	46.56	3.90	3.73	6.00	4.69	42.31	38.31	59.44	45.94	176.70	181.10	523.00	295.40
LM45	95.00	105.84	95.80	107.94	42.88	47.31	51.44	56.84	3.70	3.86	5.20	4.99	47.00	43.75	62.81	44.75	227.50	229.40	505.80	378.20
LM85	93.65	102.97	92.00	102.38	42.00	47.50	45.61	49.44	4.20	3.67	4.60	3.25	35.31	34.50	52.13	48.94	193.10	180.80	328.30	234.30
Crosses																				
LM02 x LM04	86.00	111.22	97.00	116.22	38.04	49.44	39.30	54.82	2.50	2.69	4.70	4.23	52.88	38.88	59.38	51.69	150.00	158.00	322.70	360.00
LM02 x LM05	85.10	112.00	97.50	105.38	41.27	58.31	52.75	64.25	2.60	2.80	4.00	3.20	54.88	35.88	51.19	47.38	183.70	175.60	328.40	288.40
LM02 x LM09	93.55	110.97	97.30	114.53	39.13	41.13	43.62	46.75	4.10	4.13	4.30	4.38	44.69	40.00	58.94	46.00	217.60	214.30	352.50	279.90
LM02 x LM13	103.70	112.28	104.40	119.00	47.31	43.94	56.00	56.63	3.70	3.67	4.90	5.07	40.38	38.06	53.50	49.31	213.90	185.80	450.80	418.50
LM02 x LM17	99.65	108.56	98.90	112.50	41.00	51.81	45.16	57.19	2.50	2.83	4.10	3.38	43.00	32.19	54.13	46.44	133.10	140.30	299.00	255.30
LM02 x LM21	88.50	111.06	105.10	111.50	37.31	45.63	48.19	57.56	3.50	2.82	4.40	4.33	58.06	39.13	56.94	48.69	225.10	151.40	359.00	364.10
LM02 x LM22	91.85	113.06	94.57	115.69	35.19	48.69	39.00	57.88	2.50	3.90	3.80	3.60	52.56	35.06	56.25	44.81	138.50	197.50	260.60	278.60
LM02 x LM23	90.40	116.94	94.50	120.72	43.19	53.50	40.75	53.44	2.80	3.00	5.60	4.20	53.81	40.50	60.81	43.88	195.80	194.50	426.60	295.40
LM02 x LM29	100.15	109.75	107.80	117.75	41.62	47.25	38.75	54.79	3.00	4.22	4.90	4.30	43.31	42.06	58.56	46.38	162.70	251.40	335.30	325.80
LM02 x LM45	96.37	114.47	109.40	120.44	46.38	53.69	54.69	58.75	3.00	4.61	5.60	3.90	48.13	38.81	62.56	45.06	200.00	288.10	583.00	307.40
LM02 x LM85	95.35	110.69	99.50	114.75	38.63	44.31	43.50	60.94	2.60	3.65	4.20	3.63	44.81	34.50	50.25	49.88	134.20	167.70	280.80	327.80
LM04 x LM05	87.20	109.94	65.80	116.72	38.93	47.81	44.00	62.44	3.05	3.43	3.73	3.37	57.75	36.69	56.38	45.19	208.60	182.50	279.20	284.90
LM04 x LM09	87.25	112.34	98.70	118.25	33.13	47.44	34.24	45.06	3.30	3.04	4.30	3.57	53.31	43.31	56.94	48.06	172.20	188.30	250.80	234.10
LM04 x LM13	98.75	111.22	103.40	119.03	43.94	51.56	49.62	54.31	2.70	3.68	4.98	4.78	45.44	41.94	52.25	47.06	160.90	238.00	394.50	366.20
LM04 x LM17	97.80	107.97	92.60	110.88	40.50	53.44	41.88	50.25	3.00	3.51	4.50	4.50	44.81	37.06	54.38	42.69	163.60	207.70	326.50	289.40
LM04 x LM21	87.95	104.19	89.20	109.88	36.56	42.88	35.55	49.06	3.40	3.25	4.60	3.77	53.25	37.13	58.81	42.50	189.80	154.90	287.60	235.60
LM04 x LM22	93.05	104.56	86.70	110.53	37.75	44.50	39.82	53.69	3.15	2.12	3.78	4.34	50.19	40.44	56.25	47.81	181.30	116.20	242.90	334.30
LM04 x LM23	84.05	104.50	89.10	118.94	28.13	44.50	39.76	53.75	2.30	2.67	5.00	4.67	48.19	39.31	60.44	47.69	97.50	139.60	363.80	370.20
LM04 x LM29	86.03	113.34	95.80	113.75	36.25	45.06	47.81	49.06	3.10	2.54	4.00	4.53	56.38	45.94	51.50	46.75	191.90	161.30	322.60	311.20
LM04 x LM45	78.30	105.10	93.25	113.38	31.30	43.63	45.75	54.19	2.18	2.67	4.00	3.40	64.31	38.81	62.31	47.06	127.00	139.90	349.20	259.70
LM04 x LM85	81.20	110.16	88.30	108.44	36.25	47.25	41.17	47.75	1.90	3.75	3.60	4.49	57.13	35.75	53.00	48.69	116.00	189.30	240.70	312.60
LM05 x LM09	89.75	101.56	111.50	115.03	39.06	47.56	50.00	50.81	3.00	2.89	3.13	4.25	55.25	38.63	53.88	47.69	193.50	158.30	250.50	308.60
LM05 x LM13	80.40	113.81	100.90	113.44	42.03	55.75	52.63	56.88	2.45	3.02	4.83	4.25	54.00	39.38	56.06	56.50	164.30	205.90	430.70	408.00
LM05 x LM17	92.40	107.47	93.40	113.59	45.19	53.44	38.49	47.75	4.20	2.67	3.90	3.53	45.19	35.06	54.19	47.31	255.70	149.70	241.20	238.60
LM05 x LM21	84.30	106.97	86.00	106.69	28.25	52.50	39.00	60.31	2.60	3.45	6.60	4.31	58.56	36.50	49.44	45.50	126.20	197.60	383.70	360.60
LM05 x LM22	79.20	106.44	94.00	117.69	35.62	49.81	32.82	65	2.50	2.54	4.98	3.47	46.63	39.63	51.81	43.63	124.10	150.10	239.20	294.90
LM05 x LM23	81.85	108.94	102.70	113.02	39.06	54.81	50.40	55.52	3.50	2.43	5.70	3.04	55.69	36.44	53.50	55.63	232.80	144.70	471.70	294.50
LM05 x LM29	80.00	106.31	79.80	111.28	33.88	49.31	34.50	60.69	3.00	3.15	3.40	4.13	54.44	37.69	54.63	45.81	166.00	175.80	192.20	348.40
LM05 x LM45	80.85	106.50	97.80	114.07	31.35	52.75	53.14	68.12	2.53	3.13	6.10	4.88	59.06	34.44	59.38	48.38	147.20	172.10	531.10	500.30
LM05 x LM85	86.75	101.72	94.55	108.88	39.38	45.44	42.72	50.38	3.00	2.98	4.10	2.93	50.75	35.63	58.19	48.13	193.50	150.10	309.80	214.50

Table 5.4 (Continued)

