Characterisation of Selected Bread Wheat (*Triticum aestivum* L.) Genotypes for Drought Tolerance Based on SSR Markers, Morpho-physiological Traits and Drought Indices

by

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Summary

Bread wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* L. var. *durum*) are staple cereal food crops worldwide. In South Africa, bread wheat is the second most economically important cereal after maize. Drought stress associated with climate change is a major cause of the yield gap in wheat production in South Africa. Drought tolerant wheat cultivars are yet to be developed and released in the country. Wheat improvement for drought tolerance is one of the major breeding goals in South Africa. Integrative pre-breeding techniques involving genotypic and phenotypic characterisation ensure an accurate selection of potential drought tolerant parents for breeding. Therefore, the specific objectives of the current study were: 1) to determine the genetic diversity and population structure of forty-seven diverse bread wheat genotypes introduced from the International Maize and Wheat Improvement Center (CIMMYT) using ten selected polymorphic Simple Sequence Repeat (SSR) markers, 2) to characterise fifteen bread wheat genotypes introduced from CIMMYT using physiological and morphological traits, and 3) to assess drought tolerance amongst fifteen selected bread wheat genotypes using nine drought tolerance indices.

Genetic diversity and population structure of 47 CIMMYT derived bread wheat genotypes were examined using 10 SSR molecular markers. All the SSR markers used in the study were highly polymorphic. The highest PIC values were recorded for XGWM 132, WMS 179 and WMS 30 with 0.93, 0.89 and 0.89, respectively. Cluster analysis detected 3 distinct clusters with Clusters A and C consisting of most diverse genotypes. Two distinct heterotic patterns were identified to select unique parents for crosses. Analysis of molecular variance (AMOVA) detected significant genetic diversity among populations, among individuals and within individuals with explained percentage variance of 3%, 37% and 60%, respectively. Genetic diversity and population stratification was mainly due to private alleles detected. Based on detected genetic variability, a total of 15 genotypes were selected and subjected for phenotypic characterisation. The selected genotypes included SYM2016-037, SYM2016-038, SYM2016-029, SYM2016-010 and SYM2016-012 from Cluster A, SYM2016-044, SYM2016-004, SYM2016-016, SYM2016-019, SYM2016-014, SYM2016-008, SYM2016-006 and SYM2016-047 from Cluster B and SYM2016-042 and SYM2016-027 from Cluster C.

The above selected 15 bread wheat genotypes were evaluated under field and greenhouse conditions using a randomised complete block design with 3 replications. Drought stress was imposed as follows: 1 week before 50% heading (WBH) and 1 week after 50% heading (WAH). A fully-irrigated water regime (NS, non-stress) was used as a comparative control. Genotypes were evaluated using 2 physiological and 8 morphological traits. Significant differences ($P < 0.05$) were detected among genotypes and genotype x test environment interaction. Genotype effect was significant for days to flowering, days to maturity, plant height, number of productive tillers, number of spikelets per spike, grain number and 100 grain weight. Genotype x test environment interaction was significant for canopy temperature, days to flowering, days to maturity, plant height, number of spikelets per spike, grain number, 100 seed weight and the yield. Significant correlations were detected between yield and days to flowering, days to maturity, plant height, number of productive tillers, number of spikelets per spike, grain number and 100 seed weight under greenhouse condition. The number of productive tillers per plant and the number of spikelets per spike were positively associated with yield under field evaluation. Principal component analysis revealed PC1 to be consistently associated with yield, 100 seed weight and number of spikelets per spike. Days to flowering and maturing, plant height and canopy temperature were positively associated with either PC2 or PC3 under greenhouse and field conditions. A yield penalty was noted for early flowering and maturing genotypes such as SYM2016-014, SYM2016-027 and SYM2016-029 relative to late flowering and maturing genotypes SYM2016-016, SYM2016-037 and SYM2016-006. Crossing of these complementary lines and continuous selection of progenies is essential to develop early maturing genotypes with stable and high yield potential. In this study, days to flowering and maturity, plant height, canopy temperature and 100 seed weight were favourable traits to screen genotypes for drought tolerance. Screening for drought tolerance under greenhouse condition was more reliable than under field evaluation.

The above 15 wheat genotypes were evaluated using 9 drought tolerance indices based on yield data. The drought indices used were drought resistance (DR), mean productivity (MP), harmonic mean of yield (HM), stress susceptibility index (SSI), stress tolerance index (STI), tolerance index (TOL), yield index (YI), yield reduction index (YR) and yield stability index (YSI). Analysis of variance detected significant differences among genotypes ($P < 0.001$) and genotype by water regime interaction ($P < 0.01$) affecting yield response. Significant differences were also recorded among genotypes ($P < 0.05$) for DR, HM, MP, STI, YI and YSI. Consistent mean genotype ranking was recorded for HM, MP, STI, SSI and YI enabling selection of genotypes SYM2016-006, SYM2016-016 and SYM2016-037. PC analysis detected high variation of 82.2% among genotypes, with percentage variation partitioned as follows: 42.64% for PC1, 22.37% for PC2 and 12.18% for PC3. Both PC and bi-plot analyses revealed strong associations between HM, MP, STI, YI and yield under drought stressed and non-stressed conditions. High yielding genotypes such as SYM2016-006, SYM2016-016 and SYM2016-037 scored higher values for HM, MP, STI, YI and yield under drought stressed and non-stressed conditions. DR was associated with early maturing genotypes such as SYM2016-014, SYM2016-029 and SYM2016-38. These genotypes were considered as potential parents for future wheat breeding programmes emphasizing drought tolerance.

Declaration

I, Sibusiswangaye Yolanda Mdluli, declare that

1. The research reported in this dissertation, except where otherwise indicated, is my original research.
2. This dissertation has not been submitted for any degree or examination at any other university.
3. This dissertation does not contain other scientists' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other scientists.
4. This dissertation does not contain other scientists' writing, unless specifically acknowledged as being sourced from other scientists. Where other written sources have been quoted, then their words have been re-written but the general information attributed to them has been referenced.
5. This dissertation does not contain text, graphics or tables taken from the internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the reference section.

Signed…… ……………………………Date…30/05/2018…………

Sibusiswangaye Yolanda Mdluli (Candidate)

As the candidate's supervisor(s), I/We have approved this dissertation for submission.

Signed………………………Date…01/06/2018…………….

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Dedication

This dissertation is dedicated to my
Ma Mvelase no Baba uMdluli.
The little girl you raised is becoming a strong and intelligent woman as you had dreamed and hoped. God bless you both.

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List of Abbreviations

CIMMYT	International Maize and Wheat Improvement Center
GxE	Genotype by environment interaction
NS	Non-stress water regime
PCA	Principal Components Analysis
PIC	polymorphic information content
SSR	Simple Sequence Repeat markers
UPGMA	Unweighted pair group mean arithmetic
WAH	Drought stress imposed one week after 50% flowering
WBH	Drought stress imposed one week before 50% flowering

Introduction to Dissertation

Background

Wheat is the third most cultivated cereal globally after rice and maize (Monneveux et al., 2014). According to FAO (2018), world's wheat production was estimated at 757.9 million tons in the years 2017/2018 and forecasted to decline to 746.6 million tons in the years 2018/2019. Wheat is generally categorised as either durum wheat *(Triticum turgidum L. var. durum*) or bread wheat *(Triticum aestivum* L.) (Pena, 2002). Bread wheat dominates 90% of total world wheat production (Monneveux et al., 2014).

The growth habits of bread wheat are winter, spring and facultative wheat types (Lantican et al., 2005). Winter wheat is sown before the winter and requires a period of vernalisation for flowering. Spring wheat is sown in the spring and does not require vernalisation, just as facultative wheat (Lantican et al., 2005). Grain character of bread wheat allows classification into 5 distinct groups namely, hard red winter, hard red spring, soft red winter, hard white and soft white wheats (Monneveux et al., 2014). Grading of bread wheat in accordance with milling and baking standards is based on the falling number, protein content and hectolitre mass (ARC, 2017). The falling number is related to the grain starch content which measures the activity of the alpha amylase enzyme. Protein content is related to the percentage protein within the grain and hectolitre mass is related to the flour extraction from each grain (Pena, 2002; ARC, 2017). The gluten protein is responsible for giving bread wheat flour its viscoelastic properties allowing for soft and light breads (Pena, 2002).

In Africa, the dominant wheat producing countries are Egypt, Morocco, Algeria, South Africa and Tunisia (FAO, 2012). There are 3 main wheat production zones in South Africa, namely the rain fed spring wheat production zone in the Western Cape, the rain fed winter wheat zone in the Free State and the irrigated spring wheat zones in the summer rainfall area (ARC, 2014).

Constraints to wheat production and productivity in South Africa

The ability to meet the local bread wheat demands of the South African population fall short as a consequence of biotic and abiotic stress factors (Smit et al., 2010). There are variations for the different constraints for bread wheat production depending on the environmental conditions of the aforementioned production zones (ARC, 2014). Some of the major bread wheat biotic constraints are diseases such as stem rust (*Puccinia gramminis* f sp. *tritici*) and stripe rust (*P. striiformis* f sp. *tritici*), fusarium head blight (*Fusarium graminearum*, *F. crookwellense* and *F. culmorum*), root diseases such as take-all and crown rot. The major insect pest of wheat is Russian wheat aphid (*Diuraphis noxia*) (Smit et al., 2010). On the other hand, abiotic constraints include post-harvest sprouting, soil acidity, high aluminium sensitivity linked with low soil pH, poor soil fertility, poor management practices and climate stress (Smit et al., 2010; ARC, 2017).

Drought as a threat to wheat production and productivity

Nearly 50% of present day wheat production areas experience periodic drought (Farooq et al., 2014). Reports presented by the IPCC (2014) indicate an increased frequency of cyclones, flooding, drought, heat waves and wildfires affecting crop production across various regions of the globe. Drought stress associated with climate change is the leading abiotic constraint to world's wheat production and productivity (FAO, 2016). The Near East and Africa were reported with crop and livestock losses majorly due to drought stress (IPCC, 2014). Moreover, as a consequence of the El-Nino phenomenon, which is the rise of eastern and central Pacific Ocean temperatures, drought and erratic rainfall devastates various regions in the world, notably sub-Saharan Africa (SSA) (Shiferaw et al., 2013). A trend of decreasing annual rainfall has been reported in South Africa, particularly in the arid and semi-arid regions of the country, which are more drought prone (Edossa et al., 2014). Hence, there exists a requirement for improved tolerance in bread wheat to drought stress for sustainable wheat production in the country.

Breeding bread wheat for drought tolerance

Abiotic stress in plants triggers variable responses at molecular, biochemical, physiological and morphological levels of organisation (Zandalinas et al., 2018). Differential phenotypic responses of diverse genotypes to drought stress is mainly due to genetic and environmental variations as well as the genotype by environment interaction (GXE interaction) (Bernado, 2002). Drought tolerance and yield are affected by GxE interaction as they are quantitative traits (Dabholkar, 1999; Fleury et al., 2010). This may result in inconsistencies in rankings of different genotypes across different test environments (Farshadfar et al., 2013), referred to as the "phenotype-genotype gap" (Blum, 2011; Farooq et al., 2014). Genetic characterisation, via molecular marker technology, reduces the genotype-phenotype gap and complements phenotyping of crop germplasm resources (Amelework et al., 2016). Molecular marker technologies available for genotyping include the Restriction Fragment Length Polymorphisms (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphisms (SNPs), with their modifications (Korzun, 2003).

Drought stress is most detrimental and irreversible in the reproductive growth stage (Blum, 2011). Therefore, drought tolerance screening must take into account the drought intensity, the season when drought occurs and the growth stage in cereal crops with which drought stress coincides (Mir et al., 2012). For classifying growth stages, the Zadoks decimal code growth scale is used (Zadoks et al., 1973; Tottman, 1987; Acevedo et al., 2002). Precision phenotyping in drought characterisation should ensure selection is targeted towards physiological and morphological traits with high heritability (Abdolshahi et al., 2015). Phenotyping tools should be of high throughput and associated with high yield under drought stress and non-stress conditions (Lopes et al., 2012; Mir et al., 2012).

Physiological traits well correlated with yield include cool canopy temperature (Moslem et al., 2013) and high stomatal conductance (Monneveux et al., 2014). These traits depend on the drought stress type and stress intensity (Mir et al., 2012). High stomatal conductance and a cool canopy are further correlated with each other since they are associated with high transpiration and maintained photosynthesis during drought stress (Villegas et al., 2000; Araus et al., 2002). High transpiration is further correlated with a deep root system for extraction of moisture at greater soil depth (Lopes and Reynolds, 2010; Pinto and Reynolds, 2015). For instance, under drought stress condition crops such as sorghum, upland rice and maize were reported to extend their roots as deep as 60 cm into the soil profile for water uptake (Pareek et al., 2010). Root growth through the soil profile also depends on the soil structure, texture and drought type (Lopes et al., 2012). On the other hand, morphological traits such as earliness or early flowering and maturity (Lopes et al., 2012), plant height (Abdolshahi et al., 2015), grain number (Acevedo et al., 2002; Ehdaie et al., 2012) and 1000 kernel weight (Lopes and Reynolds, 2010; Drikvank et al., 2012) are all traits well correlated with yield. Shorter days to flowering and maturity have previously been recommended for drought tolerance breeding since by shortening their life cycle genotypes prevent drought stress coinciding with vital growth stages (Villegas et al., 2000; Blum, 2011). This is otherwise referred to as drought escape (Blum, 2011). Grain yield is also notably correlated with plant height, which is associated with stem internode storage of water soluble carbohydrates in environments where soil moisture at deeper depth is unavailable (Nguyen and Blum, 2004). These morphological traits are considered as major yield components (Farshadfar, 2012).

Genotypes’ yield response under non-stress condition may considerably change when exposed to drought stress, thus introducing a degree of unpredictability (Sio-Se Mardeh et al., 2006; Sahar et al., 2016). For this reason, drought indices are used to assess and categorise the degree of drought tolerance or susceptibility of genotypes under drought stress and non-stress conditions (El-Mohsen et al., 2015). Genotypes may be grouped into 4 distinct classes based on their yield responses; Group A genotypes are high yielding under drought stress and non-stress, Group B genotypes are high yielding only under non-stress, Group C genotypes are high yielding only under drought stress and Group D genotypes express poor yield under both drought stress and non-stress (Fernandez, 1992). Drought indices used to detect these different genotype groupings are the drought resistance index (Lan, 1988), harmonic mean of yield (Jafari et al., 2009), geometric mean productivity (Kristin et al., 1997), mean productivity and tolerance index (Rosielle and Hamblin, 1981), modified stress tolerance indices 1 and 2 (Farshadfar and Sutka, 2002), stress susceptibility index (Fischer and Maurer, 1978), stress tolerance index (Fernandez, 1992), yield index (Gauzzi et al., 1997), yield stability index (Golestani-Araghi and Assad, 1998) and yield reduction index (Jafari et al., 2009). The yield stability (Golestani-Araghi and Assad, 1998) and yield reduction (Jafari et al., 2009) indices measure the extent of yield loss due to drought compared to yield under non-stress condition. Landraces were reported to have superior yield under drought stress compared to improved germplasm as expressed by low values of the tolerance index and a value for the stress susceptibility index less than 1 (Dodig et al., 2012). Sio-Se Mardeh et al. (2006) reported the use of tolerance index to select for genotypes with high yield only under drought stress. Conversely, the drought resistance index (Lan, 1988), harmonic mean of yield (Jafari et al., 2009), yield index (Gauzzi et al., 1997), mean productivity (Hohls, 2001), stress tolerance index and geometric mean productivity (Fernandez, 1992) were reported to be useful to select genotypes with stable yield across conditions of drought stress and non-stress allowing favourable classification of Group A genotypes. Therefore, the above methods are useful when used together to improve the selection efficiency of genotypes for drought tolerance breeding.

Rationale of the study

Despite being one of the main wheat producers in Africa, the South African demand for bread wheat far exceeds its supply (SAGL, 2017). Thus, most of South African wheat in 2016/2017 season was reported to be imported from the Czech Republic, Germany, the Russian Federation, Poland, Brazil, Uruguay and the United States of America (DAFF, 2014; SAGL, 2017). Drought stress is one of the main constraints that curtailed wheat production and productivity in South Africa consequentially resulting in poor yield driving greater reliance upon bread wheat imports. Moreover, the expansion in the human population, change in dietary preferences towards a greater consumption of bread and decreasing agricultural land due to urbanisation further impose greater demand for bread wheat. This trend spells disaster for South African commercial and small-holder wheat farmers, commercial milling and baking industries and employees within these institutions. Hence, improving bread wheat response to drought stress in South Africa is a beneficial strategy at the economic, environmental and social levels.

There is a limited study in South Africa on breeding wheat for drought tolerance involving genetic and phenotypic characterisation. This is on account of limited availability of genetic resources and the expense associated with genetic and phenotypic characterisation. Also, there is a degree of complication in locating a suitable molecular marker technology for bread wheat with its large hexaploid genome (2n=6x=42, AABBDD) made up to 80% repeated DNA motifs attributable to the narrow genetic base and homozygosity (Brenchley et al., 2012). SSRs have been recommended as suitable for bread wheat genetic diversity analysis since they are highly polymorphic, genome specific, co-dominant molecular markers and are fairly convenient to use (Korzun, 2003). Hence SSRs were the marker of choice in the current study. There is limited study conducted in the country that evaluated wheat genotypes for drought tolerance using non-destructive physiological traits such as the canopy temperature and stomatal conductance along with a selection of morphological traits and drought indices under controlled greenhouse environment and open field conditions.

Aim

The overall aim of this study was to determine the response of bread wheat genotypes to drought stress using phenotypic and physiological traits and molecular markers.

Objectives

The specific objectives of the study were:

i. To determine the genetic diversity and population structure of 47 diverse bread wheat genotypes introduced from CIMMYT using ten selected polymorphic SSR markers

ii. To characterise fifteen bread wheat genotypes introduced from CIMMYT using physiological and agronomic traits

iii. To assess drought tolerance amongst fifteen selected bread wheat genotypes using nine drought tolerance indices

Hypotheses

The hypotheses tested in the study included:

i) There is significant genetic diversity among the forty-seven CIMMYT bread wheat genotypes and the ten specific SSR molecular markers used in this study are highly polymorphic.

ii) There is significant phenotypic variation among the selected bread wheat genotypes under drought stress and non-stress conditions. Also, the physiological and morphological traits are well correlated with yield under drought stress and non-stress conditions.

iii) There is significant yield variation among the bread wheat genotypes under drought stress and non-stress conditions. The nine drought tolerance indices were significantly associated with yield under drought stress and non-stress conditions thus could discriminate drought tolerant from drought susceptible bread wheat genotypes.

Dissertation outline

The present dissertation comprised of the literature review and three experimental chapters which were condensed into discrete and inter-dependant papers according to the University of KwaZulu-Natal's dominant dissertation format. There are some overlaps and unavoidable repetitions of references and some introductory information between chapters.

Chapter	Title
-	Dissertation introduction
1	A review of literature
2	Genetic characterisation of selected bread wheat (*Triticum aestivum* L.) genotypes through SSR markers II
3	Phenotyping bread wheat (*Triticum aestivum* L.) genotypes for drought tolerance I
4	Assessment of drought tolerance in selected bread wheat (*Triticum aestivum* L.) genotypes using drought tolerance indices III
-	An overview of findings and implications of the study

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Chapter 1 A Review of Literature

Abstract

Drought stress, as a result of climate change, is one of the prominent causes of crop failure and yield losses. Rapid population expansion, change in food preferences and recurrent drought stress pose a demand for well-characterised and drought tolerant wheat germplasm. Integrative breeding techniques, which incorporate both genetic and phenotypic characterisation ensure accurate selection of parents for breeding. Marker-assisted breeding (MAB) assists the breeder to eliminate genotypes with inferior traits without the influence of GxE. The SSR markers, in particular, are suitable genetic markers for genetic analysis and MAB. SSRs are spread throughout the large wheat genome and are co-dominant markers. As a result, SSRs are recommended as the marker of choice in wheat genotyping. After genetic characterisation, selected genotypes are advanced for field or greenhouse phenotypic characterisation. Physiological and morphological traits significantly correlated with high grain yield such as the canopy temperature, stomatal conductance, days to 50% flowering, days to 50% maturity, plant height, grain number and 1000 kernel weight are key traits for phenotyping for drought tolerance. Moreover, selection for drought tolerant germplasm is achievable via drought selection indices, which are also well-correlated with seed yield. Selection indices such as drought resistance index, mean productivity, harmonic mean, stress susceptibility index, stress tolerance index, tolerance index, yield index, yield reduction index, and yield stability index have been successful in the detection of high-yielding, drought tolerant genotypes under drought stressed or non-stressed environments. The above integrative methods are useful selection tools for cultivar development of wheat emphasising drought tolerance.

Keywords: Drought stress, genotype by environment interaction, morphological traits physiological traits, SSR markers, wheat

1.1 Introduction

Drought stress is one of the major causes of yield failures in crop production (FAO, 2013). More than 50% of wheat production areas globally experience periodic drought (Muhammad and Ali, 2014). According to Araus et al. (2002), the major wheat production zones, which suffer moderate to severe drought stress are Argentina, Australia, Canada and the United States of America. Mir et al. (2012) reported climate change causing desertification. Moreover changing precipitation patterns limit sustainable crop production leading to loss of potential arable lands. Over the last decades, drought and heat stress have negatively and significantly impacted the sub-Saharan African (SSA) region (Shiferaw et al., 2013) due to increased semi-arid climate and the El Nino or La Nina-Southern Oscillation (ENSO) effects. An estimated 60% of SSA region remains at risk of experiencing recurrent drought (Shiferaw et al., 2013).

Drought stress can be classified in the context of meteorological, hydrological or agricultural drought stress (Araus et al., 2002). Agricultural drought stress is important to crop and livestock farmers (Passioura, 2007). Post-flowering or reproductive stage water deficit, causes permanent damage in flowering crop plants (Blum, 2011). Reduced seed number per plant was reported by Fang et al. (2010) in chickpea, whereby drought stress led to an abortion of flowers and reduced viability of pollen. Moreover, water deficit can pose a major threat to grain-filling in cereal crops (Tatar et al., 2016). In wheat, anthesis is a more sensitive stage to drought stress (Araus et al., 2002).

High population expansion, changing food preferences and recurrent drought stress will require increased food production and food supply by adapting drought tolerant and high yielding cultivars (Fleury et al., 2010; Shiferaw et al., 2013). Characterisation for drought adapted and drought tolerant germplasm is regarded to be a priority breeding goal. Unfortunately, grain yield is subject to GxE and shows low heritability (Sleper and Poehlman, 2006). Thus direct selection for yield alone cannot be relied upon for drought tolerance breeding. Morphological, molecular, biochemical, and physiological markers are important parameters for selection of drought tolerant genotypes (Araus et al., 2002).

Molecular marker technology assists the selection process and can empower the breeder to eliminate undesirable genotypes thus keeping breeding populations to a manageable size (Korzun, 2003). The changes in migration, mutation, gene linkage, selection and non-random mating alter allele and genotype frequencies. These forces determine genetic diversity and population stratification (Hamilton, 2009; Nielsen et al., 2014). Understanding genetic diversity and population structure is the foundation of any breeding endeavour.

