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INVESTIGATION INTO RESPONSE OF WHEAT GENOTYPES TO DROUGHT AND OPTIMUM CONDITIONS IN THE EASTERN CAPE PROVINCE, SOUTH AFRICA

By

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Declaration

I, Luyolo Siseko Mzileni, do hereby declare that this dissertation submitted by me for the degree of Master of Science in Agriculture (Crop Science: Plant Breeding) at the University of Fort Hare is my work to the best of my knowledge. It neither contains any information or materials previously published by another person nor materials which have been accepted for the award of any other degree at any other university. This dissertation does not contain text, tables, figures, or graphs taken from the internet, unless specifically acknowledged, and the source is being detailed in the dissertation, and in the references section. I also do hereby declare that I am fully aware of the University of Fort Hare's policy on fraudulent actions, and research ethics and I have taken every precaution to comply with the regulations. My research has been approved by the University of Fort Hare's Research Ethics Committee.

Signed:……………………………………………Date………20 April 2023……………

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Signed:…………………………………………………Date…………24 April 2023……………

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Dedication

I dedicate this dissertation to my family at large, including the late ones. Overall, to be specific, *"Father, this is for you, thank you Gadle'lithanga mfo!".*

Abstract

Wheat is generally one of the dominant crops globally, being mainly used for human food and livestock feed. Due to climate change, drought makes it challenging to produce enough wheat mostly under dryland production regions in South Africa. Drought stress has severely reduced wheat yield by up to 70%, and adversely compromised wheat grain quality. The adoption of drought-tolerant cultivars offers a sustainable and low-cost solution for increasing wheat yields and minimise importing the crop to meet national requirements. The main objective of this study was to investigate the response of different wheat genotypes to drought and optimum conditions in the Eastern Cape Province, South Africa. Forty diverse wheat genotypes were evaluated in this study. The specific objectives were: (i) to evaluate the response of wheat genotypes under optimum and drought-stressed field conditions; (ii) to determine the effect of terminal drought stress on wheat grain quality composition; and (iii) to identify appropriate drought tolerance indices that can be used as selection tools under field conditions. This study was conducted in the field using a 5x8 alpha lattice design, replicated twice under two water regimes (drought and optimum) over two consecutive winter seasons of 2020 and 2021 at two different sites namely University of Fort Hare Research Farm in Alice, and Zanyokwe irrigation scheme in Keiskamahoek. Drought stress was imposed from 50% flowering up to physiological maturity. Data on agro-physiological traits such as duration to heading (DTH); flowering (DTF); maturity (DTM); plant height (HT); spike length (SL); number of spikelets per spike (SPS); kernels per spike (KPS); and grain yield (GY (kg/ha)) was subjected to the analysis of variance using Genstat 18th edition. As the study took place over two sites, a combined ANOVA table revealed significant differences ($p<0.001$) among genotypes, and all interactions such as genotype by water regime (G*WR); genotype by seasons (G*S) for all studied traits. Notably, the extent and severity of drought differed between geographical regions and between seasons. This necessitated the adoption of the additive main effect and multiplicative interaction analysis (AMMI) for the identification of stable genotypes under two different water regimes over two sites. Regarding grain yield, superior and/or stable genotypes included G5 (4334 kg/ha under optimum, and 2871kg/ha under drought), and G22 (4418 kg/ha under optimum, and 2624kg/ha under drought) at the UFH site. G21 (3194 kg/ha under optimum, and 2938 kg/ha under drought), G33 (2552kg/ha under optimum, and 3810 kg/ha under drought), and G35 (2688 kg/ha under optimum, and 3309 kg/ha under drought) at the ZAN site. Stable genotypes across sites included G21 and G33. There were generally weak correlations between agro-physiological traits and grain yield.