	PH				KPS				TN				TSW				GY			
	Stressed		Non-stressed		Stressed		Non-stressed		Stressed		Non-stressed		Stressed		Non-stressed		Stressed		Non-stressed	
Genotype	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
LM09 x LM13	89.80	111.22	101.90	118.09	32.89	40.81	33.56	48.06	2.90	3.49	4.90	3.63	52.56	41.94	56.06	51.06	150.20	180.90	274.40	266.70
LM09 x LM17	92.05	106.25	92.60	114.38	31.75	42.69	33.69	45.12	3.50	2.54	5.35	4.50	52.63	41.63	55.81	46.44	176.00	136.60	298.40	282.50
LM09 x LM21	88.68	107.72	89.10	109.84	38.69	44.94	31.96	49	3.60	4.21	4.00	3.63	52.63	42.69	61.69	49.38	219.70	247.10	236.60	262.40
LM09 x LM22	91.80	108.50	101.00	120.50	27.75	39.62	30.29	50.31	3.50	2.99	4.55	3.70	58.19	46.31	61.38	47.63	158.40	169.10	254.10	266.30
LM09 x LM23	92.45	114.41	100.30	121.00	41.50	39.06	49.56	53.31	3.50	3.76	6.58	5.16	54.88	47.25	59.63	51.44	250.90	207.10	596.30	420.10
LM09 x LM29	91.10	111.19	97.70	118.31	29.38	37.38	24.42	48.69	2.70	2.60	3.40	4.47	55.31	41.56	54.19	53.56	134.80	120.70	132.40	350.90
LM09 x LM45	92.52	113.88	117.60	116.31	34.69	41.75	45.00	41.94	3.90	3.83	5.30	3.81	53.25	44.00	54.81	52.81	213.20	211.30	385.70	253.20
LM09 x LM85	75.60	102.91	112.80	106.56	38.00	44.63	38.61	47.62	5.20	2.03	5.35	4.63	31.63	38.06	58.38	48.00	187.50	106.80	355.60	316.30
LM13 x LM17	98.40	109.97	103.00	113.25	50.19	50.14	50.12	45.83	2.83	3.13	4.90	3.98	44.56	37.69	55.00	48.25	171.30	175.90	409.00	264.20
LM13 x LM21	91.10	106.44	100.60	109.69	44.06	47.00	47.79	48.56	3.40	2.83	3.50	4.02	48.50	39.50	49.63	42.88	211.20	157.50	246.20	250.60
LM13 x LM22	90.00	106.53	113.30	112.69	39.00	45.56	35.18	55.25	2.60	2.76	5.70	4.03	45.88	36.44	50.94	39.06	135.40	136.80	323.00	266.90
LM13 x LM23	91.80	118.16	114.40	118.56	41.50	53.00	52.88	55.69	2.30	2.93	4.80	4.50	53.81	43.31	57.25	40.63	156.00	200.10	435.90	310.60
LM13 x LM29	89.55	110.81	103.90	112.91	39.38	41.38	49.88	47.13	3.10	3.00	5.90	4.53	54.94	39.56	53.31	47.00	204.50	148.60	473.40	299.20
LM13 x LM45	96.50	115.31	99.00	112.25	45.13	41.62	38.20	50.31	3.80	3.12	3.80	3.92	45.44	43.75	57.94	46.00	230.90	171.90	255.30	271.40
LM13 x LM85	89.60	112.62	102.00	111.66	32.69	51.50	47.75	51.25	2.50	2.79	5.40	3.21	51.69	38.50	55.00	44.13	125.10	169.70	432.80	217.70
LM17 X LM21	86.36	103.66	91.00	102.09	30.10	49.23	28.50	41.5	2.70	3.37	2.60	3.23	47.50	39.81	55.75	38.63	115.20	200.20	123.90	155.50
LM17 x LM22	91.60	107.47	104.90	111.62	34.56	51.19	42.79	53.81	3.60	3.25	4.70	3.87	43.63	36.44	52.81	41.63	157.10	185.10	315.50	260.50
LM17 x LM23	80.80	100.69	85.70	106.31	24.25	50.31	32.69	52.56	3.48	3.74	5.00	3.56	43.13	36.13	53.06	39.94	109.10	203.10	264.30	224.40
LM17 x LM29	92.50	108.25	94.30	112.41	36.12	47.00	36.83	49.75	3.20	4.06	5.30	4.92	46.13	37.63	47.56	44.75	157.40	215.40	282.20	326.10
LM17 x LM45	96.75	105.12	100.60	112.06	36.46	48.37	44.64	56.75	3.20	4.08	5.10	4.25	42.25	37.06	53.88	39.13	154.00	213.60	389.90	283.70
LM17 x LM85	89.80	104.00	89.70	107.09	26.44	43.50	39.46	50.63	2.50	3.57	3.15	4.14	57.13	31.19	53.75	41.25	114.10	145.30	202.10	260.00
LM21 x LM22	91.90	101.06	92.20	106.65	34.94	43.25	40.12	51.06	3.60	2.70	4.20	4.88	58.44	36.50	55.63	45.19	218.90	127.90	280.80	328.70
LM21 x LM23	83.55	107.00	82.20	112.75	39.11	53.50	46.00	64.69	1.50	2.02	4.40	4.10	60.63	36.44	55.50	39.94	106.80	117.10	337.00	317.60
LM21 x LM29	91.05	104.44	94.90	107.25	24.31	41.44	33.40	45.56	2.50	3.03	2.00	3.47	58.06	38.06	49.50	47.75	108.80	146.90	99.20	225.40
LM21 x LM45	88.50	105.88	99.10	104.06	39.75	47.31	39.92	45.94	3.10	3.57	4.90	3.69	55.63	34.94	65.63	50.94	210.60	176.80	385.80	258.10
LM21 x LM85	84.70	101.06	90.00	100.19	34.75	44.38	35.50	51.44	3.30	2.90	3.60	3.73	45.94	36.25	64.44	39.88	151.80	140.40	244.90	232.60
LM22 x LM23	90.05	109.00	87.50	114.69	36.69	54.12	37.76	53.44	3.00	2.79	4.80	3.79	51.13	41.88	46.94	46.19	167.20	191.10	256.00	279.20
LM22 x LM29	91.90	106.13	97.50	106.84	26.88	44.63	34.12	45.88	3.10	3.19	4.70	4.14	59.56	38.81	60.63	48.38	149.10	166.60	292.40	276.20
LM22 x LM45	92.80	105.94	98.40	108.53	36.06	53.63	39.00	46.5	2.70	3.47	4.50	4.43	55.94	41.88	55.44	51.56	162.50	233.70	295.10	321.80
LM22 x LM85	83.95	105.56	95.60	109.91	27.88	43.06	40.90	49.62	3.50	2.17	6.03	3.17	56.31	41.00	49.31	46.88	164.40	114.60	375.60	223.60
LM23 x LM29	84.15	118.00	95.80	116.38	28.75	46.63	45.56	55.19	2.20	3.92	5.10	3.57	58.63	42.13	56.06	50.13	104.30	233.70	399.40	293.70
LM23 x LM45	92.30	120.44	96.38	118.53	38.81	47.87	37.45	49.44	3.40	4.17	3.20	3.69	53.88	41.06	64.31	47.00	210.80	246.90	235.10	255.00
LM23 x LM85	87.95	101.88	94.50	114.50	42.13	42.38	52.25	55.56	3.20	3.47	5.55	3.60	43.75	36.63	50.75	44.31	175.10	160.40	466.30	264.70
LM29 x LM45	83.10	104.94	103.10	109.63	31.69	44.31	35.75	51.88	2.70	2.83	4.50	3.90	50.50	40.31	62.75	51.19	129.70	151.80	307.80	309.70
LM29 x LM85	94.10	108.16	98.90	108.84	36.50	52.00	35.81	50	2.70	3.58	4.70	3.97	44.31	32.88	62.31	44.06	126.20	183.80	353.00	261.90
LM45 x LM85	82.10	109.84	94.50	108.00	31.06	42.81	36.99	45.81	2.10	4.00	4.00	2.73	46.81	42.44	58.06	49.81	90.80	217.80	252.70	186.70
Mean	89.75	108.32	96.82	111.90	37.17	47.44	41.90	52.51	3.07	3.22	4.57	4.01	50.04	38.78	55.77	46.69	168.81	177.22	326.00	295.03
CV (%)	0.90	0.90	0.90	0.90	0.80	0.80	0.80	0.80	8.80	8.80	8.80	8.80	0.70	0.70	0.70	0.70	10.90	10.90	10.90	10.90
SED	2.97	2.97	2.97	2.97	2.76	2.76	2.76	2.76	0.47	0.47	0.47	0.47	2.30	2.30	2.30	2.30	57.31	57.31	57.31	57.31
LSD (5%)	5.84	5.84	5.84	5.84	5.42	5.42	5.42	5.42	0.92	0.92	0.92	0.92	4.52	4.52	4.52	4.52	79.74	79.74	79.74	79.74