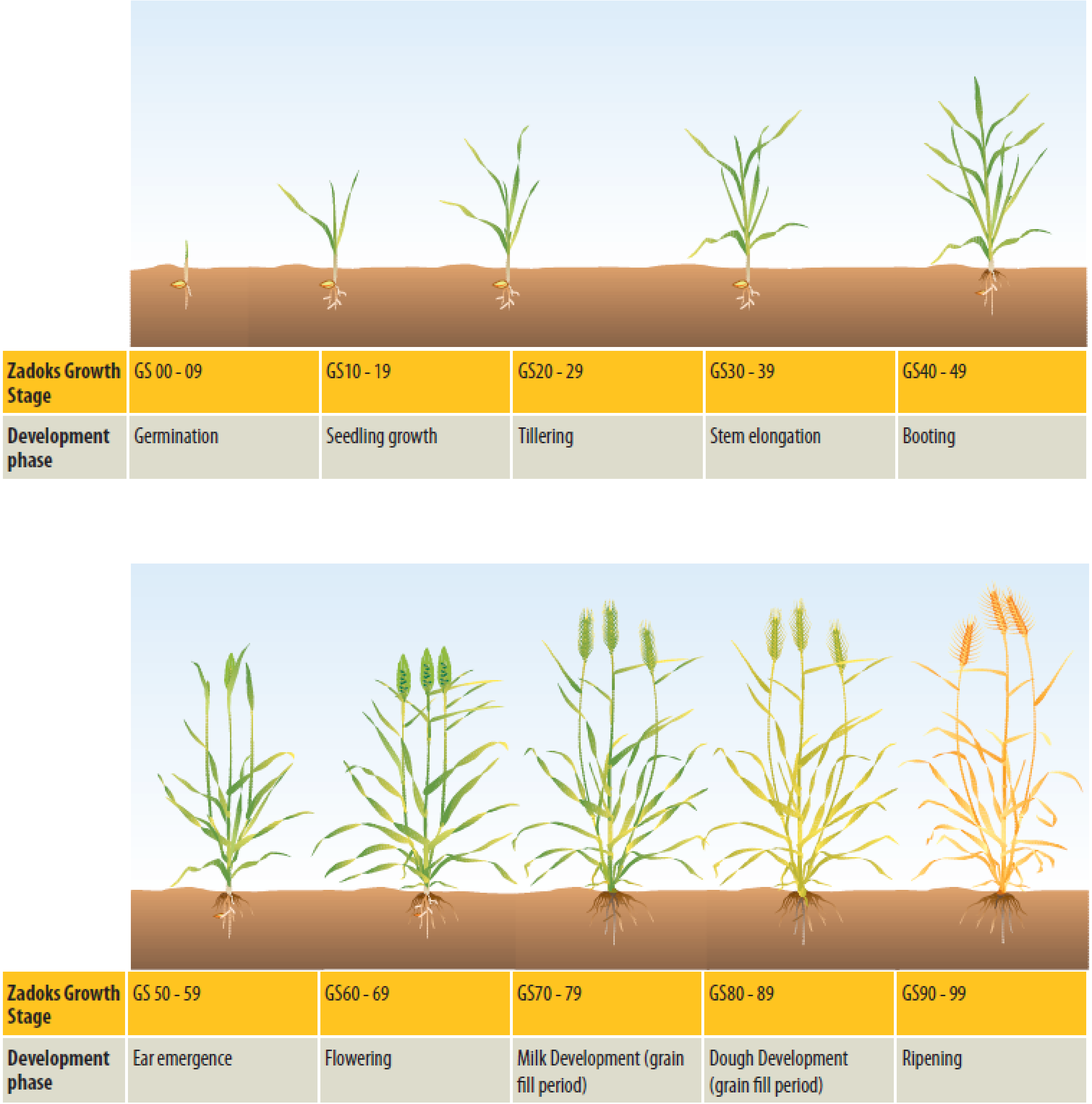
The most commonly utilised molecular markers are Restriction Fragment Length Polymorphisms (RFLP), Amplified Fragment Length Polymorphisms (AFLP), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR) And Single Nucleotide Polymorphisms (SNP) (Korzun, 2003). These are effective tools in marker-assisted breeding programmes. Among the molecular markers, SSRs present a convenient, efficient, and accurate method in investigating genetic diversity and understanding of population stratification and discovery of potential parents for designed crosses (Korzun, 2003). Similarly, selected phenotypic and drought adaptive traits well-correlated with grain yield and convenient to phenotype are important attributes in discriminating genotypes in diverse populations evaluated under diverse test environments (Abdolshahi et al., 2015). Integration of molecular and phenotypic markers improves selection accuracy and increases the rate of genetic gain for yield and yield components (Mir et al., 2012; Abdolshahi et al., 2015).

1.2 The taxonomy and botany of bread wheat

Bread wheat or common wheat (*Triticum aestivum* L., AABBDD, 2n=6x=42) belongs to the family Gramineae (Acquaah, 2007). It is an annual hexaploid and a C3 cereal crop which is predominantly self-pollinated (Acevedo, 2002). Wheat has two growth habits, namely winter and spring wheat, whereby the former requires a period of vernalisation to induce flowering whilst the latter does not (Sleper and Poehlman, 2006; Acquaah, 2007). Wheat growth stages are distinctly recognised and are recorded based on growth scales.

1.3 Classification of the cereal growth stages

The Zadoks growth scale (Figure 1.1) is utilised for understanding the wheat growth stages for research, vital fertiliser application periods and emasculation or pollination (Zadoks et al., 1973). Wheat growth stages are divided distinctly into the vegetative and the reproductive growth stages (Tottman, 1987). Important agronomical cereal growth stages are recognised as establishment, tillering, flower initiation, grain-filling and ripening (Passioura, 2007) as illustrated in Figure 1.1.



Zadoks Growth Stage	GS 00 - 09	GS10 - 19	GS20 - 29	GS30 - 39	GS40 - 49
Development phase	Germination	Seedling growth	Tillering	Stem elongation	Booting

Zadoks Growth Stage	GS 50 - 59	GS60 - 69	GS70 - 79	GS80 - 89	GS90 - 99
Development phase	Ear emergence	Flowering	Milk Development (grain fill period)	Dough Development (grain fill period)	Ripening

Figure 1.1: Illustration of the different growth stages of monocotyledonous crops according to the Zadoks growth chart as adopted from Scanlan (2017).

1.4 Demand and production of bread wheat

Wheat is the third most consumed cereal crop after rice and maize, hence it is a staple food and commodity crop (DAFF, 2014; Monneveux et al., 2014). Globally wheat productivity in the year 2017/ 2018 was estimated at 757.9 million tons (FAO, 2018). In South Africa, wheat products are the most consumed food after maize products. The main wheat production regions in the country are classified as either summer rainfall or winter rainfall wheat production regions (ARC, 2017). The Western Cape, Northern Cape and Free State are the largest wheat production regions in South Africa (DAFF, 2014). The wheat protein gluten makes the wheat grain valuable for the bread-making industry (Sleper and Poehlman, 2006). Nationally about 62 loaves of bread per person per annum are consumed (DAFF, 2014).

1.5 Drought stress in the context of plant breeding

Drought escape and drought tolerance are both modes of drought resistance (Fischer and Maurer, 1978). Whereby, drought tolerance is characterised by economical water consumption under drought stress, termed as “endurance with low water content”, hence metabolic activity is maintained throughout the drought stress event (Fischer and Maurer, 1978; Kosova et al., 2014). On the other hand, drought escape limits transpiration by limiting water loss of the canopy while maximising water absorption through roots (Fischer and Maurer, 1978; Kosova et al., 2014). Otherwise referred to as “endurance with high water content” (Fischer and Maurer, 1978; Kosova et al., 2014) which may also involve deliberate shortening of the plant life cycle by means of early germination, flowering and maturity (Fleury et al., 2010). In so doing, this prevents vital growth stages coinciding with the drought stress event (Kosova et al., 2014). This affects the plant at the molecular, biochemical, physiological and morphological levels of organisation through the soil-plant-atmosphere continuum (Kosova et al., 2014). Exploiting the different responses to drought stress of crops translates into the exploitation of genetic diversity.

1.6 Importance, benefits and complications of exploiting genetic diversity

Genetic diversity is to a breeder what colour is to a painter. Genetic diversity ensures and improves the likelihood of selecting individuals of economic interest for hybridisation and genetic advance (Henry, 2001). Genetic diversity serves as a buffer for the species against biotic and abiotic threats, which can potentially wipe out a genetically uniform species (Shah et al., 2009). Drought tolerance, just as grain yield, is a polygenic trait (Mwadzingeni et al., 2015). The phenotypic expression is the consequence of numerous genes and their interaction with other genes within or between two or more gene loci (Mwadzingeni et al., 2015). Therefore, the polygenic nature of the drought tolerance and yield characters present difficulty in breeding and genetic characterisation. A constraint associated with bread wheat genetic analysis is its large hexaploid genome (Brenchley et al., 2012). Wheat is composed of the A, B and D genomes, each of which consisted of 7 chromosomes. This brings a total of 21 chromosomes in haploid common wheat (Faheem et al., 2015). Furthermore, bread wheat has approximately 17 billion nucleotides (Brenchley et al., 2012; Mwadzingeni et al., 2017). Moreover, bread wheat has a large number of repetitive sequences, which take up about 80% of the genome (Nielsen et al., 2014). Thus, highly polymorphic molecular markers that are widely distributed within the wheat genome are effective for bread wheat genetic analysis (Mir et al., 2012; Nielsen et al., 2014).

1.7 Molecular markers as a tool for cereal genotyping

Molecular markers are recommended as selection tools to understand and narrow the genotype-phenotype gap (Amelework et al., 2016). Their reliability stems from their functionality independent of immediate environments (Mashilo et al., 2016). There is a variation in marker accuracy and ease of use in relation to the marker of choice and their application (Mir et al., 2012). The commonly used molecular markers in cereal molecular studies are the AFLPs, RFLPs, RAPDs, SSRs and SNPs (Korzun, 2003).

SSRs are recognised as efficient in the study of the vast wheat genome (Korzun, 2003). These markers are aptly referred to as Variable Number of Tandem Repeats (VNTR) since SSRs exist as two to six nucleotide repeats scattered in the genome (Henry, 2001). They are known to flank regions key for traits of interest and the di-nucleotides are cited as some of the more dependable SSRs, even though their palindromic nature results in them being read the same way backwards and forwards (Henry, 2001). Additional benefits of SSR markers are their genome specificity, their co-dominant nature and how they are genome-wide (Chen et al., 2012). Hence, the co-dominance nature of SSRs means they have the capacity to discriminate between the heterozygote or homozygote states of genes (Chen et al., 2012). Shah et al. (2009) reported SSRs as highly polymorphic in wheat. They are also known to distinguish genotypes precisely in genetic diversity characterisation of wild and improved germplasm in seed banks (Shah et al., 2009).

1.8 Analysis of genetic diversity and population structure

The Hardy-Weinberg principle is the foundation of population genetics (Andrews, 2010). The principle states that in an idealised infinite population, whereby there is an absence of mutation, migration, non-random mating, selection and linkage, allele and genotype frequencies will remain constant generation after generation (Hamilton, 2009). A deviation from this equilibrium, according to Soriano et al. (2016), can result in distinct sub-grouping within the population.

In a genetic diversity study of sorghum genotypes sampled from various regions of South Africa, Mofokeng et al. (2014) reported farmers to select particular genotypes based on their favourable brewing traits. Similarly, Desta et al. (2014) revealed the clustering of Eritrean wheat landraces was a consequence of farmers selecting particular wheat landraces for their favourable disease resistance, pest resistance and baking suitability. Artificial and natural selection procedures continuously intensify the selected alleles and bring about population structure (Nielsen et al., 2014).

Wind-blown pollen and the sharing of seed or propagules by farmers significantly increases gene flow (Nm) among sub-populations (Mofokeng et al., 2014). In plants, the degree of exchange of genetic material between sub-populations, influences the genetic differentiation, otherwise known as the genetic differentiation (F) and gene flow as noted by Morjan and Rieseberg (2004). Moreover, seed exchange was pointed out by Morjan and Rieseberg (2004) to contribute greater to the mean genetic material exchange (Nm = 6.92), than wind-blown pollen (Nm = 2.51) or even genetic material exchange by both seed exchange and wind-blown pollen (Nm = 1.82). Interestingly, the mean genetic differentiation caused by seed exchange (FST = 0.46) was greater than that for free flowing pollen (FST = 0.39) and the combination of the two mechanisms (FST = 0.32). Thus, sharing of seed or free flowing pollen contribute significantly to the prevalence of common genetic backgrounds among genotypes, rendering greater relatedness of germplasm from various geographical regions as reported in rye by Targonska et al. (2016) and in bread wheat by Mwadzingeni et al. (2017). Prior knowledge of population structure of the breeding material is a necessity for locating possible heterotic groups, capitalising on the genetic diversity and the improvement of genetic advance (Nielsen et al., 2014).

A specific set of genetic parameters are employed for dissecting genetic diversity and population stratification. According to Salem et al. (2015), a positive linear relationship is shared between the genetic diversity and the number of alleles per locus. In one study, the average number of alleles detected was 5.59 alleles per locus while the mean polymorphic information content (PIC) was 0.65 (Salem et al., 2015). While a study involving Chinese winter wheat by Chen et al. (2012) revealed a mean value of 5.05 alleles per locus realised with minimum and maximum values of 2 and 10 alleles per locus, respectively. Also the mean PIC in the same study was 0.64 and 0.63 for landraces and modern cultivars, in that order. On the other hand, Mofokeng et al. (2014), reported mean PIC and mean number of alleles per locus as 0.50 and 6.40, respectively in sorghum. This relationship thus may be used for inference of genetic diversity when selecting well-suited and highly descriptive molecular loci. Additionally, Botstein et al. (1980) as cited by Salem et al. (2015), presented standardised values of indicating the extent of genetic diversity among loci, values were allocated as highly informative, moderately informative and slightly informative for PIC values > 0.50, <0.50 but >0.25 and <or =0.25, respectively.

Standardised genetic differentiation values as discussed by Wright (1978) are low at a range of 0 to 0.05, moderate at a range of 0.05 to 0.15, high at a range of 0.15 to 0.25 and when above 0.25 genetic differentiation is considered extremely high. In a study by Mwadzingeni et al. (2017), the expected heterozygosity and genetic differentiation values ranged from 0.07 to 0.29 and 0.31 to 0.89, in that order between different genotypes of bread wheat. While a mean fixation index of 0.79 with values varying from -0.65 to 0.99 were reported by Soriano et al. (2016) in durum wheat accessions, correspondingly the mean expected heterozygosity was 0.71. Revealing moderate to extremely high genetic diversity between wheat genotypes.

The genetic distances between groups of individuals are employed for the generation of an Analysis of Molecular Variance (AMOVA) (Merimans, 2006). Genetic variation is partitioned in accordance with the different hierarchical factors, whereby polymorphic loci which tend to exhibit genetic variation within the population, which is near equivalent to the total population genetic variation may be representative of a population of highly diverse individuals (Merimans, 2006). Genetic variation partitioned between populations, within populations and within individuals was 13%, 69% and 18%, respectively as reported by Soriano et al. (2016). Whilst, Abraha et al. (2016) reported low values between populations genetic variation (3%) than among (35%) and within (69%) the different tef genotypes.

1.9 Overcoming drought phenotyping constraints through use of secondary traits

Since grain yield is characterised by markedly high GxE and poor heritability (Dabholkar, 1999; Sleper and Poehlman, 2006) direct selection for grain yield can be difficult. Lopes et al. (2012) reported grain yield to display significant GxE across 12 mega-environments. The GxE from the aforementioned study were mainly attributed to the variations across the different environments caused by differences in soil temperature and pH at grain-filling stage. Similarly, Abdolshahi et al. (2015) reported yield to exhibit GxE with a relatively poor heritability estimate detected for the yield (0.45) than tiller number (0.90), number of days to heading (0.95), number of days to ripening (0.87), plant height (0.79), grain number (0.72) and thousand grain weight (0.85).

Therefore, Abdolshahi et al. (2015) proposed indirect selection under drought stress and non-stress conditions for secondary traits. Characters which are high throughput, exhibit moderate to high correlations with yield and are relatively simply inherited are candidate secondary traits (Abdolshahi et al., 2015). Araus et al. (2002) further recommended using physiological traits for selection which have a sound correlation with yield. Some of the most utilised indicator traits are presented in Table 1.1.

Table 1.1: Selected physiological and morphological traits for drought tolerance phenotyping in wheat

Physiological and morphological traits	References
Canopy temperature (C^{o})	Lopes and Reynolds (2010); Lopes et al. (2012); Pinto and Reynolds (2015)
Stomatal conductance (mm/m^{2}s)	Araus et al. (2002); Rebetzke et al. (2013)
Plant height (cm)	Nguyen and Blum (2004), El-Rawy and Hassan (2014)
Days to flowering (Zadoks scale: GS50-59)	Lopes et al. (2012); Kosova et al. (2014)
Days to maturity (Zadoks scale: GS90-99)	Farooq et al. (2014)
1000 Seed weight (g)	Merah and Monneveux (2014)

1.9.1 Physiological parameters

The stomatal conductance and canopy temperature serve as indicator traits for genotype photosynthetic capacity as reported by Villegas et al. (2000). Grain-fill in cereals is a vital stage for accumulation of seed weight by either post-anthesis photosynthesis or dry matter accumulated during the vegetative stage which was previously stored in the stem (Tatar et al., 2016). Monitoring of photosynthesis, particularly in the reproductive stage is essential for accumulating economically beneficial yield (Farooq et al., 2014). Both the stomatal conductance and canopy temperature are highly responsive to the surrounding environment and abscisic acid (ABA) is the plant hormone responsible for controlling guard cell activity and canopy transpiration (Blum, 2011).

Factors which influence leaf stomatal conductance and canopy temperature originate either internally or externally according to Rebetzke et al. (2013). The internal factors influencing transpiration and the stomatal opening and closing are the leaf age, plant sink strength, differential requirements for photosynthesis, and stomatal number, and stomata size (Rebetzke et al. (2013). External factors include the leaf position relative to the entire crop as well as the degree of shading on the canopy, carbon dioxide (CO_2) concentration in the atmosphere, light intensity, vapour pressure, air temperature and time of day (Rebetzke et al. (2013).

The stomatal conductance is measured using a hand-held leaf porometer either in the form of a steady state leaf porometer, the null balance, dynamic diffusion porometer or a mass flow device (Pask et al., 2012). Under drought stress, stomatal resistance becomes progressively slow as stomatal openings consequently close (Farooq et al., 2014). Pang et al. (2017) reported the rate of stomatal conductance and transpiration to be retarded simultaneously when available plant soil water was limiting. Thus stomatal conductance may be used as an indicative trait for the detection of the onset of ABA-induced closure of stomata leading to arrested transport of assimilates to developing seed or high concentrations of ABA in the pods causing pod death, as reported in chickpea.

The canopy temperature is also a non-destructive physiological trait for measure of the canopy transpiration according to Pinto and Reynolds (2015). This parameter is measured using an infrared thermometer at midday when water stress is at its most intense in the day (Lopes and

Reynolds, 2010). Pask et al. (2012) reported that low canopy temperature (difference of 1-2 ^{o}C) is favourable over warmer canopies, particularly under water stress conditions. Pinto and Reynolds (2015) reported a cooler canopy in wheat was associated with continued transpiration under drought stress, open stomata and a water source at the deeper soil profile accessed by longer primary roots than drought susceptible genotypes. The warmer canopies, on the other hand, were related to vigorous water use at early growth stages and slow metabolism and poor total biomass and yield accumulation (Rebetzke et al., 2013). The canopy temperature was indicated by Mason and Singh (2014) to have a pleiotropic effect upon the plant height and days to 50% flower. These cases are indicators of source-sink relations at play.

1.9.2 Morphological parameters

Source-sink relations under water deficit

The genetic potential for any crop to produce ultimate yield, under drought stress, is severely hampered (Dorostkar et al., 2015). Source and sink relations of the crop determine the manufacturer organs and receptor organs. Plaut et al. (2004) further indicated the source organs as leafs, stems and leaf sheaths while sink organs as grains. Correspondingly, Blum (2011) confirmed the yield components to constitute the sink of most crop species. Pinto and Reynolds (2015) related the reserved stem assimilate for use by the root under severe drought stress, to reach depths of 0 to 120cm to access soil moisture. Conversely, Biswal and Kohli (2013) reported drought tolerant genotypes to respond to drought events by the increase of stem reserve assimilation as opposed to reliance upon photosynthesis and flag leaf persistence. The organs the culms, flag leaf sheaths and stem internodes were all identified by Biswal and Kohli (2013) to contribute for grain-fill photoassimilates under drought stress, whereas under non-stress the top-most flag leaves were responsible for assimilates for grain-filling.

Plant height

Plant height was recognised by Farooq et al. (2014) and Araus et al. (2002) to be linked with stem reserve storage in the internodes, in preparation for leaf senescence of the drought stressed crop. The height reducing genes (*Rht*) are widely recognised for control of this trait, with the typical stem height for wheat being classified as dwarf, short, semi-dwarf and tall for height ranges of < 50cm, 50 to 70cm, 70 to 120cm and 120cm and taller, in that order by Pask et al. (2012). The antagonistic relationship between the stem height and harvest index (Pask et al., 2012) introduces difficulty in selecting both a high-yielding and drought tolerant germplasm. Hence highly homozygous inbred lines with these complementary traits would be favourable for hybridisation. A similar conflict was noted for drought escape and the total biomass accumulated and consequently yield as reported by Blum (2005). The author reported that genotypes with a propensity of demonstrating drought escape during the reproductive stage tend to be of relatively short stature, with small leaf area and demonstrate limited tillering capabilities.

Flowering time and days to maturity

In some genotypes, the reduced photosynthetic capacity, a limited sink strength and magnitude and poor flag leaf persistence, in some genotypes, leads to the initiation of a short grain filling period under drought stress (Farooq et al., 2014). Tuberosa (2012) recommended flowering period plasticity as one of the valid mechanisms of drought escape as an attempt to complete growth prior to the drought stress season. This is prominent in cereal crops such as wheat and barley (Rebetzke et al., 2013; Kosova et al., 2014). Unfortunately, a yield penalty is paid by early maturing genotypes (Villegas et al., 2000). By virtue of a limited time for which to accumulate dry matter, relatively lower total dry matter is accumulated by early flowering and maturing genotypes, as reported by Dorostkar et al. (2015). Compared to later-maturing genotypes, lesser yield may be accumulated, as discussed by Nguyen and Blum (2004), since the availability of dry matter for partitioning to the filling grains is limited. On the other hand, drought escape was favoured by Fleury et al. (2010) and Kosova et al. (2014) describing this mechanism as a method of maintaining plant water status and integrity, under environments prone to terminal drought, which take place under reproductive stages.

Grain number and grain weight

Drought stress at the grain-filling stage in cereal crops limits the amount of carbohydrates accumulated by the developing grain (Tatar et al., 2016). Correspondingly, Ashraf (2014) related the drop in carbohydrate and sucrose content of grain crops to water deficit events. Tatar et al. (2016) further associated water deficit at stem elongation or anthesis to reduced number of grains per spike and limited seed size. Reduced grain number in wheat, on account of water deficit, may be the result of reduced pollen viability, as previously mentioned, as a consequence of poor early microspore development as a study by Ji et al. (2010) concluded. Plaut et al. (2004) also reported a reduction of the grain number and grains per spike due to water deficit. Similarly, Dorostkar et al. (2015) associated poor seed yield to the lighter thousand seed weight, in grams, from 30.35 g under non-stress to 20.88 g under drought stress in the 2010/2011 season and from 39.37 g under non-stress to 31.91 g under drought stress in the 2011/2012 season.

1.10 Selection indices for drought tolerance

Selection indices are chosen depending on whether indices demonstrate a significantly superior relationship with the grain yield under drought stress or under non-stress or both conditions (Dorostkar et al. 2015). This will allow discrimination of drought tolerant germplasm from drought susceptible germplasm in a population (Dodig et al., 2012; El-Mohsen et al., 2015) and drought selection indices must be convenient to apply in the drought tolerance study (Farshadfar et al., 2013).

Drought tolerance indices presented in Table 1.2 are those utilised in the current study. These indices include drought resistance index (DR) (Lan, 1988), harmonic mean of grain yield (HM) (Jafari et al., 2009), mean productivity (MP) and tolerance index (TOL) (Rosielle and Hamblin, 1981), stress susceptibility index (SSI) (Fischer and Maurer, 1978), stress tolerance index (STI) (Fernandez, 1992), yield index (Gauzzi et al., 1997), yield reduction index (YR) (Golestani-Araghi and Assad, 1998), and yield stability index (YSI) (El-Mohsen et al., 2015). The specific formulae incorporate the yield under water stress (Ys) or optimal moisture environments (Yp) (Dodig et al., 2012). Thus classification of genotypes may be by high yield under both non-stress and water deficit (group A), high grain yield under non-stress only (group B), high grain yield under water stress only (group C) and poor grain yield under both non-stress and water stress (group D) (Fernandez, 1992).

Table 1.2: Drought tolerance selection indices

Indices	Formulae	References
Drought resistance Index, DR	DR = Ys(Ys/Yp)/Xp	Lan (1988)
Harmonic Mean of grain yield, HM	HM = 2(Yp*Ys)/(Yp+Ys)	Jafari et al. (2009)
Mean Productivity, MP	MP = (Yp + Ys)/2	Rosielle and Hamblin (1981)
Stress Susceptibility Index, SSI	SSI = (1-Ys/Yp)/(1-Xs/Xp)	Fischer and Maurer (1978)
Stress Tolerance Index, STI	STI = (Ys*Yp)/ $(Xp)^2$	Fernandez (1992)
Tolerance Index, TOL	TOL = Yp – Ys	Rosielle and Hamblin (1981)
Yield Index, YI	YI = Ys/Xs	Gauzzi et al. (1997)
Yield Reduction, YR	YR = 1 – (Ys/Yp)	Golestani-Araghi and Assad (1998)
Yield Stability Index, YSI	YSI = Ys/Yp	El-Mohsen et al. (2015)

Key: Ys - yield for each genotype under drought stress, Yp - yield for each genotype under non-stress, Xp - yield for all genotypes under non-stress, Xs - yield for all genotypes under drought stress.

1.10.1 Harmonic Mean and Stress Tolerance Indices

Drought tolerance indices TOL, SSI, MP, STI and HM were successful in grouping maize hybrids in accordance with the above-mentioned grouping system in the study by Jafari et al. (2009). Correspondingly, significant correlations were detected between the yield under non-stress with HM and STI with coefficients of 0.80 and 0.88, and under water stress also with HM and STI, with correlation coefficients of 0.96 and 0.90, respectively (Jafari et al., 2009). The significantly high linear relationship between STI and HM reported by Jafari et al. (2009) indicated STI and HM to discriminate drought tolerant accessions. In a study by Dorostkar et al. (2015), the grain yield of diverse wheat genotypes was also significantly correlated with HM and STI under drought stress and non-stress. Dodig et al. (2012) reported a correlation between MP and TOL. Selection for TOL under drought stress led to reduced grain yield under non-stress (Dodig et al., 2012).