From the experiment, grain quality traits such as fixed protein (PF); wet gluten (WG); hectolitre mass (HLM); and thousand kernel weight (TKW) were also examined. A combined ANOVA revealed significant differences ($p<0.001$) among the interaction of genotypes by environments (G*E) for all traits except PF. This implies that the performance of wheat genotypes across sites was also different, and therefore, necessitated separate analysis of variance for each site. Significant differences ($p<0.001$) among genotypes (G), water regimes (WR), and the interaction of genotypes by water regime (G*WR) were observed for all studied quality traits except PF in both sites. G*WR showed no significant differences for TKW in the ZAN site. The stability in the performance of genotypes across water regimes was further determined. G38 was stable for wet gluten; G31 and G26 were stable for PF; G36 was stable for HLM; and G11, G15, and G29 were stable for TKW at the UFH site. G6 was stable for both WG and PF; G13 and G15 were stable for HLM; and G35, G21, and G40 were stable for TKW at the ZAN site. These results suggest that the quality of wheat grains was affected under drought stress conditions except PF.

Average grain yield data under both stressed (Ys) and optimum (Yp) conditions was used to compute a number of different drought tolerance indices. These include mean productivity (MP); geometric mean productivity (GMP); harmonic mean (HM); Tolerance index (TOL), stress susceptible index (SSI), sensitive drought index (SDI), and stress tolerance index (STI). The aim was to identify appropriate drought tolerance indices that can be used as selection tools under drought stress. MP, GMP, and HM were the more appropriate indices as they had a strong and positive correlation with grain yield under both drought and optimum conditions. However, genotypes G5, G22, G8, and G21 were more tolerant and stable as they showed high mean values. Based on the results, G19, G16, G2, and G20 were more sensitive to drought as they showed low values of MP, GMP, and HM. Overall, genotype: G5, G21, G22, and G33 are recommended for production under drought and optimum conditions, as they showed stable performance across water regimes. Principal component analysis also revealed that MP, GMP, and HM were the only indices that had positive loadings into the first principal component.

Keywords: drought stress; wheat genotypes; agro-physiological traits; AMMI; quality traits; drought tolerance indices.

List of abbreviations acronyms

DTH	Duration to heading
DTF	Duration to flowering
DTM	Duration to maturity
GY	Grain yield (Kg/ha)
Kg	Kilogram
ha	Hectare
HT	Plant height
SL	Spike length
SPS	Number of spikelets per spike
KPS	Number of kernels per spike
CT	Canopy temperature
CC	Chlorophyll content
TKW	Thousand kernel weight
HLM	Hectolitre mass (specific weight)
WG	Wet gluten content
PF	Fixed protein
ANOVA	Analysis of variance
UFH	University of Fort Hare
ZAN	Zanyokwe irrigation scheme
ufhD	UFH-drought
ufhW	UFH-optimum or well-watered
zanD	ZAN-drought
zanW	ZAN-optimum or well-watered
SRAs	Summer Rainfall Areas
WRAs	Winter Rainfall Areas
IRAs	Irrigated Areas
ICARDA	International Centre for Agricultural Research in the Dry Areas

Ys	Yield under drought stress
Yp	Yield under optimum conditions
MP	Mean productivity
GMP	Geometric mean productivity
HM	Harmonic mean
TOL	Tolerance index
SSI	Stress susceptible index
SDI	Sensitive drought index
SS	Sum of squares
MS	Mean squares
DF	Degrees of freedom
CV%	Coefficient of variance
GM	Grand mean
PCA	Principal component analysis
IPCA	Interaction principal component analysis

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CHAPTER 1

INTRODUCTION

1.0 Background information

Wheat (*Triticum aestivum* L.) is among the world's major cereal crops in terms of area under cultivation, and the proportion of the world's population that is reliant on it (Alexandratos and Bruinsma, 2012). In South Africa, wheat is the second most important staple crop after maize and has a total consumption of 3.14 million tons per annum (DAFF, 2018). Between 2000 and 2016, the total area under wheat production in the country was reduced from 934 000 ha to 508 365 ha and this reduced the total grain production from 2.43 million tons to 1.91 million tons (DAFF, 2018; FAOSTAT, 2018). A number of factors, such as erratic and poorly distributed rainfall, are attributed to the decline in the total area planted to wheat (Dube et al., 2016). The reduced total production is unable to meet annual wheat requirements for the country's population. The steadily increasing human population and diverse dietary needs and preferences are estimated to require about a 50% increase in total wheat production by 2030 (Gahlaut et al., 2017).