CV, coefficient of variation; E1, greenhouse; E2, field; GY, grain yield; KPS, kernels per spike; LSD, list significant difference; PH , plant height; SED, standard error of differences; TSW, thousand seed weight; TN, tiller number.

5.3.2 Combining Ability Tests of F2 Crosses and their Parents

Table 5.5 presents the analysis of variance and significant tests of components of genetic variance for general and specific combining ability and the σ^2gca /σ^2sca ratio (Baker, 1978) for yield and yield components from a 12 x 12 half diallel cross of bread wheat genotypes evaluated under drought stressed and non-stressed conditions. Under stressed condition significant GCA effects were observed for all traits recorded across test conditions except for grain yield from the greenhouse experiment. Only PH and TSW in the greenhouse and KPS in the field had significant SCA effects. Under well-watered conditions, PH, KPS and TSW had consistently significant GCA effects. Grain yield had significant GCA effects only under greenhouse conditions, while TN had non-significant GCA effects under both greenhouse and field conditions. Under non-stressed conditions, PH, KPS and TSW had significant SCA effects in the field, while in the greenhouse, only KPS had significant SCA effects. The highest proportion of variance due to general combining ability over the variance due to specific combining ability under stressed conditions was observed for TSW from field experiments. All proportions of σ^2_{gca} to σ^2_{sca} under greenhouse conditions were less than a unity. In the field, the ratio was above 1 for PH, KPS and TSW under stressed conditions, and for PH and TN under non-stressed condition.

Table 5.5. Analysis of variance, components of genetic variance for general and specific combining ability and σ²gca /σ²sca ratio for yield and yield components from a 12 x 12 half diallel cross of bread wheat evaluated under drought stressed and non-stressed conditions

Stressed conditions											
		Mean square									
Source of variation	DF	PH		TN		KPS		TSW		GY	
		E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
GCA	11	119.72***	76.34***	0.58*	0.65*	86.15***	106.47***	106.92***	45.16***	1690.98^{ns}	2288.60*
SCA	66	25.45*	12.06^{ns}	0.36^{ns}	0.28^{nss}	28.60^{ns}	12.98*	36.60**	5.67^{ns}	1881.37^{ns}	1178.24^{ns}
Residual	77	15.58	9.06	0.29	0.22	21.32	8.63	18.68	5.40	1715.62	1018.94
σ^2_{gca}		7.44	4.81	0.02	0.03	4.63	6.99	6.30	2.84	0.001	90.69
σ^2_{sca}		9.87	3.00	0.07	0.06	7.28	4.35	17.92	0.28	165.75	159.29
σ^2_{gca} /σ^2_{sca}		0.75	1.60	0.29	0.50	0.64	1.61	0.35	10.32	0.001	0.57
Non-stressed conditions											
Source of variation	DF	PH		TN		KPS		TSW		GY	
		E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
GCA	11	161.48***	107.24***	0.45^{ns}	0.50^{ns}	173.64***	100.87***	47.01***	30.01***	15624.38*	3966.91^{ns}
SCA	66	48.00^{ns}	12.46***	0.87^{ns}	0.30^{ns}	32.48**	19.29*	15.06^{ns}	12.74***	8752.76^{ns}	3332.57^{ns}
Residual	77	33.31	7.17	0.85	0.28	17.44	11.62	11.41	5.63	6556.23	2291.07
σ^2_{gca}		9.16	7.15	0.001	0.02	11.16	6.37	2.54	1.74	647.73	119.70
σ^2_{sca}		14.70	5.29	0.02	0.01	15.04	7.67	3.65	7.11	2196.53	1041.49
σ^2_{gca} /σ^2_{sca}		0.62	1.35	0.001	1.05	0.74	0.83	0.70	0.24	0.29	0.11

DF, degrees of freedom; E1, greenhouse; E2, Field; GCA, general combining ability; GY, grain yield; KPS, kernels per spike; ns, non-significant; PH, plant height; SCA, specific combining ability; TSW, thousand seed weight; TN, tiller number; ***, significant at 0.001; **, significant at 0.01; *, significant at 0.05; $\sigma^2_{gca,}$ variance of general combining ability; $\sigma^2_{sca,}$ variance of specific combining ability; negative variance.

5.3.3 General Combining Ability Effects of Parental Lines

Table 5.6 presents GCA estimates for the studied traits among parental genotypes of bread wheat evaluated under drought stressed and non-stressed conditions. Negative GCAs for plant height across the four test conditions were observed for parents LM17 and LM21. Further, from both sites under stressed conditions, LM05 had negative GCAs; while under non-stressed conditions, LM29, LM45 and LM85 had negative GCAs for plant height. Parents LM02 and LM23 had positive GCAs for KPS across sites and water regimes. Additionally, parent LM85 had consistent positive GCA for KPS under stressed conditions, while LM02, LM04 and LM13 had positive GCAs for KPS under non-stressed conditions. Positive GCAs were also observed for the number of productive tillers on LM17 and LM85 under stress, as well as on LM09, LM22 and LM29 under optimum conditions. LM04 and LM09 had consistently high positive GCA effects for thousand seed weight. Parents LM22 and LM23 had positive GCAs under stressed conditions in both environments, while LM29 and LM45 had positive GCA effects under well-watered conditions in the greenhouse and the field. LM02 had high positive GCA for grain yield under both stressed and non-stressed conditions in the two test environments. Notably, under field conditions, LM45 (42.38) had the highest general combining ability effect for GY, followed by LM23 (33.28) and LM17 (17.14), respectively. Under optimum conditions, LM05 and LM29 had positive GCA values in both the greenhouse and field experiments.