1.10.2 Drought Resistance and Yield Indices

The drought stress tolerance indices DR and YI were reported by El-Mohsen et al. (2015) to effectively detect drought tolerant genotypes under both drought stress and non-stress conditions. These deductions were inferred from the significant correlation detected between the indices DR and YI with yield under drought stress and non-stress deficit also (El-Mohsen et al., 2015). The drought selection indices DR and YI were detected with a high factor loading for the principal component designated for drought tolerant genotypes, along with the indices STI, MP, HM and the grain yield under non-drought and drought stress (El-Mohsen et al., 2015).

1.10.3 Stress Susceptibility and Tolerance Indices

An SSI less than 1 indicated drought tolerant genotype as reported by Fischer and Maurer (1978). Landraces tended to display lower values for TOL and SSI compared to improved germplasm, thus were more drought tolerant than modern cultivars (Dodig et al., 2012). Dodig et al. (2012) further discussed SSI must be used under severe drought stress environments. Sio-Se Mardeh et al. (2006) reported high TOL values effectively revealed drought susceptibility of accessions under both drought stress and non-stressed conditions. Essentially such accessions are classified as group D genotypes, as recognised by Jafari et al. (2009) utilising the drought selection indices TOL and SSI. A negative correlation was detected between the grain yield under drought stress for the TOL and SSI with correlation coefficients of -0.51 and -0.329, respectively, by Dorostkar et al. (2015), thus indicating for selection of appropriately drought tolerant wheat genotypes. Low values for TOL and SSI are recommended under drought stress. Moreover, genotypes which also exhibited poor seed yield under drought stress and non-stress, thus belonged to group D, were detected by high TOL and SSI drought selection indices by Dorostkar et al. (2015).

1.10.4 Yield Stability, Yield Reduction Indices and Mean Productivity

A notable correlation was detected between the drought selection index YSI with the grain yield under both non-stress and water stress according to El-Rawy and Hassan (2014). An arguably poor correlation between the seed yield under drought stress and non-stress was detected by El-Mohsen et al. (2015) for the YSI, YR, SSI and TOL. On the other hand, Hohls (2001) reported selecting for MP under drought stress, however, would not lead to limited yield either under drought stress nor non-stress. Dorostkar et al. (2015) reported a significant correlation between MP and the yield under drought stress and non-stress with correlation coefficients of 0.55 and 0.59, respectively.

1.10.5 Ranking of genotypes using selection indices

The rank mean, standard deviation of ranks and rank sum were used for ranking of genotypes by El-Mohsen et al. (2015) and by Farshadfar et al. (2012). Whereby, genotypes with low values for rank mean and rank sum ranked higher, whereas, a low value for standard deviation of rank meant stable rank across different drought tolerance indicators. The standard deviation of ranks and rank sum are given in formula a) and b), respectively.

a) Standard deviation of ranks = $\frac{\sum_{i=1}^{m}(\text{Rij-Ri mean})}{\text{n-1}}$ (El-Mohsen et al., 2015)
b) Rank sum = R*i* mean + standard deviation of ranks (Farshadfar et al., 2012; El-Mohsen et al., 2015)

Where, R*ij* is the in vivo drought tolerance or resistance index rank, R*i* mean is the rank mean across all drought tolerance or resistance indicators for each genotype. These ranking procedures ensure drought tolerant, germplasm are selected without bias by considering a single drought selection indicator (Farshadfar et al., 2012).

1.11 Conclusions and future prospects

Climate change is rapidly transforming the agricultural landscape through crop failures. The increase in regions for wheat production affected by water stress demands breeding for more drought tolerant and adapted wheat cultivars. This is not only to improve farmers' profits by obtaining stable yield under water stress but also to satisfy the need of improved tolerance and adaptation for the milling industry and the entire value chain.

Through use and understanding of integrative mechanisms, which look into the molecular, physiological (Araus et al., 2002; Abdolshahi et al., 2015) and morphological (Farooq et al., 2014) plant responses, yield can be significantly improved even with the negative impacts of drought stress. This is particularly possible by elimination or minimising GxE through multi-environmental trials (Dabholkar, 1999), employing indicator traits well-correlated with the seed yield (Dodig et al., 2012; Lopes et al., 2012; El-Mohsen et al., 2015) and DNA fingerprinting (Henry, 2001; Korzun, 2003).

Genetic characterisation ensures effective determination of heterotic groups and the degree of genetic diversity of a collection of germplasm. This incorporated with phenotypic characterisation by physiological and morphological traits and selection indices improve accuracy and repeatability of selection for drought tolerant parental germplasm under drought stress.

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Chapter 2 Genetic characterisation of selected bread wheat (*Triticum aestivum* L.) genotypes through SSR markers

Abstract

Understanding the genetic relationship and genetic diversity present among germplasm collections improves selection efficiency of parents for breeding and genetic analysis. The objective of this study was to determine the genetic diversity and population structure of 47 diverse bread wheat genotypes introduced from CIMMYT using ten selected polymorphic SSR markers. Leaf samples were collected from test genotypes and sent to INCOTEC Laboratory (Incotech South Africa Pty Ltd, Ashburton Pietermaritzburg Republic of South Africa) for SSR analysis. Data were subjected to analysis for generating a dissimilarity matrix by the Jaccard index for hierarchical clustering by the Neighbour-joining algorithm on DARwin 6.5. The F-statistics, genetic distance (GD), genetic identity (GI), gene flow (Nm) and fixation index (F), Shannon's Information Index (I), analysis of molecular variance (AMOVA) and PIC were assessed employing GenAlex Software. The mean number of detected alleles and effective alleles were 10.9 and 6.31, respectively. The highest PIC value was for the marker XGWM132 with a value of 0.93 whilst mean PIC was 0.80.The frequency of private alleles ranged from 0.036 to 0.289 whereas recorded mean number of private alleles was 10 among the tested populations. Genotypes were grouped into three distinct clusters. SYM2016-037, SYM2016-038, SYM2016-029, SYM2016-010, and SYM2016-012 from Cluster A, SYM2016-044, SYM2016-004, SYM2016-016, SYM2016-019, SYM2016-014, SYM2016-008, SYM2016-006, SYM2016-047 from Cluster B and SYM2016-042 and SYM2016-027 from Cluster C. Clusters A and C consisted of the most genetically distinct genotypes.

Keywords: bread wheat, genetic diversity, population structure, SSRs

2.1 Introduction

Genetic variation allows population adaptation and potential for withstanding biotic and abiotic stress factors (Shah et al., 2009). The genetic diversity of a population is influenced by migration, artificial or natural selection, mutations and genetic drift (Andrews, 2010). These factors determine the resultant genotype and allele frequencies within the population from generation to generation. The Hardy-Weinberg law (Hartl and Andrew, 1997) states that in a large population of randomly mating individuals with negligible evolutionary changes induced by mutations, migrations, artificial or natural selections, and where there is no linkage, the genotype and allele frequencies remain constant from generation to generation in non-overlapping generation species (Falconer and Mackay, 1996; Hartl and Andrew, 1997). The application of this principle also depends on the mode of reproduction as well as the “local population” which is isolated in time and space (Hartl and Andrew, 1997). In wheat, which is predominantly a self-fertilising crop exchange of seed from breeder to breeder as well as farmer to farmer majorly influences the genetic diversity (Mashilo et al., 2016).

The Hardy-Weinberg law is at the core of quantification of genetic diversity and differentiation of population structures in assisting to understand the deviation of the genotype frequencies away from the expected Hardy-Weinberg proportions. Thus, it serves as a “null model” (Hamilton, 2009). The non-identity of the different sub-populations is a robust measure of genic variation (Nei, 1973). Thus, the gene diversity of the total population is the function of both gene diversity within and between the subpopulations. The gene differentiation (FST) is also the same as the FST also referred to as the coefficient of differentiation (Nei, 1973). Nei (1973) reports the quantification of deviation from Hardy-Weinberg equilibrium in the population which is computed using the coefficient of differentiation of the individuals relative to the subpopulation and the coefficient of differentiation of the individuals relative to the meta-population or total population, represented as FIS and FIT, in that order.

Assortative mating or mating due to the close proximity of two or more individuals and self-fertilisation increases homozygosity which may be a property of naturally occurring species (Andrews, 2010). This may result in unequal allele frequencies of particular groups of individuals giving rise to population stratification (Morjan and Rieseberg, 2004). The most extreme procedure for increasing homozygosity would be by way of self-fertilisation, increasing the number of fixed loci. This is represented as a deviation from Hardy-Weinberg expected genotype and allele frequencies as mating is no longer occurring at random

(Hamrick, 1982). Through use of codominant and polymorphic molecular markers, one can draw inferences on the genetic component of a group of diverse germplasm (Henry, 2001).

Simple sequence markers (SSR) markers otherwise known as variable number of tandem repeats (VNTR), due to their repetitive sequences, are co-dominant molecular markers (Henry, 2001). SSR markers flank vital regions valuable for a particular trait (Henry, 2001). Tandem repeats are randomly distributed in the crop genome and their distribution patterns and frequencies can be distinct within and among populations (Henry, 2001; Amelework et al., 2016). Integrations of genetic studies with rigorous, multi-location phenotyping is informative, especially where phenotyping may be influenced by GxE (Chen et al., 2012). Molecular markers are further reliable due to their continued functionality independent of the immediate environments (Mashilo et al., 2016). Furthermore, molecular markers assist in understanding and appreciating the genotype-phenotype relations (Amelework et al., 2016). The hexaploid genome of bread wheat (*Triticum aestivum* L.; 2n=6x=42; AABBDD) can present a constraint to genetic diversity and population structure studies (Wurschum et al., 2013; Mwadzingeni et al., 2015). Selected molecular makers including SSRs with high PIC are effective genomic tools for genetic analysis and genetic differentiation of crop species useful for breeding and systematic conservation (Nielsen et al., 2014). Therefore, the objective of the study was to determine the genetic diversity and population structure of 47 diverse bread wheat genotypes introduced from CIMMYT using ten selected polymorphic SSR markers.

2.2 Materials and methods

2.2.1 Plant materials and study sites

The lines were selected from 100 accessions based on their agronomic performance and adaptation under South African growing conditions. The 47 bread wheat genotypes acquired from CIMMYT which were genotyped in this study are listed in Table 2.1.

Table 2.1: List of 47 wheat genotypes with their pedigrees used in the current study

Genotype	Pedigree
SYM2016-001	1447/PASTOR
SYM2016-002	ALTAR 84/AEGILOPS SQUARROSA (TAUS)//OPATA/3/ATTILA
SYM2016-003	BABAX/3/PRL/SARA//TSI/VEE#5/4/CROC_1/AE.SQUARROSA (224)//2*OPATA
SYM2016-004	BABAX/3/PRL/SARA//TSI/VEE#5/4/WBLL1
SYM2016-005	BAU/KAUZ//PASTOR
SYM2016-006	BUC/MN72253//PASTOR
SYM2016-007	CHIBIA/WEAVER
SYM2016-008	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*FRAME
SYM2016-009	CROC_1/AE.SQUARROSA (213)//PGO/3/NG8319//SHA4/LIRA
SYM2016-010	CROC_1/AE.SQUARROSA (205)//BORL95/3/KENNEDY
SYM2016-011	CROC_1/AE.SQUARROSA (224)//OPATA/3/RAC655
SYM2016-012	CROC_1/AE.SQUARROSA (205)//KAUZ/3/SLVS
SYM2016-013	CROC_1/AE.SQUARROSA (224)//2*OPATA/3/2*RAC655
SYM2016-014	D67.2/P66.270//AE.SQUARROSA (320)/3/CUNNINGHAM
SYM2016-015	HD30/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI
SYM2016-016	JNRB.5/PIFED
SYM2016-018	PASTOR/3/VEE#5//DOVE/BUC
SYM2016-019	SLVS/6/FILIN/IRENA/5/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER
SYM2016-020	SRN/AE.SQUARROSA (358)//MILAN/SHA7
SYM2016-021	SW89.5277/BORL95//SKAUZ
SYM2016-023	SW94.60002/4/KAUZ*2//DOVE/BUC/3/KAUZ/5/SW91-12331
SYM2016-025	TIE CHUAN 1*2/3/HE1/3*CNO79//2*SERI
SYM2016-026	VEE#8//JUP/BJY/3/F3.71/TRM/4/2*WEAVER/5/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/6/WEAVER
SYM2016-027	WORRAKATTA/2*PASTOR
SYM2016-028	LOCAL CHECK
SYM2016-029	CHAM 6
SYM2016-030	KLEIN CHAMACO
SYM2016-031	HIDHAB
SYM2016-032	DHARWAR DRY
SYM2016-033	FRTL/CMH83.2517

Table 2.1: Continued

Genotype	Pedigree
SYM2016-034	SARA/THB//VEE/3/BJY/COC//PRL/BOW
SYM2016-035	PASTOR/FLORKWA.1//PASTOR
SYM2016-036	CHAM6/ATTILA//PASTOR
SYM2016-037	CROC_1/AE.SQUARROSA (224)//OPATA/3/PASTOR/4/PASTOR*2/OPATA
SYM2016-038	CROC_1/AE.SQUARROSA (224)//OPATA/3/ALTAR 84/AEGILOPS SQUARROSA (TAUS)//OPATA/4/PASTOR
SYM2016-039	CROC_1/AE.SQUARROSA (224)//OPATA/3/ALTAR 84/AEGILOPS SQUARROSA (TAUS)//OPATA/4/PASTOR
SYM2016-040	CROC_1/AE.SQUARROSA (224)//OPATA/3/ALTAR 84/AEGILOPS SQUARROSA (TAUS)//OPATA/4/PASTOR
SYM2016-041	ATTILA/PASTOR//PASTOR
SYM2016-042	ATTILA//PGO/SERI/3/PASTOR
SYM2016-043	PASTOR//TODY/BAU/3/PASTOR
SYM2016-044	ALTAR 84/AE.SQ//2*OPATA/3/PIFED
SYM2016-045	KRICHAUFF/2*PASTOR
SYM2016-046	KABY//2*ALUBUC/BAYA
SYM2016-047	ALTAR 84/AEGILOPS SQUARROSA (TAUS)//OCI/3/VEE/MJI//2*TUI
SYM2016-048	ALTAR 84/AEGILOPS SQUARROSA (TAUS)//OCI/3/VEE/MJI//2*TUI
SYM2016-049	ALTAR 84/AEGILOPS SQUARROSA (TAUS)//OCI/3/VEE/MJI//2*TUI
SYM2016-050	MILAN/KAUZ//PRINIA/3/BABAX

2.2.2 DNA extraction, purification and amplification

Genotypes were grown to seedling stage at the University of KwaZulu-Natal (Pietermaritzburg, Republic of South Africa) Controlled Environmental Facility (CEF). Leaf tissue samples were collected for each genotype. Samples were sent for genotyping using SSR markers at the INCOTEC Laboratory (Incotech South Africa Pty Ltd, Ashburton Pietermaritzburg Republic of South Africa).

2.2.3 Polymerase chain reaction and SSR analysis

The test genotypes were distinguished by SSR markers. The 10 SSR markers, with their forward and reverse primers (Table 2.2) were selected based on their high PIC values. These SSR markers are also described in the Grain Genes Database for Triticeae and Avena species (GrainGenes).

Table 2.2: List of 10 wheat SSR markers used in the current study

Markers	Forward primer	Reverse primer	AT(ºC)	PIC	References
WMC177	AGGGCTCTCTTTAATTCTTGCT	GGTCTATCGTAATCCACCTGTA	51	0.94	Somers and Isaac (2004)
WMC78	AGTAAATCCTCCCTTCGGCTTC	AGCTTCTTTGCTAGTCCGTTGC	61	0.93	Roder et al. (1998)
WMS30	ATCTTAGCATAGAAGGGAGTGGG	TTCTGCACCCTGGGTGAT	94	0.92	Roder et al. (1998)
WMS169	ACCACTGCAGAGAACACATACG	GTGCTCTGCTCTAAGTGTGGG	94	0.90	Roder et al. (1998)
WMS304	AGGAAACAGAAATATCGCGG	AGGACTGTGGGGAATGAATG	94	0.91	Roder et al. (1998)
WMC179	CATGGTGGCCATGAGTGGAGGT	CATGATCTTGCGTGTGCGTAGG	61	0.87	Somers and Isaac (2004)
XGWM132	TACCAAATCGAAACACATCAGG	CATATCAAGGTCTCCTTCCCC	94	0.99	Roder et al. (1998)
WMC532	GATACATCAAGATCGTGCCAAA	GGGAGAAATCATTAACGAAGGG	61	0.96	Somers and Isaac (2004)
XGMW484	ACATCGCTCTTCACAAACCC	AGTTCCGGTCATGGCTAGG	94	0.89	Roder et al. (1998)
WMC153	ATGAGGACTCGAAGCTTGGC	CTGAGCTTTTGCGCGTTGAC	61	0.87	Somers and Isaac (2004)

Key: AT-annealing temperature (ºC), PIC-polymorphic information content.

SSRs were utilised in bulked amplification following the procedure of CIMMYT (2005) using the leaf tissue collected. The PCR products were fluorescently labelled and were separated through a capillary electrophoresis method. This was done using an ABI 3130 automatic sequencer (Applied Biosystems, Johannesburg, South Africa) for the pool of 47 genotypes.

2.2.4 Data analysis

Polymorphism and allele diversity analyses

The co-dominant markers were used to analyse the genetic diversity of genotypes. The genetic diversity parameters used were the number of amplified alleles (Na), the number of effective alleles detected (Ne), the allelic richness (Ar) as well as the expected heterozygosity (He). The Shannon's information index (I) was analysed according to Sherwin et al. (2006). Genetic parameters were analysed using GenAlex software (Peakall and Smouse, 2012).

The number of polymorphic loci detected was analysed according to genotypes' parental origin using genotypes' respective pedigrees. Genotypes were grouped into four populations according to their pedigree relationships: Population I were single crosses involving Pastor in their parentage, Population II were genotypes resulted from crosses with *Aegilops squarrosa* as common parent, Population III composed of crosses derived with different parentages and Population IV comprised of a mixture of lines. The marker PIC was calculated using the following formula:

a) $\text{PIC} = 1 - \sum p_{ij}^2 - [(\sum p_{ij}^2)]^2 + \sum[(p_{ij})^2]^2$ using Yasuda (1988) as reported by Desta et al. (2014)

Where, the P_{ij} represents the frequency for the *j*th alleles upon the *i*th locus. Nei's unbiased genetic distance was computed by employing GenAlex software. The genetic distance formula is given as:

b) $\text{GD}=1- \sum_{\mathrm{i-1}}^{\mathrm{n}}(\mathrm{pij})^2$ (Desta et al., 2014)

Merimans (2006) method was employed to generate the FST, genetic differentiation. Also the analysis of molecular variance (AMOVA) was done for establishing total genetic variation partitioning using GenAlex software.

Cluster analysis

For the cluster analysis, a dissimilarity matrix was constructed by the Jaccard index. The presence or absence of a fragment was given by 0 or 1, respectively. This was done in order to generate a binary data matrix. The generated matrix was used to form genetic relationships based on Neighbour-joining algorithm using the unweighted pair group mean arithmetic (UPGMA). Bootstrap analysis was done for accurate node construction whereby the bootstrap value was set at 10 000 bootstrap values. The software utilised for cluster analysis was DARwin 5.0 developed by (Perrier and Jacquemoud-Collet, 2006).

2.3 Results

2.3.1 Polymorphism and allele diversity of the SSR markers

Results generated for the different genetic parameters using the ten SSR markers are shown in Table 2.3. The mean Na value recorded was 10.9. The lowest value of Na detected was 4 for the marker WMC78, whilst the highest was 21 for the marker XGWM132. On the other hand, the Ne values ranged from 2.70 to 14.53 for the markers WMC78 and XGWM132, respectively. The mean value of genetic parameter Ne was 6.31.

The minimum and maximum observed heterozygosity was 0.02 for WMC7 and 1.00 for the markers WMS153, XGWM132, WMS179 and WMS30, respectively. The mean observed heterozygosity detected was 0.5.The expected heterozygosity was quite high, with a mean of 0.81, whilst the lowest and highest values detected were 0.64 for the marker WMC78 and 0.94 for the marker XGWM132. The marker WMS153 revealed an excess of unstable alleles with a FIS value of -0.22. On the contrary, the marker WMC78 was recorded with a high FIS value of 0.96. The mean FIS was 0.41. The PIC value was high for the marker XGWM132 with a value of 0.93. Conversely, the PIC value was the lowest for the marker WMC78 with a value of 0.63. The detected mean PIC revealed a trend of relatively high PIC value with a mean of 0.80. The mean allelic richness detected was 0.29 and these values ranged from a minimum of 0.1 for the marker XGWM132 to a maximum of 0.55 for the marker WMC78. The amplification ranged from 118 to 397 base pair (bp). The markers which had the greatest amplification range were WMS179 and XGWM132 with ranges of 216 to 397 bp and 118 to 159 bp, respectively.

Table 2.3: Genetic parameters generated by the 10 SSR markers employed in the current study

Markers	Genetic parameters							
	Na	Ne	Ho	He	FIS	PIC	Ar	Asr
WMC 177	6.00	3.99	0.04	0.76	0.94	0.75	0.34	199-212
WMC 78	4.00	2.70	0.02	0.64	0.96	0.63	0.55	248-279
WMS 30	12.00	8.93	1.00	0.90	-0.13	0.89	0.16	233-255
WMS 169	13.00	5.49	0.30	0.83	0.64	0.82	0.30	207-245
WMS 304	9.00	3.22	0.15	0.70	0.78	0.69	0.40	216-238
WMS 179	13.00	9.11	1.00	0.90	-0.12	0.89	0.13	216-397
XGWM 132	21.00	14.53	1.00	0.94	-0.07	0.93	0.10	118-159
WMC 532	7.00	4.41	0.04	0.78	0.94	0.77	0.33	176-199
XGWM 484	12.00	5.13	0.47	0.81	0.42	0.80	0.39	164-197
WMS 153	12.00	5.55	1.00	0.83	-0.22	0.82	0.23	155-202
Mean	10.90	6.31	0.50	0.81	0.41	0.80	0.29	-
SE	1.50	1.14	0.14	0.03	0.16	0.03	0.04	-

Key: Na - mean number of alleles per locus per population, Ne - mean number of effective alleles per locus per population, Ho - mean observed gene diversity within genotypes per population, He - mean expected gene diversity within genotypes per population, FIS - genetic differentiation, PIC - polymorphic information content, Ar - allelic richness, Asr - allele size range (base pairs), SE - standard error.

Population parameters

Table 2.4 presents the estimated genetic parameters for the four tested populations. The Na values ranged from 5.9 for Population I to a high of 7.6 for Population III. The mean value recorded for Na was 6.78. The mean Ne value was 4.94 and the lowest and highest values for Ne identified were 4.31 for Population II and 5.59 for Population III, respectively.

On the other hand, values for the Shannon’s information index were the highest and lowest for Populations III and II with values of 1.78 and 1.46, in that order. The mean value recorded for the index was 1.62. The mean values of the Ho and He were 0.50 and 0.78, in that order, indicating a notable difference between the two genetic parameters. The Ho values ranged from 0.47 to 0.54 for Populations IV and II, respectively. Whereas, the lowest and highest values detected for He were 0.72 for Population II and 0.83 for Population III. The minimum and maximum number of private alleles per population recorded was 5 for Population I and 10 for Population III.

Table 2.4: Genetic parameters for the 4 wheat populations

Populations	Genetic parameters						
	N	Na	Ne	I	Ho	He	Pa
Population I	9.00	5.90	4.59	1.53	0.51	0.77	5.00
Population II	12.00	6.20	4.31	1.46	0.54	0.72	8.00
Population III	12.00	7.60	5.59	1.78	0.49	0.83	10.00
Population IV	14.00	7.40	5.28	1.72	0.47	0.80	9.00
Mean	11.50	6.78	4.94	1.62	0.50	0.78	-
SE	0.30	0.47	0.37	0.08	0.07	0.02	-

Key: N - total number of alleles per locus, Na - mean number of alleles per locus per population, Ne - number of effective alleles per locus per population, Ho - mean observed gene diversity within genotypes per population, He - mean expected gene diversity within genotypes per population, I - Shannon's Information Index, Pa - number of private alleles, SE - standard error.

The pairwise comparison of the Nei unbiased genetic distances and genetic identities as well as the genetic differentiation with the gene flow are presented in Table 2.5. The highest and lowest values of the GD were recorded between Populations 3 and 2 with a GD value of 0.31, whereas Populations III and IV had a value of 0.01. Also, a higher GI was recorded between Populations III and IV with a value of 0.99, while a comparatively low GI value was recorded for the pairwise population comparison of Populations II and III with a value of 0.74.

The comparison of populations III and IV revealed a low FIS value of 0.02, whereas the FIS for the comparison of Populations II and III was quite high with a value of 0.07. The latter value between Populations II and III indicated a considerable inbreeding between these populations, however the inverse can be inferred for the former population comparisons using the FIS. The Nm values were the lowest and highest between Populations II and III with a value of 4.7 and Populations III and IV with an Nm value of 12.3, in that order.