Table 5.6 Estimates of general combining ability effects for plant height, grain yield and yield components of twelve parental genotypes of bread wheat evaluated under drought stressed and non-stressed conditions

Stressed condition										
	PH		KPS		TN		TSW		GY	
Parent	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
LM02	4.38	4.42	3.54	2.36	-0.21	0.14	-0.15	-1.21	5.32	11.39
LM04	-0.76	0.77	0.61	-1.10	-0.29	-0.24	3.19	1.16	-2.24	-10.57
LM05	-6.70	-0.75	-0.62	5.20	-0.28	-0.49	3.78	-1.97	0.34	-17.54
LM09	-0.82	1.54	-2.88	-6.23	0.47	-0.15	1.68	4.45	15.23	-10.79
LM13	3.98	3.18	5.78	-0.28	-0.14	-0.39	-1.68	0.50	7.64	-19.31
LM17	-0.51	-3.19	-5.59	1.26	0.02	0.41	-3.49	-2.20	-35.08	17.14
LM21	-2.84	-4.57	-2.05	-1.31	-0.32	-0.33	6.81	-2.05	-4.57	-31.10
LM22	0.21	-1.39	-2.69	2.61	0.17	-0.39	6.81	1.31	23.05	-5.50
LM23	0.50	6.99	1.62	0.39	-0.18	0.42	1.31	1.32	-0.22	33.28
LM29	0.21	-2.51	-2.41	-1.04	-0.05	0.06	-3.02	-1.52	-24.44	-8.95
LM45	-1.39	0.20	-0.17	-2.15	-0.25	0.61	-1.82	4.40	-9.51	42.38
LM85	3.71	-4.68	4.86	0.29	1.05	0.35	-13.41	-4.19	24.48	-0.43
Non-stressed conditions										
	PH		KPS		TN		TSW		GY	
Parent	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
LM02	3.95	4.16	3.73	4.23	-0.16	0.02	-0.41	0.05	17.79	23.42
LM04	-5.32	3.45	0.94	0.25	-0.32	0.24	0.37	0.31	-15.27	22.91
LM05	-1.17	1.39	2.32	6.11	0.16	-0.11	-1.04	1.22	16.17	38.99
LM09	4.13	4.55	-6.31	-3.77	0.40	0.32	1.45	3.57	-15.06	23.36
LM13	8.74	2.12	5.62	-0.55	0.16	0.01	-2.43	-1.85	35.62	-14.00
LM17	-1.74	-2.35	-4.83	-0.23	-0.14	0.18	-3.65	-5.15	-65.31	-15.18
LM21	-3.49	-5.76	-3.93	-0.68	-0.54	0.20	2.71	-1.49	-52.34	-2.53
LM22	-0.20	-0.42	-4.49	-1.28	0.23	0.01	-3.31	0.38	-41.77	-3.92
LM23	-1.24	6.02	2.78	0.97	-0.26	-0.36	0.35	-0.04	7.65	-22.37
LM29	1.71	-2.52	-1.86	-2.29	0.47	0.25	5.46	0.30	64.71	3.52
LM45	-1.11	-2.52	2.32	-0.44	0.01	-0.08	4.40	0.52	49.41	-3.03
LM85	-4.26	-8.12	3.71	-2.33	0.01	-0.69	-3.91	2.18	-1.60	-51.19

E1, greenhouse; E2, Field; GY, grain yield; KPS, kernels per spike; PH, plant height; TSW, thousand seed weight; TN, number of productive tillers.

5.3.4 Specific Combining Ability Effects of Crosses

Table 5.7 presents SCA effects of the 78 cross combinations obtained from a 12 x 12 half diallel cross of bread wheat under drought stressed and non-stressed conditions. The cross LM17 x LM23 had consistently low negative SCA effects for plant height, ranking in the bottom 10 of the cross combinations across the four test conditions. In addition the families LM04 x LM45, LM29 x LM45 and LM09 x LM85 had low negative SCA effects for plant height in the field and greenhouse under stressed conditions. LM02 x LM45 had relatively large SCA effects for the number of kernels per spike under stressed conditions in both sites. Under well-watered conditions, LM09 x LM23, LM02 x LM21 and LM05 x LM45 had SCA effects ranking among the top 10 in each site. Under field conditions, crosses LM29 x LM85 and LM21 x LM23 had the largest SCA effects for KPS under stressed and optimum conditions, respectively. Highest SCA effects for the number of productive tillers in the field were obtained from the crosses LM09 x LM21 and LM02 x LM13, while in the greenhouse, the crosses LM09 x LM85 and LM05 x LM21 had high SCA effects under stressed and non-stressed conditions, respectively. The cross LM13 x LM23 had consistently high SCA effects for TSW under stressed conditions in both the greenhouse and field, while SCA effects for LM21 x LM45 and LM13 x LM17 were consistently high under non-stressed conditions. SCA effects for grain yield under stressed conditions ranked consistently high for the cross LM09 x LM21, while under optimum conditions LM09 x LM23, LM05 x LM45, LM02 x LM13 and LM05 x LM21 ranked high in both environments.

Table 5.7. Specific combining ability effects of 76 cross combinations obtained from a 12 x 12 half diallel cross of bread wheat under drought stressed and non-stressed conditions