Table 2.5: Pairwise estimates of genetic differentiation (top diagonal outside brackets), gene flow (top diagonal within brackets), genetic distance (bottom diagonal outside brackets) and genetic identity (bottom diagonal within brackets) for 4 wheat populations

Populations		FIS (Nm)			
		Pop1	Pop II	Pop III	Pop IV
GD (GI)	Pop I	1	0.06 (4.7)	0.04 (4.8)	0.05 (4.8)
	Pop II	0.21 (0.81)	1	0.07 (4.7)	0.05 (4.8)
	Pop III	0.13 (0.88)	0.31 (0.74)	1	0.02 (12.3)
	Pop IV	0.17 (0.84)	0.18 (0.83)	0.01 (0.99)	1

Key: GD - genetic distance, GI - genetic identity, FIS - genetic differentiation, Nm - gene flow, Pop I - Population I, Pop II - Population II, Pop III - Population III, Pop IV - Population IV.

2.3.2 Cluster analysis

The cluster analysis for the 47 wheat genotypes based on the Jaccard coefficient of dissimilarity is presented in Figure 2.1. The mean genetic distance detected between genotypes was 0.57 which was considerably high. This may indicate a significant genetic variability between the different genotypes. According to Jaccard's coefficient of dissimilarity, the genotypes which were highly dissimilar were SYM2016-037 and SYM2016-002 with a coefficient of 0.86. The next greater genetic distance were noted between SYM2016-037 and SYM2016-029 with a value of 0.85, whilst the closely similar genotypes with a small genetic distance between them were SYM2016-026 and SYM2016-027, SYM2016-010 and SYM2016-009 as well as SYM2016-002 and SYM2016-029 all with dissimilarity value of 0.02.

The genotypes were clustered into three major groups: A, B and C indicated in black, blue and red branched lines, respectively (Figure 2.1). These subgroups were closely related as indicated by the proximity of clustering of the subgroups. Cluster A had 21 genotypes, which was the greatest number of genotypes between subgroups. Cluster A consisted of the following genotypes: SYM2016-043, SYM2016-005, SYM2016-037, SYM2016-023, SYM2016-038, SYM2016-040, SYM2016-049, SYM2016-029, SYM2016-025, SYM2016-032, SYM2016-010, SYM2016-050, SYM2016-031, SYM2016-002, SYM2016-012, SYM2016-011, SYM2016-020, SYM2016-009, SYM2016-030, SYM2016-046 and SYM2016-018. This subgroup, was further subdivided into 3 sub-groups.

Cluster B was the next bigger group consisting of 19 genotypes. This Cluster comprised of SYM2016-044, SYM2016-004, SYM2016-028, SYM2016-016, SYM2016-019, SYM2016-015, SYM2016-035, SYM2016-014, SYM2016-036, SYM2016-008, SYM2016-006, SYM2016-033, SYM2016-007, SYM2016-021, SYM2016-013, SYM2016-034, SYM2016-048, SYM2016-047 as well as SYM2016-039. Cluster B was further subdivided into 4 sub-groups.

Finally, Cluster C was the last group that contained 7 genotypes only. The genotypes allocated in Cluster C were SYM2016-042, SYM2016-045, SYM2016-041, SYM2016-003, SYM2016-001, SYM2016-026 and SYM2016-027. Only two sub-groups were contained within Cluster C.

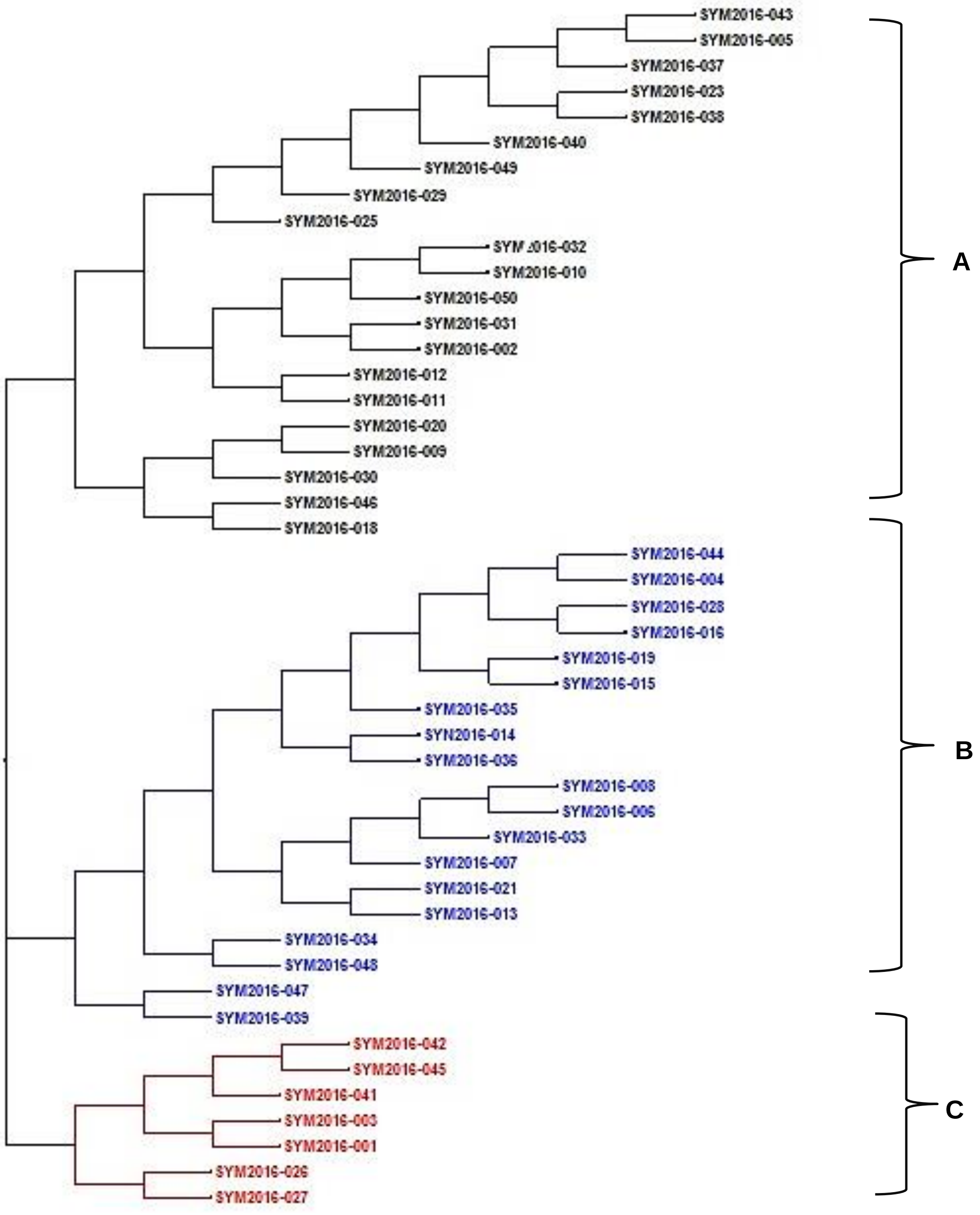

Figure 2.1: Unweighted pair group mean arithmetic dendrogram depicting 47 wheat genotypes based on Jaccard's coefficient of dissimilarity

2.3.3 Analysis of molecular variance (AMOVA)

The results of the analysis of molecular variance for the 4 populations based on parentage is presented in Table 2.6. Significant differences were detected for genetic variation allocated within individuals, across different individuals and populations ($P < 0.001$). A considerable percentage genetic variation was assigned to the genetic variation within the different genotypes, which accounted for 60% of the percentage genetic variation. Percentage genetic variation across the different individuals and 4 populations was 37% and 3%, respectively. Thus, a relatively low percentage of genetic variation was attributable to dissimilarities between the different populations, hence the clustering pattern of the 47 genotypes, as previously observed.

Table 2.6: Results of the analysis of molecular variance of the 4 populations of wheat genotypes

Sources of variation	df	SS	MS	Estimated Variation	Percentage variance	Significance levels
Among populations	3	24.106	8.035	0.106	3%	0.001
Among individuals	43	239.319	5.566	1.533	37%	0.001
Within individuals	47	117.500	2.500	2.500	60%	0.001
Total	93	380.926		4.139	100%	

Key: df - degrees of freedom, SS - sum of squares, MS - mean squares.

2.4 Discussion

2.4.1 Selected accessions based on cluster analysis

This study allowed selection of 15 genetically dissimilar wheat genotypes from a pool of 47 diverse wheat genotypes, which were genotyped using ten polymorphic SSR markers. The genotypes selected were SYM2016-037, SYM2016-038, SYM2016-029, SYM2016-010, and SYM2016-012 from Cluster A, SYM2016-044, SYM2016-004, SYM2016-016, SYM2016-019, SYM2016-014, SYM2016-008, SYM2016-006, and SYM2016-047 from Cluster B and SYM2016-042 and SYM2016-027 from Cluster C. Clusters A and C were considered to be highly genetically distinct due to their greater phylogenetic distances based on the hierarchical cluster analysis.

2.4.2 Genic distance and genetic diversity

Across populations, the mean number of realised alleles was 6.78 and values ranged from 5.9 to 7.6 (Table 2.4). The mean number of effective alleles was 4.94 and values ranged from 4.31 to 5.59. Highest and lowest genetic differentiation values were 0.02 and 0.07 among the sub-populations, in that order. Negative genetic differentiation values indicated an excess of heterozygotes detected for the loci WMS153, WMS30, WMS179 and XGWM132 with values of -0.22; -0.13; -0.12 and -0.07, in that order (Table 2.3). Standardised values of the fixation index, as reported by Wright (1978), are noted as negligible for values ranging from 0–0.005, moderate for values ranging from 0.05–0.15, great for values 0.15–0.25 and values exceeding 0.25 are considered to express populations with large genetic differentiations.

Morjan and Rieseberg (2004) described how values of gene flow may indicate the degree to which genetic material is divergent between populations. Values of gene flow which are below a unit are considered low, values equivalent to a unit are considered moderate gene flow, whereas values exceeding a unit are excessively high. The recorded gene flow, in the current study, ranged from a low of 4.7 to a high of 12.3. This indicated an excessive exchange of genes between the different wheat populations.

The mean Shannon's information index was 1.62 among the different wheat populations (Table 2.4). Populations expressing high number of private alleles reveal higher genetic diversity of the population among 47 wheat genotypes. According to population distribution, the highest mean number of private alleles expressed was 10 for Population III. Similarly a mean value of 10 private alleles were realised in a genetic structure study of 172 landraces and 20 modern cultivars of durum wheat genotyped by 44 SSR markers (Soriano et al., 2016). According to Nielsen et al. (2014), factors such as the density of markers per chromosome, marker clustering and the presence and distribution of private alleles per locus can have an effect on the allelic richness. The presence of private alleles in the current study further reveals the large degree of heterozygous loci (Andrews, 2010). Population III consisted of the highest proportion of private alleles which makes this sub-group genetically dissimilar. These can be individuals which may have undergone rare mutations to develop distinct alleles. These private alleles distinguish the mutant individuals from pool of genotypes in the meta-population. Correspondingly, Soriano et al. (2016) ascribed the genic diversity to the presence of private alleles at the different loci in a study of durum wheat.

The genetic distances between the four populations ranged from 0.01 to 0.31. The values reported by Desta et al. (2014) were markedly higher ranging from 0.01 to 0.89 and having a mean value of 0.66. This reveals a fairly low genetic diversity among the different wheat populations used in this study. The population stratification can be brought about by geographical isolation of a group of individuals, artificial and natural selection as well as genetic drift (Nielsen et al., 2014). Linkage disequilibrium can result as a consequence of the uneven frequency of alleles within the different groups of genotypes as noted by Soriano et al. (2016). There is need for a further investigation of linkage disequilibrium.

The genic identity between the different wheat genotypes had minimum and maximum values of 0.74 and 0.99 indicating high similarity between the different populations (Table 2.5). The commonly shared parents among the different genotypes which were Pastor, Altar 84, *Aegilops squarrosa* and Pifed may have also contributed to limited genetic variability between the sub-populations. The populations which were most genetically dissimilar were Populations II and III. Populations II and III expressed the highest genetic distance and the lowest genetic identity. Thus, Populations II and III were considered to be potentially distinct. These can house potential candidates to utilise as crossing parents. On the contrary, the populations which expressed a higher degree of exchange of genetic material were the Populations III and IV. Populations III and IV expressed the lowest genetic distances and the highest value for the genetic identity.

2.4.3 Loci polymorphism and effectiveness for genotype discrimination

The highest PIC detected was for the marker XGWM132 with a value of 0.93 and mean value of 0.80 (Table 2.3). In a genetic diversity study of bottle gourd, a highly diverse horticultural crop, the mean PIC was reported to be above 0.5 for the 9 molecular markers in the study conducted by Mashilo et al. (2016). On the other hand, the mean PIC value reported by Desta et al. (2014) in Eritrean wheat accessions was a high of 0.63. The value obtained in the current study was markedly higher than the latter study. This suggests the proficiency of the selected molecular markers to uniquely distinguish the different genotypes. Although the value obtained in the current study was comparatively lower than that reported for the molecular markers WMC262 located on chromosome 4A, WMC44, situated on chromosome 1B and GWM174 which is located on chromosome 5D had PIC values of 0.96; 0.954 and 0.948, respectively (Tascioglu et al., 2016). On the contrary, the PIC values reported by Nielsen et al. (2014) were comparatively lower than that of the current study. Values reported by Nielsen et al. (2014)

ranged from 0.16 and 0.38 for the genotyping of modern cultivars and landraces, with a mean value of 0.30.

2.5 Conclusions

Fifteen wheat genotypes were selected from the pool of 47 diverse wheat genotypes which were genotyped using 10 polymorphic SSR markers. These best accounted for the genetic variability among the three different genetic clusters. The genotypes selected were SYM2016-037, SYM2016-038, SYM2016-029, SYM2016-010, and SYM2016-012 from Cluster A, SYM2016-044, SYM2016-004, SYM2016-016, SYM2016-019, SYM2016-014, SYM2016-008, SYM2016-006, and SYM2016-047 from Cluster B and SYM2016-042 and SYM2016-027 from Cluster C.

The molecular markers employed exhibited high PIC values, therefore the SSR markers were effective in discriminating of the different genotypes. The majority of genetic diversity and population stratification was due to great proportion of private alleles in the loci of the genotypes grouped under the different populations. Moreover, high heterozygosity within the individual genotypes contributed immensely to the genetic diversity observed in the study. Hence it can be concluded that the tested accessions are still unstable, thus have high potential for segregation. The reserved differences between populations can be related to genotypes being introduced from the same source. Populations III and II were also considered to be distinct heterotic groups. This was mainly due to their great genetic dissimilarity which may be favourable for selection of parents for crosses.

It is recommended that a greater number of markers be utilised for the large, hexaploid genome of bread wheat. Alternatively, a different molecular marker type may be incorporated in conjunction with SSR markers to further quantify the genetic diversity and understand the genetic structure of the bread wheat accessions.

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Chapter 3 Phenotyping bread wheat (*Triticum aestivum* L.) genotypes for drought tolerance

Abstract

Drought stress occurring during the reproductive stage can result in reduced yield or complete crop failure. Therefore, development of well-characterised drought tolerant germplasm is needed for effective breeding. The objective of this study was to characterise 15 bread wheat genotypes introduced from CIMMYT using physiological and morphological traits. Genotypes were evaluated using a randomised complete block design with 3 replications under two test environments (field and greenhouse conditions) in 2016. Drought stress was imposed as follows: 1 week before 50% flowering (WBH) and 1 week after 50% flowering (WAH). Drought stress was maintained for a period of 24 and 25 days in the greenhouse and field, in that order. A fully-irrigated water regime (NS, non-stress stress) was used as a comparative control. Data collected included stomatal conductance (SC), canopy temperature (CT), number of days to 50% flowering (DF), number of days to 50% maturity (DM), plant height (PH), number of productive tillers (PT) per plant, number of spikelets per spike (SS), number of grains per spike (KS), 100 seed weight (SW) and grain yield/plot (GY). Data were subjected to a two-sample independent t-test, combined analysis of variance (ANOVA), Pearson correlation coefficient and principal component and cluster analyses. Significant differences were detected for the genotype by test environment interaction for CT, DF, DM, PH, SS, KS, SW and GY. Significant positive correlations were detected for DF, DM, PH, PT, SS, KS and SW with the GY across test environments. Results showed that yield penalty occurred for early maturing genotypes relative to late-flowering and maturing genotypes. The genotypes SYM2016-027, SYM2016-014 and SYM2016-029 were relatively drought tolerant. Early flowering and maturity as well as short plant height and low canopy temperature under short term drought stress in the greenhouse and field conditions were favourable traits for selection. Hybridisation and fixation of resultant lines is essential to develop early maturing genotypes with stable and high yield potential.

Keywords: Bread wheat, drought tolerance, GxE, phenology, yield penalty

3.1 Introduction

Bread wheat (*Triticum aestivum* L.) is an economically valuable crop globally cultivated under warm and cool season conditions dependent upon the type of cultivar (Pena, 2002; Acquaah, 2007). It is a naturally self-pollinating C3 crop belonging to the family Gramineae (Sleper and Poehlman, 2006). Extensive research and development of wheat is undertaken by the International Centre for Maize and Wheat Improvement (CIMMYT) and International Centre for Agricultural Research in the Dry Areas (ICARDA) and various national wheat breeding programmes (Sleper and Poehlman, 2006). South Africa is the leading wheat producer in sub-Saharan Africa (ARC, 2017). One of the main constraints to wheat production nationally is climate variability, in particular drought stress (Edossa et al., 2014).

High temperatures and limited soil moisture caused by global climate change result in lower bread wheat yields (FAO, 2013; FAO, 2018). The growth stage of a crop and drought intensity determine the magnitude of yield losses associated with drought stress (Fleury et al., 2010). Globally, both tropical and sub-tropical regions are vulnerable to drought stress (IPCC, 2014). More of the developing countries are located in these regions, where drought impacts agricultural production and productivity thus leading to food and job insecurity (Mir et al., 2012; FAO, 2016). An unpredictable climatic pattern is the overriding challenge faced by wheat producers in South Africa (DAFF (2010). Wheat production using irrigation water is expensive and unsustainable. Breeding for drought adapted and drought tolerant wheat cultivars is one of the most important approaches to drought management. This requires rigorous assessment and selection of promising drought tolerant wheat genotypes from a pool of germplasm collections.

Grain yield is an economic trait for agricultural production (FAO, 2015). To achieve stable and relatively high yields under drought stress and non-stressed conditions, yield and yield-related traits should be assessed and their correlations determined for reliable selection (Lopes et al., 2012; Mir et al., 2012). The use of yield components to curb the “phenotype-genotype gap” is a method to indirectly select for yield under drought stress or fully irrigated test environments (Abdolshahi et al., 2015; Walter et al., 2015). Physiological and morphological traits can be integrated for effective phenotyping for better yield expression under drought (Araus et al., 2002; Lopes et al., 2012). Therefore, the objectives of this study was to characterise 15 bread wheat genotypes introduced from CIMMYT using physiological and morphological traits to select unique genotypes for breeding or direct production.

3.2 Materials and methods

3.2.1 Plant material and study sites

The study evaluated 15 wheat genotypes acquired from CIMMYT's heat and drought nurseries (Table 3.1). From a pool of 47 genotypes, 15 bread wheat genotypes were selected to account for the genetic variation based on a result of genetic characterisation using SSR markers from the previous chapter (Chapter 2).

Table 3.1: List of bread wheat genotypes evaluated in this study and their pedigrees

Genotype	Pedigrees
SYM2016-004	BABAX/3/PRL/SARA//TSI/VEE#5/4/WBLL1
SYM2016-006	BUC/MN72253//PASTOR
SYM2016-008	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*FRAME
SYM2016-010	CROC_1/AE.SQUARROSA (205)//BORL95/3/KENNEDY
SYM2016-012	CROC_1/AE.SQUARROSA (205)//KAUZ/3/SLVS
SYM2016-014	D67.2/P66.270//AE.SQUARROSA (320)/3/CUNNINGHAM
SYM2016-016	JNRB.5/PIFED
SYM2016-019	SLVS/6/FILIN/IRENA/5/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER
SYM2016-027	WORRAKATTA/2*PASTOR
SYM2016-029	CHAM 6
SYM2016-037	CROC_1/AE.SQUARROSA (224)//OPATA/3/PASTOR/4/PASTOR*2/OPATA
SYM2016-038	CROC_1/AE.SQUARROSA (224)//OPATA/3/ALTAR 84/AEGILOPS SQUARROSA (TAUS)//OPATA/4/PASTOR
SYM2016-042	PASTOR//TODY/BAU/3/PASTOR
SYM2016-044	ALTAR 84/AE.SQ//2*OPATA/3/PIFED
SYM2016-047	ALTAR 84/AEGILOPS SQUARROSA (TAUS)//OCI/3/VEE/MJI//2*TUI

Genotypes were evaluated under greenhouse and field conditions. Both studies were undertaken in 2016 at the University of KwaZulu-Natal (UKZN), Pietermaritzburg Campus (29° 40' S; 30° 24' E and 806 m above sea level) in South Africa. Weather data was accessed from the UKZN Centre for Water Resources Research in collaboration with the Agricultural Research Council Institute of Soil, Climate and Water. Table 3.2 presents the total rainfall received during the study period (June to October 2016) was 211.59 mm and the mean minimum and maximum temperatures were 10.78°C and 22.96°C, in that order.

Table 3.2: Weather data for Ukulinga Research Farm from June to October 2016

Month	Tx (°C)	Tn (°C)	RHx (%)	RHn (%)	Rain (mm)	Rs (MJ/ m^2)	U2 (ms)	ET0 (mm)
Jun	22.96	10.48	91.74	51.12	2.54	10.47	1.23	57.21
Jul	20.72	8.62	91.00	41.22	58.17	10.85	1.19	61.38
Aug	24.56	10.62	80.38	26.90	42.93	14.32	1.33	103.37
Sept	23.50	11.91	99.50	46.13	44.45	13.12	1.35	80.08
Oct	23.09	12.27	99.72	51.81	66.04	14.68	1.35	87.45

Key: Jun - June, Jul - July, Aug - August, Sept - September, Oct - October, Tx - average maximum temperature, Tn - average minimum temperature, RHx - average maximum relative humidity, Rain - average total rainfall, RHn - average minimum relative humidity, Rs - average total radiation, U2- average wind speed (calculated from hourly data), ET0 - average total relative evapotranspiration (calculated from hourly data).

3.2.2 Experimental design and crop establishment

A randomized complete block design (RCBD) with 3 replications was used for this study. Drought stress was imposed as follows: 1 week before 50% flowering (WBH) and 1 week after 50% flowering (WAH). The total number of days of drought stress were 25 and 24 for greenhouse and field conditions, respectively. A fully-irrigated water regime was used as a comparative control (non-stress, NS). Soil moisture was monitored using Irrometer Moisture Indicator (Irrometer Riverside Company, California, and United States of America).

Seeds were directly sown. For greenhouse trial, genotypes were established in 5L capacity plastic pots filled using a sterilised pine bark growth media under drip irrigation. Under field conditions genotypes were sown in a clay loam soil using a custom-made plastic mulch rainout system, with a sub-surface drip irrigation system (Figure 3.1). The plot length was 1.5 m with intra-row and inter-row spacing of 15 cm and 45 cm, respectively. Hand weeding was practised and cutworm and aphid infestation were controlled using Karate, an insecticide. Powdery mildew infection was controlled using TILT foliar fungicide spray.

Figure 3.1 The rainout mulching system used for field evluation of wheat geneotypes

3.2.3 Data collection and data analysis

Physiological traits

Canopy temperature (CT) was measured as an important physiological variable. CT was measured using the Major Tech High-Temperature Infrared Thermometer (MT694) and values expressed in degrees Celsius (^{o}C). All CT measurements were taken from 09:00 am to 12:00 pm. Ten and 17 days into drought stress, CT was measured for NS, WBH and WAH under greenhouse and field evaluations. Measurements were taken by pointing the infrared thermometer toward the canopy, the trigger was pressed turning on the red guidance light. The position was held until a stable average reading was displayed. The procedure was done twice for each experimental unit.

Stomatal conductance (SC) was measured using the Decagon Devices Steady State Leaf Porometer. The adaxial or upper leaf surface was read. The unit of conductance was in millimoles per meter square per seconds (m.mol/m^{2}s). The SC under greenhouse condition was measured at 10 days after drought for WBH and NS from 11:00 am to 13:00 pm, while under field conditions SC was measured at 17 days into drought stress for NS, WBH and WAH, from 12:00 pm to 13:30 pm. Measurements were taken on sunny, cloudless and windless days.

Morphological traits

Morphological traits assessed were the number of days to flowering (DF), the number of days to maturity (DM), plant height in centimetres (PH), the number of productive tillers (PT) per plant, the number of spikelets per spike (SS), the number of grains per spike (KS), the 100 seed weight in grams (SW) and the yield per plot in grams per plot (GY). DF was recorded from sowing date to the day 50% of genotypes' heads were exposed (Growth Stage 59 [GS59]). This was in accordance to the Zadok growth stages (Zadoks et al., 1973). DM was recorded from the sowing date to the day 50% of plant populations displayed the complete loss of green colour of the spikes and halfway of peduncle. Five plants were sampled and tagged to collect the PH at physiological maturity (GS94) from the soil surface to the top of the ear.