	PH				KPS				TN				TSW				GY			
	Stressed		Non-stressed		Stressed		Non-stressed		Stressed		Non-stressed		Stressed		Non-stressed		Stressed		Non-stressed	
Cross	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
LM02 x LM04	-8.45	-1.04	1.85	0.25	-3.89	0.99	-7.31	-0.22	-0.24	-0.58	0.61	0.13	3.36	0.42	3.56	4.01	-21.16	-25.16	-7.19	37.20
LM02 x LM05	-3.30	0.66	1.67	-7.94	1.41	2.93	4.33	2.84	-0.07	-0.24	-0.37	-0.45	4.01	0.68	-3.07	0.43	14.98	-2.78	-22.39	-30.61
LM02 x LM09	-0.49	-1.37	-5.47	-2.18	0.94	-3.55	2.79	-4.21	0.69	0.72	-0.21	0.24	-3.77	-1.02	2.81	-3.37	32.15	27.18	36.77	-29.75
LM02 x LM13	4.82	-2.04	-2.15	3.23	1.44	-5.72	4.53	1.47	0.81	0.36	0.42	1.11	-4.57	0.28	0.66	2.30	37.48	-0.73	75.38	111.71
LM02 x LM17	3.19	-0.88	0.69	0.55	2.16	0.17	2.75	3.36	-0.55	-0.80	-0.20	-0.65	-0.76	-3.20	1.74	2.72	-20.99	-59.31	3.84	-28.73
LM02 x LM21	-3.53	2.77	8.45	3.22	-1.58	-3.34	6.61	3.90	0.63	-0.58	0.38	0.34	5.55	2.59	0.52	3.63	49.85	-32.25	69.55	75.97
LM02 x LM22	-3.02	2.91	-5.83	1.65	-3.61	-1.87	-1.91	1.64	0.06	0.76	-0.55	-0.30	1.36	-2.54	3.16	-0.32	-45.95	18.62	-25.86	-15.45
LM02 x LM23	-3.17	1.58	-3.16	2.92	2.10	1.41	-6.56	-3.35	-0.60	-0.41	1.05	0.38	4.23	1.87	5.79	-1.94	26.66	-8.57	56.36	-2.92
LM02 x LM29	5.43	-2.01	8.47	4.42	4.62	-0.13	-4.42	1.75	0.14	0.67	0.55	0.16	-6.34	4.03	3.34	-0.87	6.76	59.34	1.87	19.28
LM02 x LM45	1.65	1.77	6.68	6.61	5.68	4.05	7.27	3.45	0.07	0.81	1.14	-0.05	-1.74	-0.06	3.57	-2.24	19.54	70.02	196.20	-2.24
LM02 x LM85	2.89	1.17	0.81	4.47	-0.59	-4.17	-1.95	7.31	-0.39	0.20	-0.08	0.10	-0.17	-0.57	-4.10	3.69	-20.04	-9.28	-47.99	66.43
LM04 x LM05	3.57	1.86	-22.58	3.41	0.61	-3.57	-2.20	3.99	0.41	0.74	-0.66	-0.57	4.39	-0.98	0.29	-3.17	41.13	25.73	-60.25	-50.85
LM04 x LM09	-2.03	3.26	3.38	1.54	-3.52	6.77	-4.38	-2.94	-0.08	-0.01	-0.23	-0.86	2.37	-0.17	-1.02	-2.72	-11.93	22.82	-53.53	-92.32
LM04 x LM13	4.64	0.15	4.30	3.27	-0.40	5.91	0.37	2.10	-0.16	0.72	0.48	0.54	-2.00	1.69	-2.42	-1.37	-14.16	73.19	30.49	42.71
LM04 x LM17	6.10	1.79	1.84	-1.07	3.20	5.80	1.68	-0.62	-0.02	0.23	0.18	0.19	-1.44	-0.80	0.16	-2.45	10.85	29.69	42.66	-11.34
LM04 x LM21	0.69	-0.85	0.00	1.60	-0.79	-2.09	-3.80	-1.64	0.56	0.20	0.56	-0.50	-1.75	-1.88	0.57	-3.98	15.90	-7.11	9.44	-69.30
LM04 x LM22	2.95	-2.34	-6.25	-3.50	0.50	-2.05	1.13	0.41	0.08	-0.67	-0.60	0.16	-3.51	0.36	1.33	1.26	-1.74	-40.98	-32.25	23.45
LM04 x LM23	-4.76	-7.60	-1.10	1.15	-11.42	-3.58	-5.33	-0.08	-0.41	-0.39	0.43	0.56	-3.89	-1.78	3.58	0.46	-70.27	-41.81	4.89	55.23
LM04 x LM29	-3.92	4.84	3.92	0.43	0.79	1.69	6.86	-1.03	0.27	-0.65	-0.37	0.11	4.23	5.43	-5.55	-1.91	37.24	-9.11	0.43	-12.06
LM04 x LM45	-11.65	-4.34	-2.02	-0.44	-7.85	-2.01	0.55	1.84	-0.72	-0.78	-0.48	-0.83	11.96	-2.53	1.49	-1.65	-52.14	-56.53	-26.26	-66.74
LM04 x LM85	-6.49	3.90	-2.94	-1.84	-1.42	2.77	-2.06	-2.92	-1.06	0.66	-0.70	0.68	9.65	-1.79	-3.18	1.09	-36.89	33.97	-76.76	34.51
LM05 x LM09	6.52	-6.60	15.50	0.98	4.49	-0.04	9.57	-3.56	-0.31	0.06	-1.68	0.28	2.95	-1.61	-2.53	-2.36	11.75	-2.32	-74.77	-14.12
LM05 x LM13	-7.66	3.66	1.12	0.33	-0.24	3.16	1.56	-1.69	-0.34	0.29	0.06	0.47	5.21	2.38	2.94	8.80	-8.37	45.81	45.75	88.22
LM05 x LM17	6.75	2.21	1.97	4.30	9.96	-1.14	-3.51	-9.48	1.26	-0.39	-0.70	-0.32	-2.41	0.46	1.53	2.92	105.32	-23.45	-63.56	-58.45
LM05 x LM21	3.09	2.85	-3.88	1.07	-7.03	0.60	-2.17	3.24	-0.17	0.63	2.28	0.49	2.21	0.75	-7.26	-0.24	-45.36	40.42	84.67	59.43
LM05 x LM22	-4.85	0.46	0.37	6.31	0.44	-3.68	-7.68	5.36	-0.49	-0.02	0.32	-0.26	-8.42	2.81	-1.55	-2.19	-56.59	-2.32	-56.85	-12.20
LM05 x LM23	-0.91	-2.25	11.82	-2.11	1.59	-0.21	3.50	-4.68	0.86	-0.40	0.85	-0.61	2.26	-1.40	-1.80	9.13	67.38	-31.90	91.93	-16.76
LM05 x LM29	-3.91	-1.27	-12.76	0.61	0.48	-1.00	-8.26	4.23	0.24	0.18	-1.25	0.16	0.94	0.44	-0.88	-2.11	13.76	10.20	-150.78	28.90
LM05 x LM45	-3.05	-2.02	1.86	2.91	-5.74	0.18	6.14	9.41	-0.30	-0.09	1.34	1.11	5.35	-3.65	0.10	0.40	-29.48	-19.55	134.81	177.62
LM05 x LM85	5.11	-3.62	2.63	1.25	3.77	-5.98	-2.32	-6.66	0.11	0.10	-0.48	-0.42	1.92	1.34	3.56	1.26	42.97	-0.45	-28.54	-59.91
LM09 x LM13	-3.91	0.07	-4.82	1.58	-7.70	-1.07	-9.92	-0.07	-0.63	0.40	-0.02	-0.64	6.18	-0.87	1.07	0.94	-39.21	12.20	-75.44	-43.65
LM09 x LM17	0.76	-0.01	-5.77	1.68	-1.80	-1.17	-0.73	-1.67	-0.19	-0.87	0.60	0.16	7.43	1.20	1.29	-0.38	8.95	-45.28	28.76	-5.16
LM09 x LM21	1.82	2.60	-7.72	0.82	5.08	3.75	-1.62	2.38	0.09	1.03	-0.46	-0.67	-1.32	1.12	3.12	1.21	31.42	81.18	-27.32	-29.34
LM09 x LM22	2.10	1.52	0.44	5.73	-5.76	-3.15	-2.62	1.11	-0.24	0.07	-0.25	-0.51	5.55	3.68	6.14	-0.61	-39.05	7.95	-6.84	-31.44
LM09 x LM23	4.05	2.22	2.48	2.47	5.70	-5.24	10.25	3.56	0.12	0.57	1.57	1.02	3.86	3.59	2.45	2.52	68.78	21.79	251.57	118.24
LM09 x LM29	1.55	2.60	-1.80	4.24	-2.34	-2.22	-10.76	2.68	-0.81	-0.73	-1.40	0.01	4.23	-1.50	-3.18	3.22	-34.14	-53.60	-175.54	40.76
LM09 X LM45	2.97	4.35	14.72	1.75	-0.72	-0.10	5.58	-6.33	0.33	0.25	0.39	-0.46	1.95	0.10	-6.33	2.41	19.80	10.93	24.46	-60.14
LM09 x LM85	-11.69	-3.43	13.94	-4.46	4.07	3.92	1.16	1.03	1.56	-1.20	0.62	0.78	-14.79	-2.04	1.87	-1.29	20.24	-52.42	52.34	51.30
LM13 x LM17	2.27	1.72	0.85	1.50	8.95	1.29	5.07	-5.16	-0.34	-0.19	0.19	-0.18	2.88	0.50	3.76	3.78	13.37	-5.45	79.67	-20.54
LM13 x LM21	-0.59	-0.67	0.00	1.61	2.76	0.82	3.58	-2.27	0.41	-0.25	-0.93	-0.10	-1.93	1.17	-5.65	-2.94	32.07	-7.85	-77.34	-38.23
LM13 x LM22	-4.53	-2.44	8.96	-1.15	-2.20	-2.20	-8.37	1.85	-0.62	-0.07	0.94	0.01	-3.25	-2.96	-1.01	-6.82	-52.89	-23.70	2.37	-27.96
LM13 x LM23	-1.44	3.98	12.80	0.97	-1.99	3.71	2.93	1.73	-0.56	-0.16	-0.16	0.55	6.30	2.89	3.36	-5.94	-17.05	15.37	31.50	11.59
LM13 x LM29	-4.83	0.24	0.63	-0.22	-0.03	-3.21	4.07	-3.09	0.12	-0.23	1.14	0.25	7.36	-0.27	-0.77	-0.99	44.69	-25.14	105.83	-8.11
LM13 x LM45	2.12	3.80	-7.66	-1.37	2.03	-5.22	-11.85	-2.16	0.75	-0.37	-1.07	-0.16	-2.35	3.08	0.08	-2.05	46.55	-27.81	-165.63	-39.02
LM13 x LM85	-2.52	4.30	-0.64	1.58	-8.93	5.81	-0.34	0.45	-0.61	-0.34	0.71	-0.45	8.78	1.63	1.79	-2.81	-33.01	11.05	69.88	-44.43