The PT was recorded by counting the number of tillers of each genotype for each water regime displaying spikes with seed after maturity for all 3 replications. Five spikes were randomly sampled, tagged and used for counting SS and KS. Thereafter, 100 randomly sampled seeds were counted and weighed on a weighing scale for the SW measured in grams (g). The GY was recorded as the product of the number of productive tillers produced, the individual seed weight in grams, the number of grains obtained per spike and the number of plants per plot. The GY was measured in grams per plot.

Statistical analysis of physiological and morphological traits

A two-sample t-test was used for analysis of SC data collected under the greenhouse condition using GenStat statistical software (Payne et al., 2015) at 95% confidence limit. The physiological and morphological data were subjected to separate and combined analysis of variance (ANOVA) and significant tests were done at the 5% probability level using GenStat. Duncan's multiple range test (MRT) and least significant differences (l.s.d.) was used to compare means at the 5% probability level. Data was also subjected to the two-tailed Pearson correlation coefficient (r) analysis, at a 5% probability level. Principal component (PC) analysis was also performed for greenhouse and field trials separately. PCs with eigenvalues equal to or greater than 1 were selected according to Jeffers (1967).

3.3 Results

3.3.1 Significance tests

Evaluation of genotypes using physiological traits

Table 3.3 presents the mean square and significance tests of genotypes evaluated under 3 different water regimes under field condition. The t-test analysis detected no significant differences among genotypes for SC evaluated under NS and WBH under greenhouse condition. Suggesting SC measured under greenhouse condition did not sufficiently distinguish genotypes for drought tolerance. Significant differences were detected between water regimes for the SC measured under field condition only. This suggested SC was not affected by genotypes and genotype by water regime interaction.

Table 3.3: Mean square and signficant tests for stomatal conductance of genotypes evaluated under 3 water regimes under field condition

Source of variation	df	Mean square
Replications	1	29723
Water regimes (W)	2	428899 ***
Genotypes (G)	14	17940 ^{ns}
G x W	26	15077 ^{ns}
Residual	39	12893
Total	82	

Key: df - degrees of freedom, *** - significant differences at $P < 0.001$ probability level, ns - non-significant differences detected.

Table 3.4 presents the mean squares and significant tests of the CT for genotypes evaluated under 3 water regimes under greenhouse and field conditions. Genotype, water regime and environment effects and their interactions were assessed in a combined ANOVA for the CT measured after 10 and 17 days of drought stress. After 10 days, only differences across water regimes were significant ($P < 0.05$), whereas after 17 days significant differences ($P < 0.001$) were recorded for genotypes, water regimes and test environments and their interactions. Hence, CT measurements taken 17 days after drought stress were valuable.

Table 3.4: Mean square and significant tests for genotypes using the canopy temperature evaluated under 3 water regimes under greenhouse and field conditions

Source of variation	df	Mean square for CT1	Mean square for CT2
Replications	1.00	27.78	8.56
Genotypes (G)	14.00	1.97 ^{ns}	5.56 ***
Water regimes (W)	2.00	16.11**	53.11 ***
Environments (E)	1.00	4817.96 ***	823.16 ***
G X W	28.00	1.78 ^{ns}	4.83 ***
G x E	14.00	4.75 ^{ns}	7.83 ***
W x E	2.00	5.33 ^{ns}	60.53 ***
G x W x E	28.00	2.64 ^{ns}	4.48 ***
Residual	85.00	3.00	1.24
Total	175.00		

Key: df - degrees of freedom, CT1 and CT2 - canopy temperature in ^{o}C measured 10 and 17 days after stress was imposed, ns - non-significant differences detected, * - significant differences at $P < 0.05$ probability level, ** - significant differences at $P < 0.01$, *** - significant differences at $P < 0.001$ probability level.

Evaluation of wheat genotypes using morphological traits

Table 3.5 presents the mean squares and significant tests for genotypes using 8 morphological traits under 3 water regimes under greenhouse and field conditions. Significant differences were detected for the genotypes ($P < 0.001$) for DF, DM, PH, SS, KS and PT ($P < 0.05$). Moreover, significant differences were recorded ($P < 0.001$) for PH only. Moreover, significant differences were detected for the genotype by test environment interaction effect ($P < 0.001$) for DF, DM, PH, SS, KS, SW ($P < 0.01$) and GY ($P < 0.05$). Hence differences across test conditions were valuable.

Table 3.5: Mean square and significant tests for genotypes using 8 morphological traits across test environments

Sources of variation	df	DF	DM	PH	PT	SS	KS	SW	GY
Replications	2.0	23.4	9.2	128.6	1668.3	0.8	212.2	0.3	0.7
Genotypes (G)	14.0	837.6 ***	892.7 ***	1068.2 ***	425.9 *	15.9 ***	297.6 ***	5.7 ***	0.2 ^{ns}
Water regimes (W)	2.0	12.0 ^{ns}	912.5 ***	119.6 ^{ns}	2403.1 ***	2.6 ^{ns}	368.1 **	2.1 ^{ns}	1.1 ***
Environments (E)	1.0	30818.7 ***	31.7 ^{ns}	3818.9 ***	18831.2 ***	1451.8 ***	15156.3 ***	0.5 ^{ns}	12.2 ***
G x W	28.0	10.8 ^{ns}	43.0 ^{ns}	39.0 ***	197.8 ^{ns}	1.9 ^{ns}	39.0 ^{ns}	0.9^{ns}	0.1 ^{ns}
G x E	14.0	398.5 ***	198.2 ***	374.6 ***	243.3 ^{ns}	11.4 ***	273.1 ***	2.9 **	0.2 *
W x E	2.0	65.9 **	915.53***	425.6 ***	203.2 ^{ns}	7.1 ^{ns}	78.0 ^{ns}	1.7 ^{ns}	0.4 *
G x W x E	27.0	13.2 ^{ns}	39.6 ^{ns}	63.1 ^{ns}	169.3 ^{ns}	2.0 ^{ns}	66.6 ^{ns}	0.9 ^{ns}	0.1 ^{ns}
Residual	170.0	10.5	38.6	42.8	220.9	2.8	56.5	1.2	0.1
Total	260.0								

Key: df - degrees of freedom, DF - number of days to 50% flowering, DM - number of days to 50% maturity, PH - plant height in centimetres, PT - number of the productive tillers, SS - number of spikelets per spike, KS - number of grains per spike, SW - 100 seed weight in grams, GY - grain yield per plot in grams per plot, ns - non-significant differences were detected, * - significant differences at $P < 0.05$ probability level, **- significant differences at $P < 0.01$, ***-significant differences at $P < 0.001$ probability level.

3.3.2 Mean response of genotypes assessed using physiological traits

Performance of genotypes using the stomatal conductance assessed across greenhouse and field test conditions

Environmental conditions may have contributed to SC and CT readings. During September 2016, total rainfall received was 44.45mm, mean relative evapotranspiration was 80.08mm and mean minimum and maximum temperatures were 11.91 ^{o}C and 23.50 ^{o}C.

Table 3.6 presents mean values for SC under greenhouse and field conditions. The mean value for SC was 301.3 m.mol/m^{2}s while minimum and maximum SC values were 21.5 m.mol/m^{2}s to 1053 m.mol/m^{2}s, in that order. On the other hand, mean SC was 184mmo/m^{2}s under field condition and minimum and maximum values were 24.90 m.mol/m^{2}s to 668.40 m.mol/m^{2}s, respectively. The lowest SC value was recorded for the genotype SYM2016-029 (178 m.mol/m^{2}s), while the highest was recoded for SYM2016-014 with 358 m.mol/m^{2}s.

Table 3.6: Mean stomatal conductance values of genotypes evaluated under 3 water regimes under greenhouse and field conditions

Genotypes	Greenhouse	Field
SYM2016-004	546.7	250.6
SYM2016-006	228.0	339.6
SYM2016-008	326.4	238.5
SYM2016-010	163.0	197.7
SYM2016-012	125.6	239.4
SYM2016-014	368.0	357.5
SYM2016-016	244.1	326.2
SYM2016-019	322.5	313.3
SYM2016-027	445.5	267.1
SYM2016-029	297.2	177.6
SYM2016-037	255.3	300.6
SYM2016-038	247.6	272.6
SYM2016-042	356.3	348.9
SYM2016-044	389.7	306.0
SYM2016-047	203.7	320.9
Mean	301.3	283.8
CV (%)	13.2	9.1
l.s.d. (P < 0.05)	184	132.6

Key: CV (%) - coefficient of variation expressed as a percentage, l.s.d.- least significant difference at the P < 0.05 probability level.

Performance of genotypes using the canopy temperature based on genotype x test environment interaction

For the CT measured 10 days after drought stress, CT mean value was 22.70 °C and minimum and maximum values were 22.05 °C and 23.5 °C for SYM2016-027 and SYM2016-044, respectively. Conversely, when CT was measured 17 days after drought stress, the mean CT value measured was 19.43°C, whilst minimum and maximum values were18.23 °C and 20.63 °C for SYM2016-044 and SYM2016-019, in that order. Table 3.7 presents mean CT values for wheat genotypes evaluated across 2 test conditions. Genotypes SYM2016-004 and SYM2016-047 with 16 °C and 16.08 °C, in that order. These were relatively lower CT mean values when measured 17 days after drought stress under the greenhouse condition. However, under field condition, lowest recorded CT values were 19.13 °C and 19.63 °C for SYM2016-044 and SYM2016-042, respectively.

Table 3.7: Mean canopy temperature values for genotypes evaluated under 3 water regimes under greenhouse and field conditions

Genotypes	Greenhouse		Field	
	CT1	CT2	CT1	CT2
SYM2016-004	16.78 ^{ab}	16.00 ^{a}	28.27 ^{d}	23.31 ^{n}
SYM2016-006	16.53 ^{a}	16.53 ^{abc}	27.78 ^{d}	21.81 ^{jklm}
SYM2016-008	17.13 ^{ab}	16.22 ^{ab}	27.68 ^{d}	23.09 ^{mn}
SYM2016-010	17.34 ^{abc}	18.09 ^{defg}	28.63 ^{d}	22.80 ^{lmn}
SYM2016-012	18.36 ^{abc}	16.73 ^{abcd}	27.17 ^{d}	20.59 ^{ij}
SYM2016-014	17.81 ^{abc}	17.21 ^{abcde}	27.56 ^{d}	20.82 ^{ij}
SYM2016-016	17.55 ^{abc}	16.64 ^{abcd}	26.88 ^{d}	20.99 ^{ijk}
SYM2016-019	17.97 ^{abc}	18.84 ^{fgh}	27.97 ^{d}	22.42 ^{klmn}
SYM2016-027	16.34 ^{a}	17.40 ^{abcdef}	27.77 ^{d}	21.38 ^{jkl}
SYM2016-029	17.42 ^{abc}	17.56 ^{bcdef}	28.45 ^{d}	22.32 ^{klmn}
SYM2016-037	17.62 ^{abc}	18.57 ^{efgh}	28.35 ^{d}	21.43 ^{jkl}
SYM2016-038	16.55 ^{a}	17.83 ^{cdefg}	28.46 ^{d}	22.03 ^{jklmn}
SYM2016-042	19.05 ^{bc}	18.22 ^{efgh}	27.38 ^{d}	19.63 ^{hi}
SYM2016-044	19.53 ^{c}	17.33 ^{abcde}	27.46 ^{d}	19.13 ^{gh}
SYM2016-047	16.97 ^{ab}	16.21 ^{ab}	28.36 ^{d}	21.78 ^{jklm}
Mean	17.53	17.29	27.88	21.57
CV (%)	2.40	1.60	2.40	1.60
l.s.d. ($P < 0.05$)	1.99	1.28	1.99	1.28

Key: CT1 and CT2 - canopy temperature in °C measured 10 and 17 days after stress was imposed, CV (%) - coefficient of variation expressed as a percentage, l.s.d.- least significant difference at the $P < 0.05$ probability level.

Means in a column followed by the same letter are not significantly different at the 5% probability level.

Performance of genotypes using the canopy temperature based on genotype x water regime interaction

Table 3.8 summarises the mean CT for genotypes measured 17 days after across 3 water regimes. Genotypes which expressed low CT under NS were SYM2016-042 and SYM2016-014 with values of 16.475 °C and 16.663 °C, in that order. Under WBH, comparatively cool CT was detected for SYM2016-012 and SYM2016-006 both and SYM2016-042 with 19.14 °C and 18.83 °C, in that order, whilst under WAH, SYM2016-047 and SYM2016-044 with 17.43 °C and 17.68 °C were recorded with relatively cooler CT, respectively. Therefore, it was noted CT was comparatively cooler under NS than WBH and WAH.

Table 3.8: Mean canopy temperature for genotypes measured 17 days after across 3 water regimes

Genotypes	WBH	WAH	NS
SYM2016-004	20.06 ^{ghijklmno}	19.23 ^{defghijklm}	19.68 ^{ghijklmno}
SYM2016-006	19.14 ^{cdefghijklm}	19.45 ^{efghijklmn}	18.93 ^{cdefghijk}
SYM2016-008	20.39 ^{ijklmno}	19.08 ^{cdefghijklm}	19.50 ^{efghijklmn}
SYM2016-010	20.83 ^{klmno}	20.90 ^{lmno}	19.61 ^{fghijklmno}
SYM2016-012	19.14 ^{defghijklm}	19.10 ^{cdefghijklm}	17.75 ^{abcdef}
SYM2016-014	20.36 ^{ijklmno}	20.03 ^{ghijklmno}	16.66 ^{ab}
SYM2016-016	20.55 ^{jklmno}	18.17 ^{abcdefg}	17.74 ^{abcdef}
SYM2016-019	19.70 ^{ghijklmno}	23.15 ^{p}	19.04 ^{cdefghijkl}
SYM2016-027	19.95 ^{ghijklmno}	19.83 ^{ghijklmno}	18.40 ^{bcdefgh}
SYM2016-029	20.58 ^{jklmno}	20.90 ^{lmno}	18.34 ^{bcdefg}
SYM2016-037	20.06 ^{ghijklmno}	21.20 ^{no}	18.73 ^{cdefghij}
SYM2016-038	20.80 ^{klmno}	20.33 ^{hijklmno}	18.68 ^{cdefghij}
SYM2016-042	18.83 ^{cdefghij}	21.48 ^{o}	16.48 ^{a}
SYM2016-044	19.80 ^{ghijklmno}	17.68 ^{abcde}	17.23 ^{abc}
SYM2016-047	21.02 ^{mno}	17.43 ^{abcd}	18.54 ^{cdefghi}
Mean	20.08	19.86	18.35
CV (%)		1.60	
l.s.d. ($P < 0.05$)		1.56	

Key: WBH - water stress 1 week before 50% flowering, WAH - water stress 1 week after 50% flowering, NS - non-stress water regime, CV (%) - coefficient of variation expressed as a percentage, l.s.d.- least significant difference at the $P < 0.05$ probability level.

Means in a column followed by the same letter are not significantly different at the 5% probability level.

Performance of genotypes using the canopy temperature based on genotype x water regime x test environment interaction

Table 3.9 presents the mean canopy temperature of genotypes measured 17 days after drought stress evaluated across 3 water regimes and two test environments Under greenhouse condition, genotypes with the lowest CT under NS were SYM2016-019 and SYM2016-027 with values 14.53 °C and 14.6 °C, respectively. However, lowest CT recorded was 18.25 °C and 18.4 °C for SYM2016-004 and SYM2016-006, respectively under WBH. Whereas, lowest CT was 13.65 °C for both genotypes SYM2016-008 and SYM2016-047, respectively under WAH.

Under field condition, the lowest CT recorded was 17.10 °C and 17.25 °C for SYM2016-042 and SYM2016-044, respectively under NS. On the other hand, the lowest values for CT were 19.10 °C and 19.83 °C for SYM2016-042 and SYM2016-012 under WBH. However, lowest values recorded under WAH were 19.50 °C and 21.20 °C for SYM2016-044 and SYM2016-047, respectively. Therefore, CT was cooler under greenhouse test condition and cooler for NS water regimes.

Table 3.9: Mean canopy temperature measured 17 days after drought stress of genotypes evaluated across 3 water regimes and 2 test environments

Genotype	Greenhouse			Field		
	WBH	WAH	NS	WBH	WAH	NS
SYM2016-004	18.25	14.50	15.25	21.88	23.95	24.10
SYM2016-006	18.40	15.55	15.65	19.88	23.35	22.20
SYM2016-008	19.85	13.65	15.15	20.93	24.50	23.85
SYM2016-010	18.70	18.15	17.43	22.95	23.65	21.80
SYM2016-012	18.45	16.35	15.40	19.83	21.85	20.10
SYM2016-014	19.20	17.30	15.13	21.52	22.75	18.20
SYM2016-016	19.30	15.05	15.58	21.80	21.28	19.90
SYM2016-019	19.30	22.70	14.53	20.10	23.60	23.55
SYM2016-027	19.80	17.80	14.60	20.10	21.85	22.20
SYM2016-029	19.55	17.50	15.63	21.60	24.30	21.05
SYM2016-037	19.20	20.85	15.65	20.93	21.55	21.80
SYM2016-038	19.75	18.50	15.25	21.85	22.15	22.10
SYM2016-042	18.55	20.25	15.85	19.10	22.70	17.10
SYM2016-044	18.95	15.85	17.20	20.65	19.50	17.25
SYM2016-047	19.25	13.65	15.73	22.78	21.20	21.35
Mean	19.10	17.18	15.60	21.06	22.55	21.10
CV (%)				1.60		
l.s.d. (P < 0.05)				2.21		

Key: CV (%) - coefficient of variation expressed as a percentage, l.s.d.- least significant difference at the P < 0.05 probability level, ND - non-stress water regime, WBH - water stress 1 week before 50% flowering, WAH - water stress 1 week after 50% flowering.

3.3.3 Mean response of genotypes for morphological traits

Table 3.10 summarised the mean values of genotypes assessed using morphological traits under greenhouse and field test environments. The overall mean DF value was 71.61 while minimum and maximum values recorded were 47.33 and 93.00 for genotypes SYM2016-027 and SYM2016-008, respectively. Under greenhouse condition, early DF were recorded for genotypes SYM2016-027 and SYM2016-004 with values of 47.33 days and 47.89 days, respectively. Conversely, under field condition, genotypes SYM2016-004 and SYM2016-044 with values 75.33 and 77.60, respectively, were also early flowering types.

Mean DM recorded was 133.01 while values ranged from 117.89 to 145.67 for genotypes SYM2016-004 and SYM2016-012, in that order over all trials. Early maturing genotypes were SYM2016-004 and SYM2016-014 with values of 117.89 and 122.44 days, in that order, under greenhouse condition. Whilst early maturing genotypes under field condition were SYM2016-014 and SYM2016-027 with 120.35 and 125.00 days, respectively. Early flowering and maturity are important attributes of crops for drought escape, making the above genotypes ideal for drought tolerance breeding.

On the other hand, mean PH detected was 87.61 cm and minimum and maximum values were 58.01 cm and 100.1 cm for genotypes SYM2016-027 and SYM2016-008, in that order. Shorter PH was recorded for SYM2016-027 and SYM2016-014 with 58.01 cm and 65.55 cm, in that order under greenhouse evaluation. Whereas, SYM2016-014 and SYM2016-029 were the shortest genotypes under field tests with 81.11 cm and 81.76 cm, respectively.

Mean value for PT was 35.92 and values ranged from 18.11 for SYM2016-010 and 58.28 for SYM2016-047. The mean value detected for SS was 18.54 and minimum and maximum values recorded were 14.07 and 22.40 for SYM2016-047 and SYM2016-037, respectively. A comparatively higher SS was recorded for SYM2016-016 with a value of 18.49 and for SYM2016-042 with 18.16 under greenhouse trial. Under field condition, SYM2016-042 and SYM2016-037 expressed high SS values of 22.21 and 22.4, in that order.

The mean KS value was 44.87 and values ranged from 26.93 and 63.93 both for genotype SYM2016-027. The genotypes with the highest KS were SYM2016-012 with 46.03 and SYM2016-019 with 44.04, under greenhouse condition. Under field condition, SYM2016-027 and SYM2016-019 had greater KS of 63.93 and 62.84, respectively.

Table 3.10: Mean values for 8 morphological traits of genotypes evaluated under 3 water regimes under greenhouse and field conditions

Genotype	Greenhouse								Field							
	DF	DM	PH	PT	SS	KS	SW	GY	DF	DM	PH	PT	SS	KS	SW	GY
SYM2016-004	47.9 a	117.9 a	91.3 ghijkl	19.0 a	15.3 abcd	35.3 bcd	4.2 abcdef	222.8 ab	75.3 ef	126.6 bcd	97.1 klm	43.8 efghi	21.5 mnopq	52.8 ijk	4.9 cdefgh	1059.3 g
SYM2016-006	57.3 c	127.8 cde	93.2 hijklm	43.4 efghi	17.1 efgh	38.1 bcdef	5.2 efghi	648.6 cdefg	79.1 gh	138.3 ijkl	91.9 ghijkl	43.2 efghi	20.4 lmnop	50.0 hij	4.9 defghi	867.0 fg
SYM2016-008	68.6 d	139.7 ijklm	100.1 m	28.8 abcdef	17.3 efghi	39.8 cdef	5.1 efghi	426.6 bcdef	93.0 l	142.2 jklm	97.7 klm	44.1 efghi	20.2 klmno	46.3 fghi	5.1 efghi	812.8 defg
SYM2016-010	68.8 d	136.7 ghijk	91.9 ghijkl	18.1 a	16.3 bcdef	36.5 bcde	5.6 ghi	294.4 abc	85.3 jk	130.7 defg	98.2 lm	36.6 bcdefgh	20.0 klm	50.0 hij	4.6 bcdefg	701.5 defg
SYM2016-012	85.2 jk	145.7 m	86.5 efgh	25.2 abcd	18.0 fghi	46.0 fghi	5.3 fghi	407.4 bcdef	88.4 k	143.0 klm	96.8 jklm	38.0 bcdefgh	21.2 mnopq	60.4 klm	4.4 bcdefg	837.5 efg
SYM2016-014	48.0 a	122.4 abc	65.6 b	23.6 abc	14.2 a	30.8 ab	3.7 abcd	203.7 ab	78.8 gh	120.3 ab	81.1 de	45.7 fghi	18.9 Ijkl	45.5 fghi	4.3 bcdef	849.2 fg
SYM2016-016	72.7 e	144.3 lm	86.9 efghi	34.8 abcdefgh	18.5 hijk	39.2 cdef	5.9 hi	587.5 cdefg	79.0 gh	143.5 klm	92.7 hijkl	50.1 hi	21.6 mnopq	48.6 ghij	4.9 defghi	1032.8 g
SYM2016-019	65.8 d	137.4 hijk	84.0 ef	29.8 abcdefg	16.6 cdefg	44.0 efgh	4.0 abcde	355.6 bcd	81.0 ghi	128.0 cde	84.9 efg	43.9 efghi	19.8 jklm	62.8 lm	4.3 bcdef	833.7 efg
SYM2016-027	47.3 a	125.0 bcd	58.0 a	22.7 abc	14.8 ab	26.9 a	3.1 a	146.6 a	83.4 ij	125.0 bcd	90.5 fghijk	39.1 cdefgh	20.5 lmnop	63.9 m	4.3 abcdef	897.4 fg

Key: DF - days to 50% flowering, DM - number of days to 50% maturity, PH - plant height in centimetres, PT - number of productive tillers per plot, SS - number of spikelets per spike, KS - number of grains per spike, SW - hundred seed weight in grams, GY - yield per pot in grams per plot, WBH - water stress 1 week before 50% flowering, WAH - water stress 1 week after 50% flowering, ND - non-stress water regime, CV (%) - coefficient of variation expressed as a percentage, l.s.d.- least significant difference at the $P < 0.05$ probability level.

Means in a column followed by the same letter are not significantly different at the 5% probability level.

Table 3.10: Continued.