Table 5.7. (Continued)

	PH				KPS				TN				TSW				GY			
	Stressed		Non-stressed		Stressed		Non-stressed		Stressed		Non-stressed		Stressed		Non-stressed		Stressed		Non-stressed	
Crosses	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
LM17 x LM21	-2.92	1.43	-1.25	-2.17	-4.16	1.07	-6.66	-7.99	-0.45	-0.05	-1.65	-0.95	-1.74	3.87	0.94	-3.89	-41.64	21.72	-119.46	-110.64
LM17 x LM22	-0.52	3.39	8.90	1.61	0.40	1.44	8.30	1.75	0.23	0.10	0.11	-0.23	-4.32	-0.57	1.33	-0.96	-8.90	11.41	75.11	-11.53
LM17 x LM23	-10.02	-8.60	-7.56	-7.46	-12.20	-0.96	-8.20	-0.05	0.46	0.32	0.21	-0.46	-3.20	-1.91	-0.36	-3.33	-41.58	5.26	-59.89	-51.85
LM17 x LM29	0.53	2.57	-0.63	3.10	3.76	0.43	0.08	0.88	0.06	0.50	0.71	0.58	-0.26	0.18	-6.06	0.06	19.85	28.51	-5.20	41.57
LM17 x LM45	4.78	-1.50	2.28	2.26	0.40	-0.45	3.65	5.62	-0.01	0.26	0.40	0.10	-4.35	-1.22	-3.52	-5.62	-8.03	0.71	49.15	-3.97
LM17 x LM85	0.10	0.56	-4.59	0.83	-8.14	-4.17	0.43	1.17	-0.77	0.11	-1.37	0.41	15.40	-3.29	1.00	-2.39	-21.74	-26.50	-80.70	20.63
LM21 x LM22	4.22	-1.88	-2.24	0.31	0.72	-3.83	6.47	-0.83	0.40	-0.22	-0.10	0.82	1.75	-1.66	0.10	1.26	31.68	-29.80	46.09	52.55
LM21 x LM23	-2.84	-1.14	-9.50	2.65	2.61	4.90	5.95	12.24	-1.34	-1.17	-0.10	0.12	5.55	-2.74	-1.96	-4.67	-65.13	-64.77	18.52	37.19
LM21 x LM29	3.52	-0.10	1.53	1.61	-8.11	-2.45	-2.51	-3.15	-0.46	-0.29	-2.30	-0.84	2.93	-0.53	-8.16	1.71	-49.95	-23.96	-182.48	-63.19
LM21 x LM45	0.97	0.39	2.34	-2.07	3.63	1.16	-0.24	-5.03	0.07	-0.02	0.49	-0.42	0.27	-4.49	4.20	4.84	27.35	-20.11	50.80	-33.68
LM21 x LM85	-0.57	-1.24	-2.74	-2.40	0.12	-0.62	-2.69	2.15	0.21	-0.33	-0.63	0.04	-4.53	0.62	7.65	-5.11	-5.24	-15.43	-32.17	-10.86
LM22 x LM23	0.82	-1.00	-7.95	-1.17	0.28	3.93	-1.63	-1.58	-0.07	-0.14	-0.04	-0.10	-2.64	1.63	-7.20	1.51	-13.89	14.02	-59.49	-7.08
LM22 x LM29	1.52	-0.28	0.38	-4.55	-5.45	-0.86	-1.13	-5.41	-0.09	0.12	0.06	-0.07	5.73	-0.84	6.29	2.27	-18.78	0.53	13.68	-18.42
LM22 x LM45	2.43	-1.40	-2.11	-3.36	0.05	5.88	-0.49	-7.04	-0.55	0.15	-0.25	0.41	1.89	1.38	-2.67	5.40	-29.87	41.56	-36.88	24.08
LM22 x LM85	-4.16	1.40	-0.88	1.56	-6.66	-3.53	3.38	-2.24	0.18	-0.80	1.46	-0.43	7.14	4.31	-4.15	1.82	-1.72	-36.41	101.55	-25.84
LM23 x LM29	-4.93	6.39	1.42	1.22	-5.86	-0.38	3.92	3.35	-0.63	0.58	0.26	-0.57	6.41	1.45	-0.21	3.34	-48.26	43.45	36.94	-5.10
LM23 x LM45	3.22	7.89	-1.39	2.89	0.51	-1.39	-8.44	-4.65	0.50	0.57	-1.75	-0.25	1.45	-0.45	4.27	0.16	33.72	30.60	-180.74	-46.94
LM23 x LM85	1.14	-7.49	0.76	2.40	5.30	-5.74	8.32	3.15	0.24	0.23	0.78	0.07	-3.80	-1.09	-4.65	-1.42	24.20	-14.83	108.41	11.09
LM29 x LM45	-7.13	-4.01	3.66	-1.56	-2.54	-0.25	-6.00	1.52	-0.32	-0.90	-0.25	-0.37	-1.99	-0.61	2.51	2.92	-34.18	-53.46	-71.25	-0.50
LM29 x LM85	6.14	2.40	3.49	1.20	3.76	8.59	-3.97	1.32	-0.39	0.21	0.13	0.12	-3.30	-4.25	6.72	-3.10	-11.54	19.54	31.93	0.10
LM45 x LM85	-5.86	3.14	-4.30	-0.14	-5.37	-2.85	-7.04	-5.12	-1.05	0.37	-0.67	-0.93	-1.02	4.47	-1.30	2.59	-71.43	27.55	-121.67	-78.26

E1, greenhouse; E2, field; GY, grain yield; KPS, kernels per spike; PH , plant height; TSW, thousand seed weight; TN, tiller number.

5.4 Discussion

5.4.1 Analysis of Variance and Mean Performance of Genotypes

The presence of significant main effects of the genotypes reveal that the materials evaluated differed for the recorded traits. This finding was expected since drought tolerant lines differing in agronomic characteristics were considered for the study (Mwadzingeni et al., 2016). Imposing drought stress was also effective as evidenced by a significant decline in plant height, yield and yield components due to stress. These decreases are supported by previous studies that reported a decline in yield, yield components and plant height due to drought stress (Kilic and Yagbasanlar, 2010; Farooq et al., 2014). Significant interactions of the genotypes, water regimes and test environments is a common phenomenon among quantitatively inherited traits that are largely influenced by environmental conditions (Purchase et al., 2000). Among the crosses, selection for transgressive segregates at F2 is possible because some crosses like LM09 x LM23 performed better than both parents across test environments, which may be an indication of over-dominance gene action as was also observed on similar traits by Ajmal and Khaliq (2011). Such selection can also be achieved under specific water regimes, as in the case of LM05 x LM23 observed under stressed conditions. Likewise, some crosses exhibited partial dominance by consistently having performances that are above their mid parent values but less than the better parent values. In the case of plant height, low values will be more favourable as exhibited by the cross LM05 x LM29 which was consistently shorter under stressed conditions. The continuous distribution of phenotypic values reveal the polygenic nature of plant height, grain yield and yield components as supported by Zhang et al. (2010) who identified their quantitative trait loci.

5.4.2 Combining Ability

Significant GCA effects indicates the influence of additive gene action, while significant SCA effects show the existence of non-additive gene action (Masood et al., 2014). Under stressed conditions, all traits studied were influenced by additive gene action as shown by the existence of significant GCA effects. These findings are in agreement with those by Joshi et al. (2004) who observed significant GCA effects for the same traits at the F2 generation. Further, PH and TSW from the greenhouse and KPS under field conditions were also influence by non-additive gene action since they exhibited significant SCA effects. Under non-stressed conditions, the

significance of GCA effects for PH, KPS and TSW reveal that these traits were influenced by additive gene action in the greenhouse and field. Additionally, under field conditions, non-additive gene action could have influence on PH, KPS and TSW resulting in significant SCA effects. Predominance of non-additive gene action was controlled all traits under stressed conditions in the greenhouse because the proportions of variance due to general combining ability over the variance due to specific combining ability were less than a unity. Similar findings for these traits were also reported on F1 crosses by Ahmad et al. (2013) who recommended for selection to be done at letter generations. However, under field conditions, predominance of additive gene action was observed for PH, KPS and TSW under stressed conditions, as well as for PH and TN under non-stressed condition as indicated by the value of $\sigma^2_{gca}/\sigma^2_{sca}$ above one. These findings are supported by Adel and Ali (2013) who reported of the influence of both additive and non-additive genes on studied traits.

5.4.3 General Combining Ability of the Twelve Parental Genotypes

Plant height should be reduced to improve adaptability to drought stressed conditions (Monneveux et al., 2012). To breed for reduced plant height, parents with low negative GCA should be prioritized. Parents LM17 and LM21 could, therefore, be useful in this case since they exhibited consistently negative GCA effects across all test conditions. Further, LM05 could be equally useful under stressed conditions in both sites, while LM29, LM45 and LM85 can contribute to plant height reduction under non-stressed conditions. For yield components, parents with positive GCA effects transmit additive genes during breeding (Dholariya et al., 2014), especially those exhibiting consistently high GCA effects under both stressed and non-stressed conditions. Parents ML02 and LM23 are good general combiners for the number of kernels per spike across drought stressed and non-stressed conditions due to existence of positive GCA effects, while LM04, LM05 and LM13 could similarly be useful under non-stressed conditions. To improve the number of productive tillers under stress LM17 and LM85 could contribute additive genes towards the traits in designed crosses. Consistent positive GCA effects for TSW shown on parents LM04 and LM09 on all test conditions and for parents LM22 and LM23 under stressed conditions in both environments make them the genotypes of choice to improve the trait and potentially to maintain high levels of drought tolerance. Finally, parent LM02 with positive

and consistent GCA effects for grain yield under both stressed and non-stressed conditions could serve as a key parent for grain yield improvement when breeding for wide adaptation. Parents LM17, LM23 and LM45 which also had high positive GCA effects for grain yield could be useful for breeding. There is high likelihood of selecting advanced respective traits at the F2 generation out of crosses from all these parents (Masood et al., 2014).

5.4.4 Specific Combining Ability Effects

Superior crosses with high specific combining ability, arising from good general combiners for particular traits are important targets for selection of transgressive segregates (Al-Naggar et al., 2015). In the case where low values or scores are required as in the case of plant height, crosses with low negative SCA effects will be more desirable. In this study, the cross LM17 x 23 that had consistently low negative SCA effects for plant height could be drought adaptable across target environments. Other crosses that could improve drought stress adaptability through reduced plant height includes LM04 x LM45, LM29 x LM45 and LM09 x LM85 with consistently low and negative SCA effects under stressed conditions. High positive specific combining ability effects for yield and yield components show that the parents crossed are good combiners and their cross progenies are good sources of improved variability for the trait under study. In this study, crosses LM02 x LM45, LM29 x LM85 and LM21 x LM23 can be selected for improved KPS while the cross LM13 x LM23 could have a better combination of genes for TSW than the parental genotypes. Likewise, under stress, grain yield can be selected for from crosses between LM09 and LM21.

5.5 Conclusions

Some F2 crosses such as LM09 x LM23 and LM05 x LM23 consistently out-yielded both parents, hence, can be useful for selection of transgressive segregates. LM05 x LM29 had consistently low negative SCA effects for plant height which can also be a target for selection. Under drought stressed conditions, all traits were controlled by additive gene action as shown by the existence of significant GCA effects. Plant height and thousand seed weight from the greenhouse and KPS under field conditions were also influenced by non-additive gene action since they exhibited significant SCA effects, while PH, KPS and TSW were also controlled by

additive genes under optimal conditions. Based on the proportion of GCA to SCA, non-additive gene interaction was more important on all traits under stressed conditions in the greenhouse, while in the field, additive gene action was important for PH, KPS and TSW under stressed conditions, and for PH and TN under non-stressed condition. Parents LM05, LM17 and LM21 can contribute genes towards reduced plant height since they exhibited consistently negative GCA effects across all test conditions under either stressed or both stressed and non-stressed conditions. Parents ML02 and LM23 are good general combiners for increased number of kernels per spike across test conditions, while LM04, LM05 and LM13 were good general combiners under non-stressed conditions. LM17 and LM85 are good general combiners for the number for productive tillers. On the other hand LM04, LM09, LM22 and LM23 could consistently contribute additive genes towards TSW under either drought stressed or both stressed and non-stressed conditions. LM02 could serve as a key parent for grain yield improvement when breeding for wide adaptation. Among the crosses, good specific combiners included LM17 x 23, LM04 x LM45, LM29 x LM45 and LM09 x LM85 which could be useful for selection of reduced plant height. Crosses LM29 x LM85 and LM21 x LM23 are good specific combiners for KPS under field conditions, while LM13 x LM23 had good SCA for TSW. Finally, LM09 x LM21 was a good specific combiner for drought stressed yield. These crosses can be useful for selection of desirable segregates at the F2 generation.