Genotype	Greenhouse								Field							
	DF	DM	PH	PT	SS	KS	SW	GY	DF	DM	PH	PT	SS	KS	SW	GY
SYM2016-029	52.8 b	134.4 efghi	73.1 c	31.8 abcdefg	15.1 abc	35.3 bcd	3.6 ab	302.7 abc	79.8 gh	126.6 bcd	81.8 de	44.6 efghi	20.8 mnopq	53.0 ijk	3.8 abcd	648.6 cdefg
SYM2016-037	66.3 d	130.3 defg	85.1 efg	36.1 bcdefgh	16.1 bcde	33.6 abc	4.7 bcdefgh	362.2 bcd	78.4 fgh	142.6 jklm	89.6 fghij	45.7 fghi	22.4 q	55.8 jkl	4.7 bcdefgh	1137.6 g
SYM2016-038	57.2 c	131.7 defgh	81.9 de	22.1 ab	14.9 abc	36.2 bcde	4.4 bcdefg	242.7 ab	86.2 jk	127.3 cd	89.0 fghi	46.7 ghi	21.1 mnopq	45.9 fghi	4.8 bcdefgh	719.4 defg
SYM2016-042	58.2 c	137.7 hijkl	90.4 fghijk	22.7 abc	18.2 ghij	42.9 defgh	4.5 bcdefg	366.4 bcde	80.8 ghi	130.8 defg	91.3 ghijkl	43.5 efghi	22.2 pq	46.0 fghi	6.1 i	839.5 efg
SYM2016-044	59.9 c	139.8 ijklm	94.1 ijklm	27.9 abcde	16.9 defgh	41.2 cdefg	5.3 fghi	425.6 bcdef	77.6 fg	135.9 fghij	93.9 ijklm	41.0 defgh	20.2 lmn	55.1 jk	4.2 abcdef	638.3 cdefg
SYM2016-047	51.1 b	129.4 def	75.6 cd	27.8 abcde	14.1 a	34.7 bcd	3.7 abc	205.1 ab	81.4 hi	129.3 def	94.3 ijklm	58.3 i	22.1 npq	49.2 ghij	4.2 abcdef	1161.4 g
Mean	60.5	133.4	83.9	27.6	16.2	37.4	4.6	320.6	81.8	132.7	91.4	44.3	20.9	52.4	4.6	855.1
CV (%)	0.7	0.2	1.4	12.0	0.5	3.4	1.3	3.0	0.7	0.2	1.4	12.0	0.5	3.4	1.3	3.0
l.s.d. (P < 0.05)	5.2	10.0	10.5	24.0	2.7	12.1	1.7	3.3	5.2	10.0	10.5	24.0	2.7	12.1	1.7	3.3

Key: DF - days to 50% flowering, DM - number of days to 50% maturity, PH - plant height in centimetres, PT - number of productive tillers per plot, SS - number of spikelets per spike, KS - number of grains per spike, SW - hundred seed weight in grams, GY - yield per pot in grams per plot, WBH - water stress 1 week before 50% flowering, WAH - water stress 1 week after 50% flowering, ND - non-stress water regime, CV (%) - coefficient of variation expressed as a percentage, l.s.d.- least significant difference at the P < 0.05 probability level.

Means in a column followed by the same letter are not significantly different at the 5% probability level.

Mean SW was detected at a value of 4.59 g while minimum and maximum values were 3.10 and 6.06 g for the genotypes SYM2016-027 and SYM2016-042, in that order over both trials. High SW were recorded for SYM2016-016 and SYM2016-010 with 5.867 g and 5.59 g, in that order, under the greenhouse condition. Genotypes SYM2016-042 and SYM2016-008 had the highest SW values of 6.057 g and 5.144 g, respectively under field condition. During the study it was observed that grains of some genotypes were shrivelled and relatively light-weight due to drought stress though some genotypes maintained plump seeds (Figure 3.2: A and B) which may have influenced SW and GY.

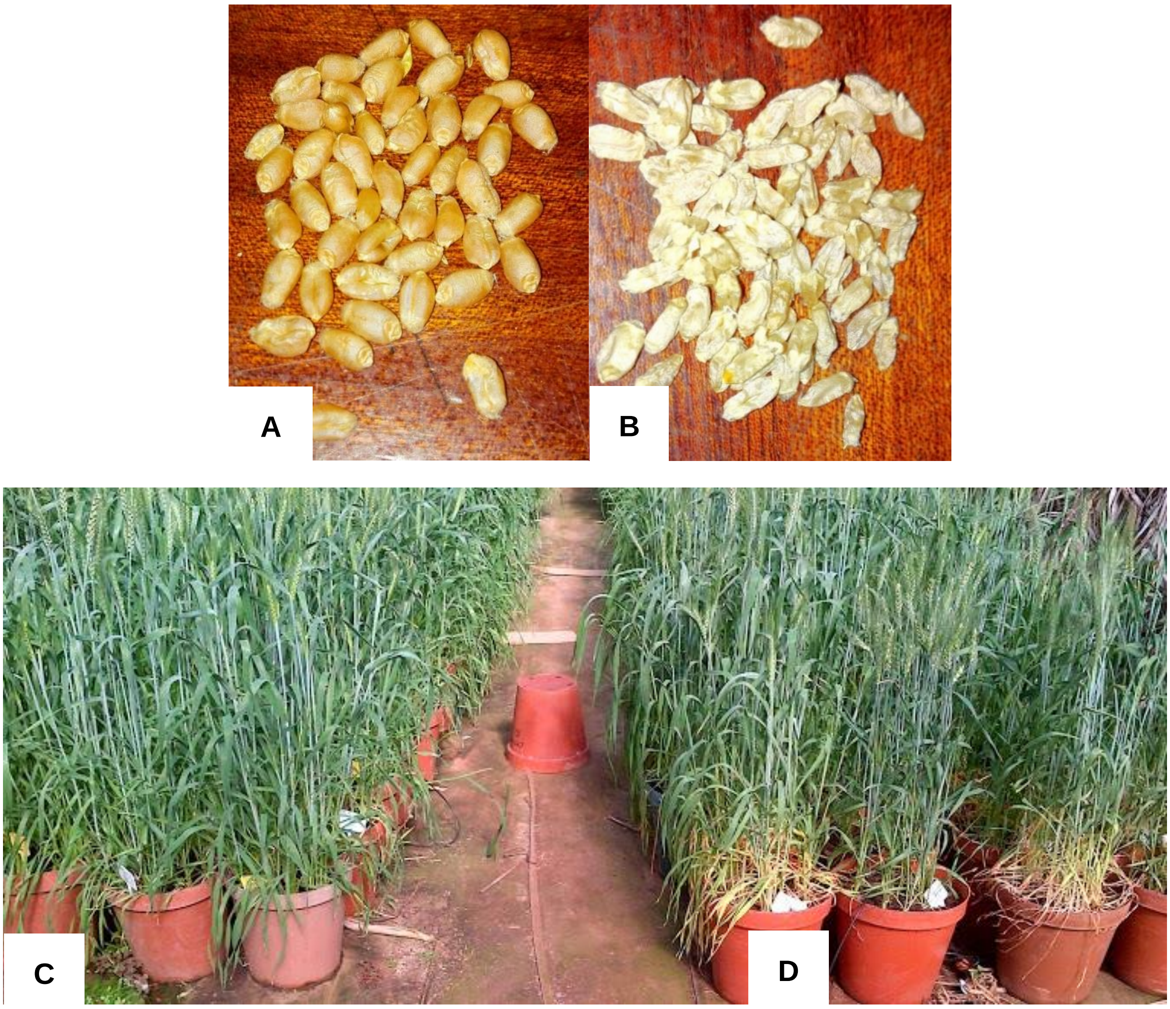

Figure 3.2: Plump (A) and shrivelled (B) grains and leaf health under non-stress (C) and drought stress (D), respectively.

Mean GY recorded across trials was 523.60 g per plot. The minimum and maximum GY was 146.56 g per plot and 1161.45 g per plot for SYM2016-027 and SYM2016-047, respectively.

A relatively high GY was recorded for SYM2016-006, with 648.63 g per plot and for SYM2016-016 with 587.49 g per plot, under greenhouse condition. However, SYM2016-047 and SYM2016-037 were detected with the highest GY of 1161.45 g per plot and 1137.63 g per plot, in that order under the field condition.

3.3.4 Correlation of physiological and morphological traits across greenhouse and field conditions

Table 3.11 presents the correlations of physiological and morphological traits used for evaluation of genotypes under 3 water regimes under greenhouse and field conditions. GY was significantly correlated with DF, DM, PH, PT, SS, KS and SW with a coefficients of 0.55, 0.54, 0.63, 0.74, 0.80, 0.571 and 0.75 respectively, under greenhouse condition. Whereas, under the field condition, GY was significantly correlated with SS and PT with correlation coefficients of 0.59 for both traits. Under greenhouse condition DF had a significant correlation with the DM, PH, SS, KS and SW with correlation coefficients of 0.83, 0.52, 0.75, 0.70 and 0.74 in that order DM expressed a significant correlation with SS, KS and SW with correlation coefficients of 0.75, 0.75 and 0.63, respectively. PH had strong and significant association with SS, KS and SW with coefficient values of 0.67, 0.69 and 0.79, in that order. SS was significantly correlated with KS and SW with a correlation coefficient of 0.77 for both traits. Under field condition, significant correlations were detected among SC and CT2, CT1 and CT2 and PH and DM, with correlation coefficients of -0.53, 0.63 and 0.52, in that order.

Table 3.11: Combined correlations of the physiological and morphological traits under 3 water regimes under greenhouse (bottom diagonal) and field (top diagonal) conditions

Traits		Field										
		SC	CT1	CT2	DF	DM	PH	PT	SS	KS	SW	GY
Greenhouse	SC	1	-0.46 ^{ns}	-0.53 *	-0.42 ^{ns}	0.004 ^{ns}	-0.24 ^{ns}	0.42 ^{ns}	0.06 ^{ns}	-0.21 ^{ns}	0.35 ^{ns}	0.39 ^{ns}
	CT1	-0.01 ^{ns}	1	0.63 **	-0.03 ^{ns}	-0.43 ^{ns}	-0.09 ^{ns}	0.10 ^{ns}	0.05 ^{ns}	-0.03 ^{ns}	-0.30 ^{ns}	-0.01 ^{ns}
	CT2	-0.11 ^{ns}	0.32 ^{ns}	1	0.24 ^{ns}	-0.16 ^{ns}	0.12 ^{ns}	0.06 ^{ns}	-0.13 ^{ns}	-0.04 ^{ns}	-0.12 ^{ns}	0.13 ^{ns}
	DF	-0.65 **	0.35 ^{ns}	0.11 ^{ns}	1	0.27 ^{ns}	0.36 ^{ns}	-0.23 ^{ns}	-0.16 ^{ns}	-0.06 ^{ns}	0.12 ^{ns}	-0.30 ^{ns}
	DM	-0.56 *	0.55 *	0.16 ^{ns}	0.83 ***	1	0.52 *	-0.04 ^{ns}	0.36 ^{ns}	0.02 ^{ns}	0.28 ^{ns}	0.19 ^{ns}
	PH	-0.17 ^{ns}	0.30 ^{ns}	-0.13 ^{ns}	0.52 *	0.44 ^{ns}	1	-0.17 ^{ns}	0.30 ^{ns}	-0.05 ^{ns}	0.34 ^{ns}	0.18 ^{ns}
	PT	-0.30 ^{ns}	-0.06 ^{ns}	-0.07 ^{ns}	0.18 ^{ns}	0.17 ^{ns}	0.17 ^{ns}	1	0.40 ^{ns}	-0.41 ^{ns}	-0.01 ^{ns}	0.59 *
	SS	-0.26 ^{ns}	0.49 ^{ns}	0.04 ^{ns}	0.75 **	0.75 **	0.67 **	0.30 ^{ns}	1	-0.06 ^{ns}	0.38 ^{ns}	0.59 *
	KS	-0.34 ^{ns}	0.61 *	0.07 ^{ns}	0.70 **	0.75 **	0.69 **	0.13 ^{ns}	0.77 ***	1	-0.50 ^{ns}	0.03 ^{ns}
	SW	-0.45 ^{ns}	0.32 ^{ns}	-0.10 ^{ns}	0.74 **	0.63 **	0.79 ***	0.23 ^{ns}	0.77 ***	0.574 *	1	0.15 ^{ns}
	GY	-0.36 ^{ns}	0.22 ^{ns}	-0.15 ^{ns}	0.55 *	0.54 *	0.63 **	0.74 **	0.80 ***	0.571 *	0.75 **	1

Key: SC - stomatal conductance in m.mol/m^{2}s, CT1 and CT2 - canopy temperature in ^{o}C measured 10 and 17 days after stress was imposed, DF - number of days to 50% flowering DM – number of days to 50% maturity, PH - plant height in centimetres, PT–number of productive tillers, SS - number of spikelets per spike, KS - number of grains per spike, SW - 100 seed weight in grams, GY - yield per plot in grams per plot, * - significant differences detected at P < 0.05 probability level, ** - significant differences detected at P < 0.01 probability level, *** - significant differences detected at P < 0.001 probability level.

3.3.5 Principal component analysis (PCA)

Table 3.12 presents the PC analysis for genotypes using physiological and morphological traits across greenhouse and field conditions. There were 3 and 4 principal components (PCs) accounting for 77.84% and 76.92% of the percentage variation under greenhouse and field evaluations, in that order. The explained variation accounted by PC1 was 51.79%, 14.92% by PC2 and 11.13% by PC3, under the greenhouse condition. The factor loadings, under the greenhouse condition were 0.38 for SS, 0.36 for DF, 0.36 for SW, 0.36 for DM, 0.36 for KS, 0.34 for GY and 0.32 for PH positively correlating with PC1. High factor loadings were recorded for PC2 for CT1 and CT2 with 0.49 and 0.50, respectively, under greenhouse condition. On the other hand, SC was recorded with the highest factor loading for PC3 which was 0.64, for the greenhouse condition.

Under field conditions, however, the explained percentage variation for each PC was 25.97% for PC1, 20.28% for PC2, 18.10% for PC3 and 12.56% for PC4. For PC1, comparatively high factor loadings were recorded for SC with a value of 0.43, GY with 0.39, SS with 0.38 and PT with 0.33. Trait factor loadings of PC2 were higher than PC1. PH with a value of 0.53, DF with 0.46 and DM with 0.46 were all traits detected with high factor loadings for PC2. CT was solely associated with PC3, with values of 0.51 for CT measured on day 10 and 0.53 for CT measured on day 17.

Table 3.12: Principal component analysis for 15 wheat genotypes using physiological and morphological traits under greenhouse and field conditions

Variables	Greenhouse			Field			
	PC1	PC2	PC3	PC1	PC2	PC3	PC4
SC	-0.22	0.07	0.64	0.43	-0.29	-0.25	0.10
CT1	0.22	0.49	0.20	-0.28	-0.15	0.51	0.06
CT2	0.02	0.50	-0.40	-0.26	0.07	0.53	0.11
DF	0.36	0.09	-0.21	-0.17	0.46	0.03	0.23
DM	0.36	0.21	-0.18	0.25	0.46	-0.05	-0.23
PH	0.32	-0.10	0.41	0.12	0.53	0.16	-0.09
PT	0.16	-0.50	-0.30	0.33	-0.30	0.34	0.10
SS	0.38	0.01	0.16	0.38	0.11	0.32	-0.25
KS	0.35	0.20	0.16	-0.21	0.00	-0.13	-0.73
SW	0.36	-0.13	0.12	0.35	0.27	-0.02	0.40
GY	0.34	-0.37	0.02	0.39	-0.10	0.36	-0.31
Eigenvalues	5.70	1.64	1.22	2.86	2.23	1.99	1.38
Explained variance (%)	51.79	14.92	11.13	25.97	20.28	18.11	12.56
Explained cumulative variance (%)	51.79	66.71	77.84	25.97	46.25	64.36	76.92

Key: SC - stomatal conductance in m.mol/m^{2}s, CT1 and CT2 - canopy temperature in ^{o}C measured 10 and 17 days after stress was imposed, DF - number of days to 50% flowering, DM – number of days to 50% maturity, PH - plant height in centimetres, PT- number of productive tillers, SS - number of spikelets per spike, KS - number of grains per spike, SW - 100 seed weight in grams, GY - yield per plot in grams per plot, PC1 - first principal component, PC2 - second principal component, PC3 - third principal component, PC4 - forth principal component.

3.3.6 Discussion

Implications of selection resulting from genotype by test environments interaction

The interaction of the genotypes with the growing environment may result in non-heritable or heritable changes in the genotypes. The latter can influence the population gene frequency. In such an instance, the environment poses selection pressure (Dabholkar, 1999). The ranking of genotypes can change across test environments due to cross-over interaction (Acquaah, 2007), as can be noted with genotypes under the greenhouse and field evaluations in this study. This is the reason of assessing genotypes over more than one test environment, as reliability and accuracy would be reduced when relying on a single test environment. This phenomenon necessitates the *modus operandi* of CIMMYT (Rajaram et al., 2002) of multi-location trails using mega-environments delineated by water availability, whether the test environment is tropical or a winter wheat production zone and consumer or industry preferences for grain quality, among other factors.

Source-sink relations and yield potential

Genotypes expressed differences in days to flowering, days to maturity, plant height, grain number and seed weight all of which indicated yield and was largely determined by differential efficiency in both the genotypes' photosynthetic capacity, dry matter partitioning, especially for grain-filling and genotypes' capacity for amelioration of plant phenology. The study carried out by Villegas et al. (2000) in durum wheat genotypes, also indicated the genotypes' photosynthetic capacity and the plasticity of the length of the growth cycle are vital to durum wheat tolerance to reproductive stage drought stress tolerance. The genotype groupings were similar for days to flowering, days to maturity, plant height, number of spikelets per spike, seed weight and yield. Early flowering and maturing genotypes were recorded with short plant height, low seed weight, relatively few number of spikelets per spike and a consequentially poor yield. Whereas, late flowering and maturing genotypes had tall plant height, high seed weight and a resultant superior yield. There seemed to be a yield penalty for short, early flowering and maturing genotypes similar to that outlined by Nguyen and Blum (2004) and Villegas et al. (2000), for early maturing and short statured wheat genotypes.

In this study, the seed weight shared a positive linear relationship with yield. The positive linear relationship of yield with seed weight was similar to that reported by Merah and Monneveux (2014) and (Ehdaie et al., 2012). This explained why individual genotypes that had lighter grain had poor yield, which can be a consequence of poor grain-filling for early maturing genotypes.

Source-sink relations need to be modified to achieve high grain yield from biomass accumulation (Passioura, 2012). Wheat grain acts as the sink and the vegetative structures such as the leaves, leaf sheaths and stems act as the source of photosynthates to the grain upon grain-filling (Plaut et al., 2004). A strong sink may improve grain-filling relative to a line with a limited or moderate sink strength (Plaut et al., 2004) as witnessed with stable and high yield of genotypes SYM2016-006, SYM2016-008, SYM2016-016 and SYM2016-037 across test environments in this study. This attribute becomes beneficial particularly under drought-prone environments, as the competition between drought-adjusting leaves and the demand of grain-filling and roots becomes inevitable (Plaut et al., 2004). The silking and kernel blistering stages which determines yield, as seen in maize (Nielsen, 2016), are also susceptible to being debilitated by severe drought stress. Since turgor pressure is needed to elongate the silks for pollination and grain filling in the blistering stage the processes may be jeopardised should water deficit occur, also during the kernel milk stages. Similar drought stress at flowering and grain-filling or in this study, limited grain number, seed weight, plant height, number of productive tillers and ultimately the yield for genotypes across test environments. Grain damage due to pest infestation and pre-harvest sprout was apparent for the greenhouse and field condition, respectively. Pre-harvest sprout impedes seed starch content due to the activity of the enzyme alpha amylase (α amylase), this diminishes seed quality (Ashraf, 2014). A limited variation of PT indicated this trait as possibly a candidate trait of high heritability. The close association of this trait with grain yield may make it good for selection purposes under varying test environments and water stresses.

The contribution of physiological processes to drought stress tolerance

Prior to anthesis, the flag leaf is the main photosynthesis organ serving as the source organ (Merah and Monneveux, 2014). As growth approaches anthesis and grain-filling, the contribution of photosynthates of the flag leaf to the developing grains is limited, more so under water deficit (Lopes and Reynolds, 2010). Gradually, ear and peduncle photosynthesis takes over (Merah and Monneveux, 2014). The persistence of the flag leaf, otherwise identified as the stay green character, is an indicator of drought tolerance in some genotypes compared to those which utilise stem reserves and senesce the flag leaf early into drought stress (Biswal and Kohli, 2013). For the photosynthetic capacity of the flag leaves to be maintained, the length of the growth cycle needs to be increased (Biswal and Kohli, 2013), as observed with the genotypes with longer growth cycles SYM2016-012 and SYM2016-016. This indicates the need for photosynthesis relates to morphological traits. Particularly to achieve higher yield.

Limited transpiration or evaporative cooling causes the canopy to warm up under drought stress relative to non-stress (Moslem et al., 2013). Canopy temperature may also be related to the rooting depth or root plasticity, which may vary with each genotype of wheat crop (Lopes and Reynolds, 2010) also with maintaining stomatal conductance and photosynthesis (Monneveux et al., 2014). Some crops with a lower canopy temperature under drought stress are adapted to capture more moisture when moisture at the top soil is depleted (Pareek et al., 2010). In this way the metabolic activities to maintain growth and development do not cease or decline (Lopes et al., 2012; Mir et al., 2012). In this study, the phenology was far more prevalent as the contributor to lowered canopy temperature. The stomatal density of the leaves and the leaf area may also have impacted canopy temperature and stomatal conductance. Genotypes with low canopy temperature had short plant height, early flowering and maturing which tended to have lower grain weight and poor seed yield, as previously mentioned in this study. Pask et al. (2012) correspondingly reported the phenology as a notable contributor in the expression of the canopy temperature. Limited variability was detected for the stomatal conductance, in this study. Seeing as replicated single leaf measurements are taken as opposed to the entire canopy, the steady state diffusion porometer may result in high leaf-to-leaf variability and lead to inaccuracies (Rebetzke et al., 2013).

3.4 Conclusions

Genotypes were subject to interaction with their respective test environments. Thus, selection for suitable drought tolerant and adapted candidates was somewhat complicated. Genotypes considered early maturing were SYM2016-014, SYM2016-027 and SYM2016-029. Genotypes with a relatively high yield also across test environments were SYM2016-016, SYM2016-037 and SYM2016-006. Traits valuable for drought screening under greenhouse and field conditions, in this study, were early flowering and maturity as well as short plant height and low canopy temperature under short term drought stress. The infrared thermometer, was a high-throughput and non-destructive technology thus would be preferable especially when working with more genotypes. Increasing the number of replications for the purposes of repeatability would be preferable for accuracy as the canopy temperature is influenced by environmental factors. The genotypes SYM2016-027, SYM2016-014 and SYM2016-029 were relatively drought tolerant. Early flowering and maturity as well as short plant height and low canopy temperature under short term drought stress in the greenhouse and field conditions were favourable traits for selection. Hybridisation and fixation of resultant lines is essential to develop early maturing genotypes with stable and high yield potential.

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Chapter 4 Assessment of drought tolerance in selected bread wheat (*Triticum aestivum* L.) genotypes using drought tolerance indices

Abstract

Phenotypic characterisation necessitates high throughput selection tools especially for drought tolerance, which is a complex trait. Drought tolerance indices are based on yield response under drought stressed and non-stress conditions. The objective of this study was to assess drought tolerance amongst 15 selected bread wheat genotypes using 9 drought tolerance indices. Genotypes were evaluated under drought stressed and non-stressed conditions. Yield data of genotypes was collected to determine drought tolerance indices. The following indices were calculated: drought resistance (DR), mean productivity (MP), harmonic mean of yield (HM), stress susceptibility index (SSI), stress tolerance index (STI), tolerance index (TOL), yield index (YI), yield reduction index (YR) and yield stability index (YSI). Yield and drought indices data were subjected to analysis of covariance (ANCOVA) and analysis of variance (ANOVA), respectively. Indices data was also subjected to mean ranking, Pearson and Spearman's rank correlation analyses, principal component (PC) and biplot analyses. All analyses was done using GenStat software. Significant differences were detected for genotypes ($P < 0.001$) and genotype by water regime interaction ($P < 0.01$) effects for yield, while significant differences were recorded among genotypes ($P < 0.05$) for indices such as DR, HM, MP, STI, YI and YSI. Mean ranking was consistent for the following indices: HM, MP, STI, SSI and YI enabling selection of for the genotypes SYM2016-006, SYM2016-016 and SYM2016-037 with drought tolerance. PC analysis detected high total percentage variation (82.2%) among genotypes. Percentage variation was partitioned as follows: 42.64% for PC1, 22.37% for PC 2 and 12.18% for PC3. PC and biplot analyses detected associations between HM, MP, STI, YI and yield under drought stress and non-stress. High yielder genotypes such as SYM2016-006, SYM2016-016 and SYM2016-037 had exceptional expression for HM, MP, STI, YI and yield under drought stress and non-stress conditions. Conversely, DR was associated with early maturing genotypes such as SYM2016-014, SYM2016-029 and SYM2016-38. These genotypes were considered as potential parents for future cross combinations.

Keywords: Drought tolerance indices, drought escape, multivariate analyses, wheat, yield stability

4.1 Introduction

Water stress at flowering limits grain yield, ultimately leading to complete crop failure (Farooq et al., 2014). Drought tolerance indices associated with yield levels are important selection parameters in breeding wheat for drought tolerance (Cabello et al., 2013). The yield response of genotypes under drought stress and non-stress conditions were classified by Fernandez (1992) into 4 distinct classes, namely, genotypes with superior yield under drought stress and non-stress conditions as Group A genotypes; genotypes with superior yield under non-stress only as Group B genotypes; genotypes with superior yield under drought stress only as Group C genotypes and genotypes with inferior yield under non-stress and drought stress conditions as Group D genotypes. Genotypes with superior seed yield under non-stress and drought stress conditions are favoured for their yield stability (Dodig et al., 2012; Cabello et al., 2013). Drought indices have been used in drought tolerance studies in various crops such as potato (Cabello et al., 2013), common bean (Ramirez and Kelly, 1998), barley (Eivazi et al., 2013), maize (Farshadfar and Sutka, 2002; Jafari et al., 2009), wheat (Dodig et al., 2012; Drikvank et al., 2012) and tef (Abraha et al., 2017).

The most widely used selection indices included drought resistance index (Lan, 1988), mean productivity and stress tolerance index (Rosielle and Hamblin, 1981), harmonic mean of yield (Jafari et al., 2009), modified stress tolerance index 1 and 2 (Farshadfar and Sutka, 2002) stress susceptibility index (Fischer and Maurer, 1978), geometric mean productivity and stress tolerance index (Fernandez, 1992), tolerance index (Rosielle and Hamblin, 1981), yield index (Gauzzi et al., 1997), yield stability index (Golestani-Araghi and Assad, 1998) and yield reduction (Jafari et al., 2009).

Overall selection criteria for drought tolerant genotypes include low values for yield reduction, stress susceptibility and stress tolerance indices; whilst high values of drought resistance, harmonic mean of yield, mean productivity, stress tolerance, yield and yield stability indices (Cabello et al., 2013). Stress susceptibility index was reported by El-Mohsen et al. (2015) to comparing yield reduction of individual genotypes relative to the mean yield reduction of all test genotypes. Hence, genotypes with a value lower than the latter are regarded as yield stable expressing a value less than 1 (Ramirez and Kelly, 1998; Drikvank et al., 2012). Mean productivity, stress tolerance index, yield stability index and harmonic mean of yield are known to discriminate Group A genotypes (Dodig et al., 2012; El-Rawy and Hassan, 2014). Whilst, tolerance index only distinguishes genotypes of Group C (Farshadfar et al., 2012).The use of the tolerance index alone is not considered effective (Dorostkar et al., 2015). Stress susceptibility, stress tolerance and yield stability indices distinguish genotypes in the same manner, thus may be used interchangeably (El-Rawy and Hassan, 2014).

Using a number of diverse drought indicators may generate large amounts of data and result in inconsistent ranking of genotypes (Abdolashahi et al., 2013). Multivariate analyses such as principal component analysis (Drikvank et al., 2012; Abraha et al., 2017) and biplot analysis (Farshadfar et al., 2012) improve inferences about drought tolerance of genotypes by dimension reduction. These techniques allow grouping genotypes into superior or inferior classes based on yield levels and drought tolerant or susceptible genotypes (Eivazi et al., 2013; El-Mohsen et al., 2015). Despite some limitations, drought tolerance indices are important selection criteria for drought tolerance based on yield response under drought stressed and non-stressed conditions. Therefore, the objective of this study was to assess drought tolerance amongst 15 selected bread wheat genotypes using 9 drought tolerance indices.

4.2 Materials and methods

4.2.1 Plant materials and study sites

Fifteen bread wheat genotypes, selected in Chapter 3 were evaluated under drought stressed and non-stressed conditions under controlled environment condition at the University of KwaZulu-Natal, Pietermaritzburg Campus.

4.2.2 Experimental design and crop establishment

The study was conducted using a randomised complete block design (RCBD) with 3 replications. Detailed crop establishment, growing conditions and treatments are described in Chapter 3. Drought stress treatments were imposed 1 week before and 1 week after 50% flowering which is considered as stress regime (drought stress) and non-stress condition as the control.

4.2.3 Data collection and statistical analysis

Grain yield

Grain yield data collection was recorded as the product of the number of productive tillers produced, the seed weight per plant (expressed in grams) and the number of grains obtained per spike and the number of plants per plot. The original yield data was not normally distributed hence was subjected to transformation using LOG base 10 transformation. To reduce the error variance and improve accuracy, an analysis of covariance (ANCOVA) is performed between a response variable and with one or more independent variables which are significantly correlated (Yang and Juskiw, 2011). In the present study the number of productive tillers was incorporated as a covariate for analysis of grain yield data. The number or productive tillers shared a high positive correlation with grain yield as summarised in Chapter 3. Furthermore, in a study by Maman et al. (2004) path analysis revealed the yield of grain sorghum and pearl millet as highly and directly influenced by number of panicles per square meter. Hence this trait was selected as a suitable covariate in the current study. ANCOVA was performed for yield per plot in grams per plot, at the 5% probability level. Mean comparisons were performed by the least significant differences (l.s.d.) and Duncan's multiple range test (DMRT) procedures each at the $P < 0.05$ probability level. The maximum and minimum values and coefficient of variation (CV) presented as percentages were also calculated. Data were analysed using GenStat 18th Edition statistical software package (Payne et al., 2015). A distinction was made between mean yield for all genotypes under non-stress and drought stress conditions, symbolised by Xp and Xs, in that order, and for grain yield for each genotype under non-stress and drought stress, symbolised by Yp and Ys, respectively.

Drought tolerance indicators

Grain yield data was utilised to calculate drought tolerance indicators. Drought indices formulae were presented in detail in Table 4.1. Data for drought indices was analysed using a two-way ANOVA at the 5% probability level. Means, mean comparisons, l.s.d. (at the 5% probability level), maximum and minimum values and CV as percentages were calculated as described for yield. Data were analysed using GenStat 18th Edition statistical software package (Payne et al., 2015). Selection for genotypes exhibiting exceptional drought tolerance and high grain yield was deduced following the criteria indicated in Table 4.1

Table 4.1: Formulae and selection criteria for 9 drought selection indices as adopted from Farshadfar et al. (2012) and El-Mohsen et al. (2015)

Indices	Formula and reference	Selection criteria in desirable direction
DR	DR = Ys(Ys/Yp)/Xp (Lan, 1988)	High
HM	HM = 2(Yp*Ys)/(Yp+Ys) (Jafari et al., 2009)	High
MP	MP = (Yp + Ys)/2 (Rosielle and Hamblin, 1981)	High
SSI	SSI = (1-Ys/Yp)/(1-Xs/Xp) (Fischer and Maurer, 1978)	Low SSI< 1=above-average tolerance
STI	STI = (Ys*Yp)/ $(Xp)^2$ Fernandez (1992)	High
TOL	TOL = Yp – Ys Rosielle and Hamblin (1981)	Low
YI	YI = Ys/Xs Gauzzi et al. (1997)	High
YR	YR = 1 – (Ys/Yp) Golestani-Araghi and Assad (1998)	Low
YSI	YSI = Ys/Yp El-Mohsen et al. (2015)	High

Key: DR - drought resistance index, HM - harmonic mean of yield, MP - mean productivity, SSI - stress susceptibility index, STI - stress tolerance index, TOL - tolerance index, YI - yield stability index, YR - yield reduction, YSI - yield stability index, Xp - yield for all genotypes under non-stress, Xs - yield for all genotypes under drought stress, Yp - yield for each genotype under non-stress, Ys - yield for each genotype under drought stress.

Genotype ranking using drought tolerance indices

Ranking of genotypes was performed using Spearman ranking technique using GenStat 18th Edition statistical software package (Payne et al., 2015). Thereafter, rank mean, standard deviation of ranks and rank sum were analysed according to El-Mohsen et al. (2015) and Farshadfar (2012). The formulae applied are presented in a) and b) below. A low value of rank sum, standard deviation of ranks and mean rank indicates superior genotypes.

The standard deviation of ranks and rank sum are given in formula a) and b), respectively.

a) Standard Deviation of Ranks = $\frac{\sum_{i=1}^{m}(\mathrm{Rij}\text{-}\mathrm{Ri\ mean})}{\mathrm{n}\text{-}1}$ (Farshadfar, 2012; El-Mohsen et al., 2015)

b) Rank Sum = R*i* mean + Standard Deviation of Ranks (Farshadfar, 2012; El-Mohsen et al., 2015)

Where, R*ij* is the in vivo drought tolerance or resistance index rank, R*i* mean is the rank mean across all drought tolerance or resistance indicators for each genotype.

All data was further subjected to pairwise Pearson's correlation coefficient analysis with a two-sided test at the 5% probability level analysed using GenStat 18th Edition Statistical Software Package (Payne et al., 2015).

Multivariate analysis

Data was subjected to principal component (PC) and biplot analyses, whereby principal components were chosen based on eigenvalues greater than 1 (Jeffers, 1967). PC and biplot analyses were conducted using GenStat 18th Edition Statistical Software Package (Payne et al., 2015).

4.3 Results

4.3.1 Significance tests

Grain yield

Table 4.2 shows the significance test and mean squares for grain yield for 15 wheat genotypes evaluated under drought stress and non-stress conditions. Significant differences were detected among genotypes, the covariate and genotype by water regime interactions.

Table 4.2: Mean squares and significance tests for grain yield of 15 wheat genotypes evaluated under drought stress and non-stress conditions

Sources of variation	df	Mean square
Replication	2	122915
Genotypes (G)	14	51093 ***
Water regimes (W)	1	15094 ^{ns}
G x W	14	25616 **
Covariate	1	2239776 ***
Residual	51	10082
Total	83	

Key: df - degrees of freedom, ns - non-significant differences detected, *** - significant at $P < 0.001$ probability level, ** - significant at $P < 0.01$ probability level.

Drought tolerance indices

Table 4.3 displays significance tests as well as mean squares for 15 wheat genotypes evaluated under drought stress and non-stress conditions involving 9 drought tolerance indices. Significant differences were detected for the genotype effect for the majority of the drought indices except for drought indices SSI, TOL and YR.

Table 4.3: Signifcant tests and mean squares for 15 wheat genotypes using 9 drought tolerance indices evaluated under drought stress and non-stress conditions

Sources of variation	df	DR	HM	MP	SSI	STI	TOL	YI	YSI	YR
Replication	2.0	5.0	34893.0	43297.0	54.1	0.7	19629.0	0.0	0.1	0.2
Genotypes (G)	14.0	0.9**	72642.0***	74722.0**	28.4^{ns}	1.8***	99114.0^{ns}	0.3**	0.2**	0.3^{ns}
Residual	22.0	0.3	11674.0	17902.0	28.0	0.3	163865.0	0.1	0.0	0.7
Total	38.0									

Key: df - degrees of freedom, DR - drought resistance index, HM - harmonic mean of yield, MP - mean productivity, SSI - stress susceptibility index, STI - stress tolerance index, TOL - tolerance index, YI - yield stability index, YSI - yield stability index, YR - yield reduction, ns - non-significant differences detected, *** - significant at $P < 0.001$ probability level, ** - significant at $P < 0.01$ probability level, * - significant at $P < 0.05$ probability level.

4.3.2 Mean responses of genotypes for grain yield and drought indices

Table 4.4 presents mean values and mean comparison of genotypes for Yp, Ys and 9 drought tolerance indices of wheat genotypes evaluated under drought stress and non-stress conditions. Minimum and maximum values recorded for mean Yp were for genotypes SYM2016-047 and SYM2016-044 with values of 254.5 g.plot^{-1} and 705.9 g.plot^{-1}, respectively. The overall mean Yp recorded was 448.6 g.plot^{-1}. Superior Yp was detected for genotypes SYM2016-044 and SYM2016-008 with values of 705.9 g.plot^{-1} and 627.5 g.plot^{-1}, respectively. The lowest and highest values of Ys were relatively poor compared to Yp with values of 283 g.plot^{-1} and 576.2 g.plot^{-1} for genotypes SYM2016-027 and SYM2016-016, in that order. Whilst mean Ys was recorded as 423.7 g.plot^{-1} and genotypes SYM2016-016 and SYM2016-012 presented above-average Ys with values of 576.2 g.plot-1 and 560.0 g.plot-1, respectively.

Table 4.4: Mean values for 15 wheat genotypes evaluated using grain yield and 9 drought tolerance indices under non-stress and drought stress conditions

Genotype	Yp	Ys	DR	HM	MP	SSI	STI	TOL	YI	YR	YSI
SYM2016-004	436.7 ^{abcde}	416.1 ^{abcd}	3.7 ^{f}	219.2 ^{abc}	225.6 ^{a}	-13.5 ^{a}	0.3 ^{a}	-7.6 ^{a}	0.5 ^{ab}	-0.1 ^{a}	1.1 ^{a}
SYM2016-006	269.3 ^{a}	422.0 ^{abcd}	0.7 ^{abcd}	696.7 ^{f}	701.4 ^{d}	14.8 ^{c}	2.6 ^{e}	-14.5 ^{a}	1.6 ^{d}	0.0 ^{a}	0.9 ^{a}
SYM2016-008	627.6 ^{ef}	399.2 ^{abcd}	1.0 ^{bcde}	417.7 ^{cd}	555.4 ^{bcd}	12.8 ^{abc}	1.3 ^{abc}	516.7 ^{a}	0.7 ^{ab}	0.6 ^{a}	0.4 ^{a}
SYM2016-010	508.1 ^{cde}	497.6 ^{bcde}	2.0 ^{ef}	343.2 ^{abcd}	346.2 ^{abc}	13.1 ^{abc}	0.7 ^{ab}	-48.3 ^{a}	0.7 ^{ab}	-0.1 ^{a}	1.1 ^{a}
SYM2016-012	542.3 ^{cdef}	560.0 ^{def}	1.7 ^{cdef}	273.1 ^{abcd}	406.1 ^{abc}	14.0 ^{abc}	0.6 ^{ab}	166.6 ^{a}	0.7 ^{ab}	0.3 ^{a}	1.3 ^{a}
SYM2016-014	296.7 ^{ab}	353.4 ^{abc}	2.6 ^{ef}	199.9 ^{ab}	237.0 ^{a}	-13.7 ^{a}	0.3 ^{a}	78.0 ^{a}	0.5 ^{ab}	0.1 ^{a}	1.0 ^{a}
SYM2016-016	443.5 ^{abcde}	576.2 ^{def}	0.6 ^{ab}	628.0 ^{ef}	674.1 ^{d}	14.1 ^{abc}	2.4 ^{de}	249.5 ^{a}	1.3 ^{cd}	0.3 ^{a}	0.7 ^{a}
SYM2016-019	427 ^{abcde}	390.3 ^{abcd}	1.2 ^{bcde}	376.7 ^{bcd}	449.1 ^{abcd}	-11.2 ^{ab}	1.0 ^{abc}	240.3 ^{a}	0.8 ^{abc}	0.3 ^{a}	1.0 ^{a}
SYM2016-027	423.4 ^{abcd}	283 ^{a}	1.4 ^{bcdef}	144.5 ^{a}	298.1 ^{ab}	13.7 ^{abc}	0.3 ^{a}	366.3 ^{a}	0.4 ^{a}	0.3 ^{a}	1.0 ^{a}
SYM2016-029	260.1 ^{a}	294.5 ^{ab}	1.6 ^{bcdef}	300.9 ^{abcd}	316.2 ^{ab}	10.5 ^{ab}	0.6 ^{ab}	-65.7 ^{a}	0.8 ^{abc}	-0.3 ^{a}	1.5 ^{a}
SYM2016-037	544.2 ^{cdef}	350.1 ^{abc}	0.3 ^{a}	455.6 ^{de}	597.2 ^{cd}	13.3 ^{abc}	1.8 ^{cde}	341.3 ^{a}	1.0 ^{bc}	0.2 ^{a}	2.1 ^{a}
SYM2016-038	441.9 ^{abcde}	439.8 ^{abcde}	2.3 ^{ef}	257.3 ^{abcd}	281.0 ^{a}	-13.5 ^{a}	0.4 ^{a}	40.9 ^{a}	0.8 ^{abc}	0.1 ^{a}	1.1 ^{a}
SYM2016-042	548.2 ^{cdef}	441.8 ^{abcde}	2.0 ^{def}	409.4 ^{bcd}	448.4 ^{abcd}	12.5 ^{abc}	1.0 ^{abc}	222.2 ^{a}	0.7 ^{ab}	0.4 ^{a}	0.5 ^{a}
SYM2016-044	705.9 ^{f}	490.8 ^{bcde}	0.6 ^{abc}	448.4 ^{de}	604.3 ^{cd}	13.3 ^{abc}	1.523 ^{bcd}	424.9 ^{a}	0.9 ^{abc}	0.4 ^{a}	0.8 ^{a}
SYM2016-047	254.5 ^{a}	440.8 ^{abcde}	2.1 ^{ef}	229.3 ^{abc}	386.5 ^{abc}	14.5 ^{bc}	0.5 ^{ab}	233.5 ^{a}	0.6 ^{ab}	0.4 ^{a}	1.2 ^{a}
Mean	448.6	423.7	1.3	360.0	435.1	13.2	1.0	182.9	0.8	0.2	1.0
CV (%)	2.6	2.6	28.6	13.4	12.3	9.2	20.7	19.8	4.9	8.4	23.3
l.s.d. ($P < 0.05$)	168.0	168.0	0.9	183	226.6	14.6	0.9	685.5	0.5	1.4	2.0

Key: DR - drought resistance index, HM - harmonic mean of yield, MP - mean productivity, SSI - stress susceptibility index, STI - stress tolerance index, TOL - tolerance index, YI - yield stability index, YSI - yield stability index, YR - yield reduction, Yp - yield for each genotype under non-stress, Ys - yield for each genotype under drought stress, Yp-mean grain yield of each genotype under non-stress, Ys-mean grain yield of each genotype under stress, CV (%) - coefficient of variation expressed as a percentage, l.s.d. ($P < 0.05$) - least significant difference at 5% probability level.

Means in a column followed by the same letter are not significantly different at the 5% probability level.

Mean value for drought tolerance indicator DR was 1.3, whilst DR values ranged between 0.3 and 3.7 for genotypes SYM2016-037 and SYM2016-004, respectively (Table 4.4). Genotypes identified with superior DR values were SYM2016-004 and SYM2016-014 with values of 3.7 and 2.6, in that order. The mean value detected for drought selection index HM was 360.0 and values ranged from 144.5 for genotype SYM2016-027 to a maximum of 696.7 for genotype SYM2016-006. Genotypes which expressed superior HM values were SYM2016-006 and SYM2016-016 with values of 696.7 and 628.0, in that order. Conversely, drought tolerance index MP was detected with a mean of 435.1, whilst minimum and maximum values were 225.4 and 701.4 for genotypes SYM2016-004 and SYM2016-006, in that order. The highest values for MP were 701.4 and 674.1 for genotypes SYM2016-006 and SYM2016-016, respectively. For drought tolerance indicator SSI, mean SSI, minimum and maximum values were 13.2 and -13.7 for genotype SYM2016-014 and 14.8 for genotype SYM2016-006, in that order. Superior SSI values were realised for genotypes SYM2016-014 as well as SYM2016-004 with values of -13.7 and -13.5, respectively. For STI drought tolerance indicator, values ranged between 0.3 for genotypes SYM2016-004, SYM2016-014, SYM2016-027 and 2.6 for genotype SYM2016-006, whilst the mean STI was recorded as 1.0. The highest values for drought selection index were 2.6 and 2.4 for genotypes SYM2016-006 and SYM2016-016, respectively (Table 4.4).

Conversely, minimum and maximum values for TOL drought tolerance index were -65.7 and 516.7 for genotypes SYM2016-029 and SYM2016-008, respectively. TOL mean value was 183.0. Genotypes with lowest values were SYM2016-029 and SYM2016-010 with values -65.7 and -48.3, respectively. For drought tolerance index YI, the detected mean value was 0.8. Minimum and maximum values were for genotypes SYM2016-027 and SYM2016-006 which had YI values of 0.4 and 1.6, in that order. On the other hand, the highest values were for genotypes SYM2016-006 and SYM2016-016 with 1.6 and 1.3, in that order. Drought tolerance index, YSI, the mean value was 1.0 and values ranged from 0.4 for genotype SYM2016-008 to 2.1 to SYM2016-037. Superior genotypes detected were SYM2016-037 and SYM2016-029 with values of 2.1 and 1.5, respectively. The mean value detected for drought tolerance index YR was 0.2, whereas values ranged from -0.3 to 0.6 for genotypes SYM2016-029 and SYM2016-008, in that order. Whist genotypes recorded with superior YR values were SYM2016-029 and SYM2016-010, respectively (Table 4.4).

4.3.3 Mean rank, rank sum and standard deviation of ranks for wheat genotypes assessed through drought selection indices

Results of Spearman’s rank of wheat genotypes based on 9 drought indices and grain yield are presented in Table 4.5. Wheat genotypes detected with low values for rank mean, standard deviation of ranks and sum of ranks were regarded as superior. Lowest values for mean rank were 6 for genotypes SYM2016-010 and SYM2016-016, whereas highest values were detected for genotypes SYM2016-027 and SYM2016-047 with values of 12 and 10, respectively. The genotypes with lowest for standard deviation of ranks, both with a value of 2, were SYM2016-019 and SYM2016-027. Maximum values were detected for genotypes SYM2016-006 and SYM2016-016 each with a value of 6. On the other hand, lowest values for sum of ranks were 68 and 70 for genotypes SYM2016-010 and SYM2016-006, whilst maximum values were 134 and 108 for genotypes SYM2016-027 and SYM2016-047, respectively. Overall, genotypes were ranked differently for each of the variables.

Table 4.5: Spearman ranks for 15 wheat genotypes using 9 drought selection indices and grain yield under drought stress and non-stress conditions

Genotype	Yp	Ys	DR	HM	MP	SSI	STI	TOL	YI	YR	YSI	Mean rank	Std dev rank	Sum of rank
SYM2016-004	9	9	1	13	15	2	14	4	13	3	5	8	5	88
SYM2016-006	13	8	12	1	1	15	1	3	1	4	11	6	6	70
SYM2016-008	2	10	11	5	5	7	5	15	11	15	15	9	5	101
SYM2016-010	6	3	5	8	10	8	8	2	9	2	7	6	3	68
SYM2016-012	5	2	7	10	8	12	9	7	8	8	3	7	3	79
SYM2016-014	12	12	2	14	14	1	15	6	14	6	8	9	5	104
SYM2016-016	7	1	14	2	2	13	2	11	2	10	13	7	5	77
SYM2016-019	10	11	10	7	6	4	6	10	6	9	10	8	2	89
SYM2016-027	11	15	9	15	12	11	13	13	15	11	9	12	2	134
SYM2016-029	14	14	8	9	11	5	10	1	5	1	2	7	5	80
SYM2016-037	4	13	15	3	4	9	3	12	3	7	1	7	5	74
SYM2016-038	8	7	3	11	13	3	12	5	7	5	6	7	3	80
SYM2016-042	3	5	6	6	7	6	7	8	10	14	14	8	4	86
SYM2016-044	1	4	13	4	3	10	4	14	4	13	12	7	5	82
SYM2016-047	15	6	4	12	9	14	11	9	12	12	4	10	4	108

Key: DR - drought resistance index, HM - harmonic mean of yield, MP - mean productivity, SSI - stress susceptibility index, STI - stress tolerance index, TOL - tolerance index, YI - yield stability index, YSI - yield stability index, YR - yield reduction, Yp - yield for each genotype under non-stress, Ys - yield for each genotype under drought stress, std dev ranks - standard deviation of ranks

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4.3.4 Correlation among drought tolerance indices and grain yield

Results of the correlation analysis of grain yield and 9 drought tolerance indices for 15 wheat genotypes evaluated under drought stress and non-stress conditions are presented in Table 4.6.

Spearman's rank correlation

A significant correlation was detected between Yp and Ys with Spearman's rank correlation coefficient of 0.4. A positive correlation was detected between Yp and drought indices HM, MP, STI, TOL and YR with correlation coefficients of 0.44, 0.38, 0.42, 0.5, and 0.46, respectively. Conversely, negative significant correlation was recorded between Yp and drought indices DR and YSI with values of -0.36 and -0.38, in that order. A significant correlation was realised between Ys and drought index SSI with a correlation coefficient of 0.39.

Among the different drought tolerance indices, DR had negative significant correlation with HM, MP, SSI, STI, TOL and YI with -0.8, -0.87, -0.54, -0.86, -0.56 and -0.7, in that order. In contrast, HM was recorded with positive significant correlations with drought indices MP, SSI, STI and YI with correlation coefficients of 0.93, 0.42, 0.98 and 0.84, while a negative correlation with YSI was detected with a value of -0.4. Similarly, MP had positive correlations with SSI, STI, TOL, YI and YR, with 0.64, 0.97, 0.43, 0.75 and 0.4 while a negative correlation was detected for YSI with -0.45, respectively. SSI was significantly associated with STI with correlation coefficients of 0.55. Whilst, significant correlations were noted between STI and YI and YSI with correlation coefficients of 0.81 and -0.4, respectively. TOL had significant correlations with YR and YSI, with correlation coefficients of 0.86 and -0.43, respectively. Whereas, YR had a significant correlation with YSI, with a value of -0.6.

Pearson correlation

Indices TOL and YR were significantly associated with only Yp with coefficients of 0.61 and 0.51, respectively. Moreover, TOL was significantly correlated with YR with a coefficient of 0.91. Furthermore, significant pairwise correlations were detected between drought tolerance indicators. Drought index DR was negatively correlated with HM, MP, SSI, STI, TOL and YI with coefficients of -0.69, -0.85, -0.62, -0.78, -0.54 and -0.53, in that order. On the other hand, HM had markedly high coefficients of 0.92, 0.97 and 0.94 for indices MP, STI as well as YI, in that order.