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GENERAL OVERVIEW AND IMPLICATIONS OF THE STUDY

Research findings in brief

Screening Bread Wheat Genotypes for Drought Tolerance Using Phenotypic and Proline Analyses

Ninety-six diverse wheat genotypes including 88 lines from the International Maize and Wheat Improvement Center (CIMMYT)'s heat and drought nurseries, and eight local checks were evaluated under greenhouse and field conditions during 2014/15 and 2015/16. The following phenotypic traits were collected after stress imposed during the heading to anthesis period: the number of days to heading (DTH), days to maturity (DTM), productive tiller number (TN), plant height (PH), spike length (SL), spikelet per spike (SPS), kernels per spike (KPS), thousand seed weight (TSW), grain yield (GY) and proline content (PC). Analysis of variance, Pearson's correlation coefficient, principal component and stress tolerance index were calculated. The main findings were as follows:

- Proline content significantly increased under stress, but weakly correlated with agronomic traits under both optimal and water limited conditions.
- Positive correlation was observed between grain yield and proline content under-drought stressed conditions.
- Twelve genotypes; LM02, LM04, LM05, LM09, LM13, LM17, LM21, LM22, LM23, LM29, LM45 and LM85, with high grain yield under drought stressed conditions and favourable adaptive traits were selected for breeding.

Variance components and heritability of yield and yield components of wheat under drought-stressed and non-stressed conditions

Data assembled from the above study were subjected to combined analysis of variance and variance components were analysed following the General Linear Model (GLM)'s variance component analysis procedure by considering the seasons and water regimes as fixed factors, while the genotypes and sites were treated as random factors. The main outcomes were as follows:

- High levels of genotypic variance (σ^2_g) were estimated for spike length (73%), number of spikelets per spike (44.19%), plant height (51.26%), number of kennels per spike (32.98%), number of days to heading (44.24%) and thousand seed weight (22.98%), resulting in high broad-sense heritability estimates of > 0.50.
- Genotypic variation was relatively moderate for the number of days to maturity, grain yield and number of productive tillers per plant, contributing to 15.03%, 8.46% and 6.13% of the total variation, respectively.
- The heritability estimates of the later traits were 20% ≤ H^2 < 50% which may limit their selection gains under drought-stressed environments.

Genome-wide association analysis of agronomic traits in wheat under drought-stressed and non-stressed conditions

A population of 93 diverse bread wheat genotypes was genotyped using the Diversity Arrays Technology sequencing (DArTseq) protocol. Population structure analysis and genome-wide association mapping were undertaken based on 16,383 silico DArTs loci with < 10% missing data. The main outcomes were:

- Inter-chromosomal linkage disequilibrium showed the existence of linkage decay as physical distance increased.
- A total of 62 significant (P < 0.001) marker-trait associations (MTAs) were detected explaining more than 20% of the phenotypic variation observed under both drought-stressed and non-stressed conditions.
- Significant (P < 0.001) MTA event(s) were observed for DTH, PH, SL, SPS, and KPS; under both stressed and non-stressed conditions, while additional significant (P < 0.05) associations were considered for TSW, DTM and GY under non-stressed condition.

Combining ability and gene action controlling yield and yield components in wheat under drought stressed and well-watered conditions

The above twelve selected parents and their 66 half diallel F2 families were evaluated using a 6 x 13 lattice design with two replications under field and greenhouse conditions during April to

October 2016. Plant height, productive tiller number, kernels per spike, thousand seed weight and grain yield were recorded. The core findings of this study were:

- Parents LM17 and LM21 had negative GCA effects for PH, hence, could be usefulness in breeding for reduced plant height.
- High GCA effects were observed on LM02 for GY; LM02 and LM23 for KPS; and LM04 and LM09 for TSW.
- Crosses LM17 x LM23, LM04 x LM45, LM29 x LM45 and LM09 x LM85 had negative SCA values for plant height, hence these families could be selected for reduced PH.
- The families LM02 x LM45, LM29 x LM85 and LM21 x LM23; LM13 x LM23; and LM09 x LM21 were better specific combiners for drought stressed KPS, TSW and GY, respectively. These are ideal crosses for further selection and genetic improvement for these traits.

Implications of Findings of this Study for Drought Tolerance Wheat Breeding

Germplasm Development

The success of any breeding program depends on the availability of genetic variability for target traits. CIMMYT has dedicated drought tolerance pre-breeding programs that develop and distribute heat and drought tolerant nurseries for evaluation and breeding across the world. Such materials could provide a combination of genes that confer adaptability to target drought scenario. The capacity of the plant to maintain cell stability and to extract and save water under stress depends on compatible osmolytes like proline. Therefore, screening germplasm using phenotypic and biochemical markers aids selection of candidate genotypes for drought tolerance breeding. The positive correlation observed between grain yield and proline content under drought stressed conditions provides some clue that proline accumulation is a useful trait that can be considered as a tool for effective selection of drought tolerant genotypes. The CIMMYT lines evaluated in this study proved to have sufficient phenotypic variability that could be exploited by breeders in improving drought tolerance when developing wheat varieties. The selected wheat genotypes; LM02, LM04, LM05, LM09, LM13, LM17, LM21, LM22, LM23, LM29, LM45 and LM85, with high grain yield under drought stressed conditions and favourable adaptive traits are valuable genetic stocks for drought tolerance breeding.

Variance Component Analyses and Heritability Estimation

The usefulness of breeding materials depends on the repeatability or heritability of key traits in target environments. This reflects on the capacity to transfer such traits to next generations during breeding. This also indicates the usefulness of such germplasm sets in tracking genes for the respective traits. High heritability values obtained for spike length, number of spikelets per spike, plant height, number of kennels per spike, number of days to heading and thousand seed weight in the present study imply the significance of these traits for enhanced selection and improved genetic gains. The genetic determinants of these traits can be tracked using molecular techniques.

Genome-wide Association Analysis

Traditional plant breeding has made significant strides in improving wheat adaptability to marginal growing environments through phenotypic selection. The use of marker assisted selection (MAS) is expected to improve the effectiveness of selection and trait introgression. Several efforts are being directed at pinpointing the genomic loci that influence key agronomic traits. The wheat genome is however very huge and complex, hence, such efforts should continuously be put to associate more markers and genes with key agronomic traits. The 65 significant marker-trait associations (MTAs) that were detected in this study could be useful in initiating MAS and targeted trait introgression of wheat under drought-stressed and non-stressed conditions. Further, such genomic resources can be vital for fine mapping and cloning of the underlying genes. Validation of these markers has been recommended before large-scale application.

Combining Ability and Gene Action

Following phenotypic evaluation, all superior lines may not be useful in transferring traits to progenies during breeding. Knowledge on the nature and magnitude of combining ability and gene action is important for selection of good general combiners that can contribute additive genes and good specific combiners from which superior lines can be selected. Progeny testing serves as a guide in selection of an appropriate breeding methodology to realise genetic gains. Significant GCA effects of parents and SCA effects of progenies observed on different traits in

this study imply that some of the parents and corresponding progenies evaluated can contribute additive and non-additive genes towards reduced plant height, and improved grain yield and yield components under both stressed and non-stressed conditions. Further, transgressive segregants for the studied traits can be selected at the F2 and subsequent generations to develop pure line cultivars. Future studies can target to develop homozygous and homogenous populations from superior F2 families and segregating lines for genetic analyses and cultivar development using the doubled haploid technique.