Table 4.6: Non parametric Spearman’s rank correlation (top diagonal) and parametric Pearson’s correlation (bottom diagonal) correlations for 15 wheat genotypes using 9 drought tolerance indices and grain yield subjected to drought stress and non-stress conditions

Variables	DR	HM	MP	SSI	STI	TOL	YI	YR	YSI	Yp	Ys
DR	1	-0.80 ***	-0.87 ***	-0.54 *	-0.86 ***	-0.56 **	-0.70 **	-0.33 ^{ns}	0.29 ^{ns}	-0.36 *	0.00 ^{ns}
HM	-0.69 **	1	0.93 ***	0.42 *	0.98 ***	0.22 ^{ns}	0.84 ***	0.18 ^{ns}	-0.40 *	0.44 ^{ns}	0.33 ^{ns}
MP	-0.85 ***	0.92 ***	1	0.64 **	0.97 ***	0.43 *	0.75 ***	0.40 *	-0.45 *	0.38 *	0.33 ^{ns}
SSI	-0.62 **	0.42 ^{ns}	0.58 *	1	0.55 **	0.26 ^{ns}	0.37 *	0.29 ^{ns}	-0.09 ^{ns}	-0.03 ^{ns}	0.39 *
STI	-0.78 ***	0.97 ***	0.96 ***	0.47 ^{ns}	1	0.32 ^{ns}	0.81 ***	0.25 ^{ns}	-0.40 *	0.42 *	0.32 ^{ns}
TOL	-0.54 *	0.11 ^{ns}	0.44 ^{ns}	0.35 ^{ns}	0.24 ^{ns}	1	-0.05 ^{ns}	0.86 ***	-0.43 *	0.50 **	-0.05 ^{ns}
YI	-0.63 **	0.94 ***	0.83 ***	0.36 ^{ns}	0.91 ***	-0.11 ^{ns}	1	-0.18 ^{ns}	-0.03 ^{ns}	0.16 ^{ns}	0.27 ^{ns}
YR	-0.36 ^{ns}	0.12 ^{ns}	0.41 ^{ns}	0.26 ^{ns}	0.22 ^{ns}	0.90 ***	-0.09 ^{ns}	1	-0.60 **	0.46 *	0.18 ^{ns}
YSI	-0.02 ^{ns}	-0.23 ^{ns}	-0.19 ^{ns}	-0.03 ^{ns}	-0.15 ^{ns}	-0.27 ^{ns}	-0.01 ^{ns}	-0.49 ^{ns}	1	-0.38 ^{ns}	-0.25 ^{ns}
Yp	-0.28 ^{ns}	0.15 ^{ns}	0.31 ^{ns}	0.22 ^{ns}	0.16 ^{ns}	0.61 *	-0.06 ^{ns}	0.51 *	-0.28 ^{ns}	1	0.40 *
Ys	-0.06 ^{ns}	0.39 ^{ns}	0.36 ^{ns}	0.21 ^{ns}	0.32 ^{ns}	0.00 ^{ns}	0.37 ^{ns}	0.21 ^{ns}	-0.32 ^{ns}	0.37 ^{ns}	1

Key: DR - drought resistance index, HM - harmonic mean of yield, MP - mean productivity, SSI - stress susceptibility index, STI - stress tolerance index, TOL - tolerance index, YI - yield stability index, YR - yield reduction, YSI - yield stability index, Yp - yield for each genotype under non-stress, Ys - yield for each genotype under drought stress, ns - non-significant differences detected, *** - significant at P < 0.001 probability level, ** - significant at P < 0.01 probability level, * - P < 0.05 probability level.

Drought indicator MP, similarly was significantly correlated with STI, YI and SSI with exceptionally high coefficients of 0.96, 0.84 and 0.58, respectively. Also, stress indicator STI was significantly correlated with YI, with a coefficient of 0.91 (Table 4.6).

4.3.5 Principal component analysis (PCA)

Table 4.7 presents principal component analysis for 15 wheat genotypes assessed using 9 drought tolerance indices and grain yield. Total percentage variation recorded was 82.2% with PC1, PC2 and PC3 accounting for 47.67%, 22.37% and 12.18% of the explained variance, respectively. Drought tolerance indices HM, MP, STI and YI contributed positive and higher factor loadings for PC1 with values of 0.39, 0.43, 0.41 and 0.34, in that order. Whilst positive values of drought tolerance indices TOL and YR were associated with Yp under PC2 with factor loadings of 0.50, 0.51 and 0.41, respectively. In contrast, positive values of DR were associated with positive values of Ys for PC3 with proportions of 0.38 and 0.63, in that order.

Table 4.7: Principal component analysis for 15 wheat genotypes assessed using 9 drought tolerance indices and grain yield under drought stress and non-stress condition

Variables	PC1	PC2	PC3
DR	-0.373	0.015	0.375
HM	0.390	-0.233	0.140
MP	0.431	-0.061	-0.039
SSI	0.275	0.030	-0.257
STI	0.406	-0.192	0.005
TOL	0.215	0.499	-0.283
YI	0.340	-0.380	0.099
YR	0.204	0.511	-0.014
YSI	-0.118	-0.285	-0.524
Yp	0.174	0.408	0.128
Ys	0.186	0.029	0.626
Eigenvalues	5.244	2.460	1.340
Explained variance (%)	47.670	22.370	12.180
Explained cumulative variance (%)	47.670	70.040	82.220

Key: DR - drought resistance index, HM - harmonic mean of yield, MP - mean productivity, SSI - stress susceptibility index, STI - stress tolerance index, TOL - tolerance index, YI - yield stability index, YR - yield reduction, YSI - yield stability index, Yp - yield for each genotype under non-stress, Ys - yield for each genotype under drought stress, PC1 - principal component 1, PC2 - principal component 2, PC3 - principal component 3.

4.3.6 Biplot analysis

Figure 4.1 presents a biplot analysis based on PC analysis of 15 wheat genotypes using 9 drought tolerance indices and grain yield under drought stress and non-stress conditions. PC1 and PC2 accounted for 70.04% variation and each component contributed at 47.67% and 22.37%, respectively. An acute angle between vectors was regarded as indicating close association of traits. An acute angle between vectors for all drought tolerance indices apart from DR and YSI which were closely associated. An overlap of vectors for Yp with TOL and YR was noted. A similar overlapping of vectors was also noted for Yp and SSI.

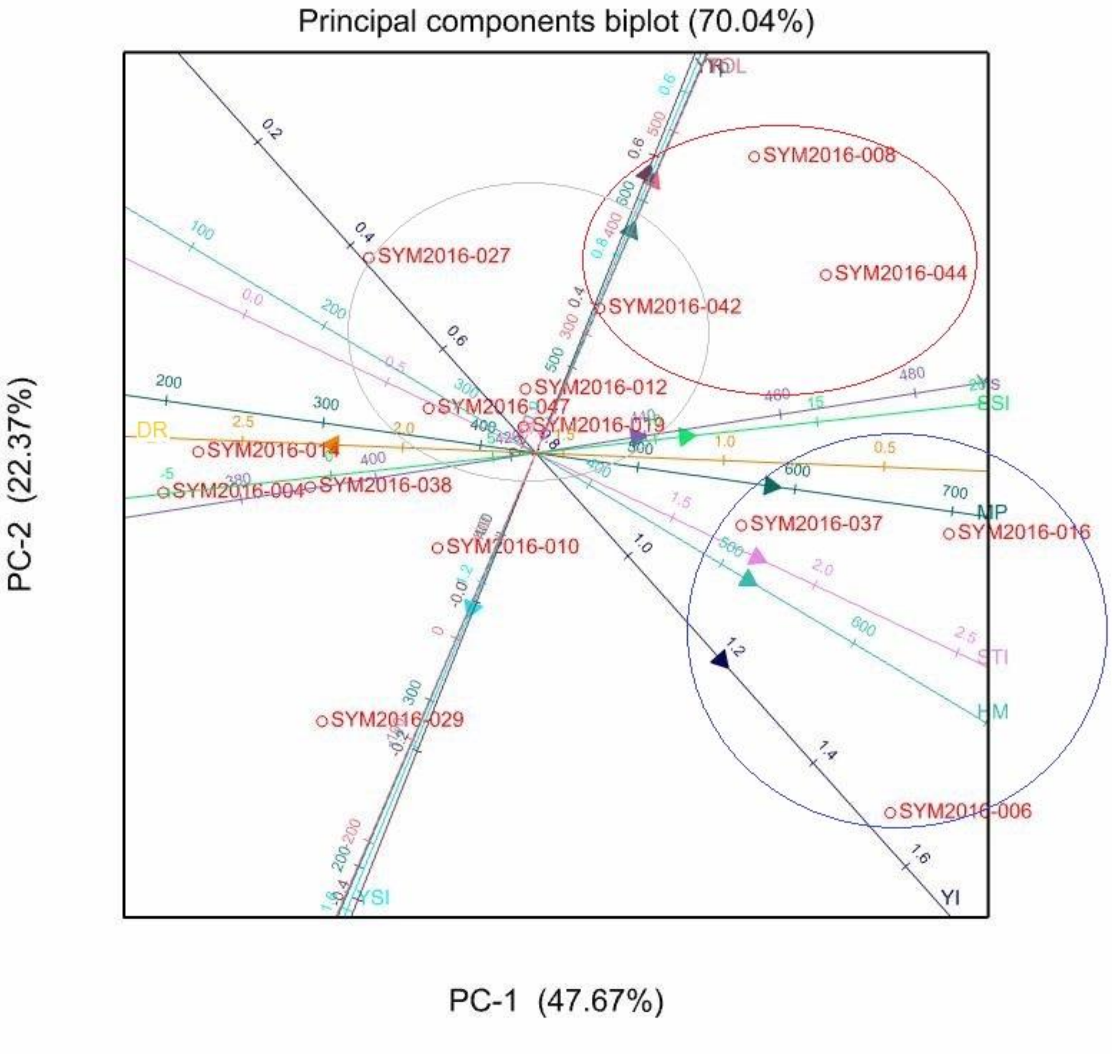

Figure 4.1: Principal components biplot for 15 wheat genotypes using 9 drought selection indices and grain yield under drought stress and non-stress conditions

Key: DR - drought resistance index, HM - harmonic mean of yield, MP - mean productivity, SSI - stress susceptibility index, STI - stress tolerance index, TOL - tolerance index, YI - yield stability index, YR - yield reduction, YSI - yield stability index, Yp - yield for each genotype under non-stress, Ys - yield for each genotype under drought stress, PC1 - principal component 1, PC2 - principal component 2

The genotype responses to drought stress were classified according to Fernandez (1992). Genotypes SYM2016-006, SYM2016-016 and SYM2016-037 were considered Group A and were clustered around vectors of SSI, MP, STI and HM. Conversely, genotypes of Group B were SYM2016-008, SYM2016-042 and SYM2016-044 which were closely associated with TOL, YR and Yp. Whilst, genotypes SYM2016-027, SYM2016-047, SYM2016-012 and SYM2016-019 were classified under Group C. However, genotypes SYM2016-004, SYM2016-010, SYM2016-014, SYM2016-029 and SYM2016-038 were all closely associated with YSI and DR and were regarded as Group D.

4.4 Discussion

4.4.1 Comparison of the different drought tolerance indices

Significant genotype by water regime interaction was detected under greenhouse condition (Table 4.2). Establishing a significant effect for genotypes and water regime interaction was vital to warrant further detailed genetic analyses as reported by Sahar et al. (2016) and Abraha et al. (2017).

Non-significant Pearson correlation coefficients of 0.37 was detected between seed yield under non-stress and drought stress conditions suggesting that high yield potential under non-stress could not assure high seed yield under drought stress condition. Similar findings were reported by Sio-Se Mardeh et al. (2006), Drikvank et al. (2012) and Sahar et al. (2016). This was contrasted with significant correlation between grain yield under drought stress and non-stress detected from Spearman’s rank correlation.

Significant variation between genotypes was detected from the ANOVA for drought indices DR, HM, MP, STI, YI as well as YSI (Table 4.3). Significant Spearman’ rank correlations were detected between grain yield under non-stress and drought indices HM, MP, STI, TOL, YR and YSI. Moreover, Ys was significantly correlated with SSI according to Spearman’ rank correlations. Pearson correlation, the parametric test, only detected significant correlations between grain yield under non-stress and TOL and YR. Arguably, in a study by Jafari et al. (2009), drought tolerance indices MP and STI were identified to exhibit significant correlations of 0.9 and 0.88 and 0.89 and 0.9 with seed yield under non-stress and drought stress, in that order.

In the current study, principal component and biplot analyses revealed PC1, PC2 and PC3 to be regarded as components associated with yield across water regimes, grain yield under non-stress conditions and grain yield under drought stress, respectively. This was noted by deliberate grouping of drought tolerance indices HM, MP, SSI and STI together for PC1, drought indices TOL and YR with Yp under PC2, as well as drought index DR and Ys under PC3.

4.4.2 Grain yield across water regimes

Selection for variables with high values in PC1 was considered favourable in the current study. This component was associated with high and stable seed yield under stress and non-stress conditions. Similarly, Drikvank et al. (2012) reported PC1 as being regarded for selection of superior seed yield under drought stress and non-stress conditions, however this component was associated with high values of drought tolerance indices MP, GMP, STI as well as high Ys and Yp. Furthermore, drought index STI was reported by Ramirez and Kelly (1998), as cited by Dorostkar et al. (2015), as a suitable indicator and was recommended as a principal selection index, this was followed by selection index SSI. El-Mohsen et al. (2015) likewise, reported PC1 to be associated with seed yield under drought stress and non-stress, however PC2 was related with grain yield under drought stress and poor tolerance to drought stress.

4.4.3 Grain yield under non-stress

In contrast, low PC2 was favourable to prevent selection of genotypes with high yield under non-stress only, thus jeopardising yield under stress. Non-significant differences were detected for the drought indices TOL and YR from the ANOVA, limiting the reliability of these drought indices for characterisation. In contrast, Abraha et al. (2017) reported TOL along with HM, GMP, K1STI K2STI, MP, STI and SSI to have significant differences for genotype effect. Interestingly, significant positive pairwise coefficients of correlation were detected between TOL and YR. Thus, in the current study, high values of TOL and YR were associated with high yield reduction under non-stress. Abdolashahi et al. (2013) also reported TOL to be associated with high yield under drought stress. Also, noted from poor pairwise correlation coefficients between Yp and Ys, high grain yield under non-stress cannot assure high grain yield under drought stress, as reported by Abraha et al. (2017). Similar findings were reported by Dodig et al. (2012)

4.4.4 Grain yield under drought stress

Low PC3 was considered favourable, in the current study, to prevent selection of genotypes with high yield under stress yet providing poor yield under non-stress condition. Significant genotypic diversity was detected in the ANOVA for the drought index DR, indicating the reliability of this trait for selection. A negative factor loading was detected between DR and PC1 (Table 4.7). A similar relationship was illustrated from the biplot analysis with vectors for DR and the drought indices associated with PC1 (Figure 4.1). However, findings by Jafari et al. (2009) contradict this finding, indicating DR and SSI are suitable for selection of high seed yield under drought stress and non-stress. Whereas, Farshadfar et al. (2012) reported significant association between grain yield under stress and non-stress conditions with DR. In the current study, drought index DR was associated with genotypes with poor seed yielders belonging to Group D according to Fernandez (1992), as illustrated in Figure 4.1. Interestingly, early flowering and maturity, in the current study were antagonistic with superior grain yield as reported in Chapter 3.

4.4.5 Screening for drought tolerant and high yielding wheat genotypes

Genotypes with high seed yield both under drought stress and non-stress belonging to Group A, as classified by Fernandez (1992), were SYM2016-006, SYM2016-016 and SYM2016-037. Correspondingly, these genotypes were associated with MP, HM, STI, YI, Ys and Yp. HM, MP, STI and YI also demonstrated significant variation among different genotypes for the ANOVA. Genotypes SYM2016-004, SYM2016-014 and SYM2016-038 were detected with relatively similarly poor yield mean values according to Duncan’s multiple range test associated with similar ranking for different drought indices. These genotypes were classified under Group D (Fernandez, 1992) as indicated in Figure 4.1. In spite of this, early flowering and maturity detected for SYM2016-029, SYM2016-014 and SYM2016-038 was regarded a favourable strategy to minimise the impact of drought stress on wheat genotypes (Araus et al., 2002; Farooq et al., 2014).

Genotypes with high yield and late flowering and maturity were clustered with Yp, Ys, drought tolerance indices HM, MP, STI and YI (Figure 4.1). Whereas, genotypes characterised by poor yield and early flowering and maturing were clustered with DR and YSI (Figure 4.1). This indicated beneficial phenotypic diversity resulting in a heterotic pattern, as diversity encourages exceptionally high heterosis after hybridisation (Bernado, 2002; Said, 2014).

4.5 Conclusions

High phenotypic diversity was observed between the genotypes investigated in the present study. Superior grain yield across drought stress and non-stress was noted for SYM2016-006, SYM2016-016 and SYM2016-037. Conversely, genotypes SYM2016-029, SYM2016-014 and SYM2016-038 had short maturity period which were vital for drought escape (Farooq et al., 2014; Abraha et al., 2017). These groups of genotypes were regarded valuable for future genetic analysis.

Drought indices MP, HM, STI and YI was most suitable for detection of high yield under drought stress and non-stress conditions, whereas genotypes with a favourable short maturity period were detected by DR.

Spearman's rank correlation was more effective in detecting associations among drought indices and yield, than Pearson correlation analysis. Principal components and biplot analyses effectively summarised information and detected associations among the different indices and wheat genotypes providing simplified visualisation of these relationships.

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An overview of findings and implications of the study

Introduction and study objectives

Bread wheat (*Triticum aestivum* L.) is one of the world's most important commodity crop. About 50% of wheat production zones in the world experience drought stress associated with climate change. An estimated 60% of sub-Saharan Africa (SSA) is experiencing recurrent drought. South Africa is water scare country with a decreasing trend in total annual rainfall hindering potential crop production. Drought stress is detrimental to wheat production and productivity in the country. Drought tolerant wheat cultivars are yet to be developed and released in South Africa. Currently, South African wheat demand exceeds domestic supply consequently more wheat is imported from the major wheat producing countries such as the Czech Republic and Germany. Wheat import minimizes profit gains along the wheat value chains such as commercial and small-holder farmers, millers and bakers in the country. Thus, developing and deploying drought tolerant and drought adapted wheat cultivars is an important breeding goal of the wheat industry for enhancing local production and maximising yield gains. Integrative pre-breeding techniques such as genotypic and phenotypic characterisation ensure an accurate selection of potential drought tolerant parents for breeding. Genetic diversity and population structure analysis improve understanding of newly introduced germplasm. This can improve selection efficiency and breeding timeframe. Hence, the objectives of this study were:

i. To determine the genetic diversity and population structure of forty-seven diverse bread wheat genotypes introduced from CIMMYT using ten selected polymorphic SSR markers

ii. To characterise fifteen bread wheat genotypes introduced from CIMMYT using physiological and agronomic traits

iii. To assess drought tolerance amongst fifteen selected bread wheat genotypes using nine drought tolerance indices

Research findings in brief

Genetic characterisation of selected bread wheat genotypes through SSR markers

This study analysed the genetic diversity and population structure of forty-seven bread wheat genotypes introduced from CIMMYT using ten SSR molecular markers. The core findings of this chapter were:

- Molecular markers used were highly polymorphic, with highest PIC values recorded for XGWM 132, WMS 179 and WMS 30 with 0.93, 0.89 and 0.89, respectively.

- Genotypes were grouped into 3 distinct clusters, whereby Clusters A and C consisted of the most genetically distinct genotypes.

- Among the 4 populations, populations II and III expressed a distinct heterotic pattern favourable for selection of parents for crosses.

- Analysis of molecular variance (AMOVA) indicated significant genetic diversity attributed to among populations (3% explained variance), among individuals (37% explained variance) and within individuals (60% explained variance).

- A majority of genetic diversity and population stratification was due to great proportion of private alleles in the loci of the genotypes grouped under the different populations.

- A total of 15 genotypes were selected for phenotypic characterisation based on observed variability.

- Genotypes selected for phenotypic characterisation were SYM2016-037, SYM2016-038, SYM2016-029, SYM2016-010, and SYM2016-012 from Cluster A, SYM2016-044, SYM2016-004, SYM2016-016, SYM2016-019, SYM2016-014, SYM2016-008, SYM2016-006, SYM2016-047 from Cluster B and SYM2016-042 and SYM2016-027 from Cluster C.

Phenotyping bread wheat (*Triticum aestivum* L.) genotypes for drought tolerance

Fifteen bread wheat genotypes selected through SSR markers were evaluated under field and greenhouse conditions using a randomised complete block design (RCBD) with 3 replications. Drought stress was imposed as follows: 1 week before 50% heading (WBH) and 1 week after 50% heading (WAH). A fully-irrigated water regime (ND, non-drought stress) was used as a comparative control. Genotypes were evaluated using 2 physiological and 8 morphological traits. The core findings of this chapter were:

- Analysis of variance (ANOVA) detected significant differences among genotypes and due to the genotype x test environment interaction. Genotype effect was significant for days to flowering, days to maturity, plant height, number of productive tillers, spikelets per spike, grain number and grain weight. Genotype x test environment interaction was detected for canopy temperature, days to flowering, days to maturity, plant height, spikelets per spike, grain number, 100 seed weight and grain yield.

- Correlation analysis detected significant positive correlations ($P < 0.05$) between yield with days to flowering ($r = 0.55$), days to maturity ($r = 0.54$), plant height ($r = 0.63$), number of productive tillers ($r =0 .74$), spikelets per spike ($r = 0.80$), grain number ($r = 0.571$) and 100 seed weight ($r = 0.75$) under the greenhouse condition. The number of productive tillers and the spikelets per spike were positively associated with yield under field evaluation each with $r = 0.59$.

- According to the principal component analysis (PCA), PC1 was consistently associated with yield, 100 seed weight and spikelets per spike, whereas days to flowering and maturity, plant height and canopy temperature were positively associated with either PC2 or PC3 under greenhouse condition and field test.

- Yield penalty was noted for early flowering and maturing genotypes relative to late-flowering and maturing genotypes.

- Early flowering and maturing genotypes were SYM2016-014, SYM2016-027 and SYM2016-029, whereas genotypes with a relatively high yield across test environments were SYM2016-016, SYM2016-037 and SYM2016-006. Hybridisation and fixation of resultant lines is essential to develop early maturing genotypes with stable and high yield potential.

- Favourable traits for drought tolerance screening in this study were days to flowering and maturity, plant height, canopy temperature and 100 seed weight.

- Screening for drought tolerance under greenhouse condition was more reliable than under field evaluation.

Assessment of drought tolerance in selected bread wheat (*Triticum aestivum* L.) genotypes using drought tolerance indices

Fifteen bread wheat genotypes were evaluated using 9 drought tolerance indices based on yield data. The following indices were used: drought resistance (DR), mean productivity (MP), harmonic mean of yield (HM), stress susceptibility index (SSI), stress tolerance index (STI), tolerance index (TOL), yield index (YI), yield reduction index (YR) and yield stability index (YSI). The core findings of this chapter were:

- ANOVA detected significant differences among genotypes ($P < 0.001$) and genotype by water regime interaction ($P < 0.01$) affecting yield response, while significant differences were recorded among genotypes ($P < 0.05$) for indices such as DR, HM, MP, STI, YI and YSI.

- Mean genotypes ranking was consistent for the following indices: HM, MP, STI, SSI and YI enabling selection of the genotypes SYM2016-006, SYM2016-016 and SYM2016-037 with drought tolerance.

- Spearman’s rank correlation detected significant differences ($P < 0.05$) among genotypes for yield response under drought stressed and non-stressed conditions.

- The PC analysis detected high total percentage variation of 82.2% among genotypes. Percentage variation was partitioned as follows: 47.67% for PC1, 22.37% for PC 2 and

12.18% for PC3. PC and bi-plot analyses detected associations between HM, MP, STI, YI and yield under drought stressed and non-stressed conditions.

- High yielding genotypes such as SYM2016-006, SYM2016-016 and SYM2016-037 had favourable expression for HM, MP, STI, YI and yield under drought stressed and non-stressed conditions. Conversely, DR was associated with early maturing genotypes such as SYM2016-014, SYM2016-029 and SYM2016-38. These genotypes were considered as potential parents for future crosses.

Implications of research findings to bread wheat drought tolerance research in South Africa

- Genotypes SYM2016-006, SYM2016-016 and SYM2016-037 and genotypes SYM2016-014, SYM2016-029 and SYM2016-38 had distinct heterotic pattern. SYM2016-006, SYM2016-016 and SYM2016-037 expressed exceptionally high yield yet despite late maturity. Genotypes SYM2016-014, SYM2016-029 and SYM2016-38 expressed favourable drought escape with poor seed yield. Hybridisation and fixation of the resultant lines is essential to develop early maturing genotypes with stable and high yield potential.

- High genetic and phenotypic diversity was observed for the tested bread wheat genotypes for selection. The diversity of these genotypes is favourable, particularly for incorporation of genes for drought escape and low canopy temperature.

- The canopy temperature is recommended as a high throughput and non-destructive phenotyping tool. Future studies must incorporate heat and drought responses of genotypes in terms of the canopy temperature and involving vital root traits.

- Further phenotyping across representative test environments is recommended including existing and obsolete bread wheat cultivars released in South Africa.

- A more rigorous genetic analysis involving large number of markers is recommended to validate the association of traits selected in the current study with genes of economic interest.