Erratic and unevenly distributed rainfall results in drought stress and can severely affect wheat cultivation in arid and semi-arid environments (Chen et al., 2012). In the summer rainfall areas of South Africa, drought stress limits dryland wheat production (Dube et al., 2016). Otieno and Ochieng (2004) reported that in terms of agricultural crop production, the country is categorized as water stressed. The average precipitation in the country is approximately 450 mm per annum (Otieno and Ochieng, 2004), which is below the mean annual rainfall required for profitable wheat production. Hossain et al., (2012) reported that all plant development stages are affected by drought, from germination, vegetative and reproductive growth to grain filling and physiological maturity of the crop. Nutrient uptake and utilization by plants are reduced by drought due to impaired membrane permeability and active transport, and reduced transpiration rate resulting from repressed root absorbing power.

Arid and semi-arid environments often experience terminal drought stress (TD), which occurs during the last phase of the wheat growth cycle, that is from anthesis to the grain filling stage, resulting in reduced grain quantity and quality (Hristov et al., 2010; Mohammadi et al., 2010; Mohammadi and Amri 2013; Rozbicki et al., 2015). A 70 percent decline in yield due to terminal drought stress has been previously reported (Lizana and Calderini, 2013; Semenov et al., 2015). In South Africa, approximately 75 percent of wheat is produced under dryland conditions, and approximately 25 percent under irrigation, with the Western Cape producing nearly 50 percent of the total production. Under dryland wheat production, several factors such as plant genotype, growth stage, severity, and duration of stress combined with environmental factors can affect plants' response to drought stress (Sarto et al., 2017). This necessitates urgent development of high-yielding, drought-tolerant, and water-use-efficient wheat genotypes to improve both production and productivity (Dube et al., 2016; Mwadzingeni et al., 2016; Tshikunde et al., 2018).

1.2 Problem statement

Wheat production is faced with numerous biotic and abiotic constraints, drought being the major abiotic constraint resulting from climate change. Drought is defined as a condition whereby there is inadequate moisture in the soil at a particular time to meet the needs of the crop. Drought stress reduces the productivity and quality of crops and limits the successful realization of the yield potential of a given genotype. Under dry-land wheat production systems, drought stress is the main cause of yield loss and may lead to complete crop failure under severe conditions. In South Africa, drought stress is a major limiting factor to plant growth and sustainable wheat production due to the large reliance on rainwater. Due to the impact of global warming, the incidence of drought will increase in the future, and this will lead to more depressed yields even in previously productive regions of the country.

In South Africa, agricultural lands devoted to wheat production are continuously replaced with alternative crops due to drought, among other factors. This has resulted in decreased wheat production and heavy reliance on wheat imports to meet local demand. According to Grain SA, the country relies on wheat imports which are about 40-50 percent of local production of 1.91 million tons. Importing such huge quantities of wheat requires a lot of foreign currency, which could be saved through the production of drought-tolerant cultivars.

1.3 Justification

This study will help to sustain wheat productivity and production through the identification of drought-tolerant genotypes in South Africa. The two potential strategies that can be used to manage drought include the use of irrigation and the cultivation of drought-tolerant varieties. Considering emerging farmers, irrigation is an impractical option because it requires substantial investment in irrigation infrastructure, coupled with the limited availability of fresh water in South Africa (Agricultural Policy Action Plan, 2015-2019). The use of borehole water often results in the accumulation of salts, leading to the salinization of fields (FAO, 2000). The use of drought-tolerant varieties is cheap, and it is also an environmentally friendly option. Genetic variability for drought tolerance has been widely reported to exist in the wheat genome (Powell et al., 1996; Russell et al., 1997; Davila et al., 1999; Nachit et al., 1993).

The use of drought-tolerant cultivars is advantageous in terms of yield gain as reported in the semi-arid wheat yield trials (SAWYT) programs. Manes et al., (2012), reported a 0.7 percent gain (37 kg ha^{-1} yr^{-1}) from about 2.07 to 2.7 t ha^{-1} in the period from 1994 to 2010, by comparing the performance of checks and drought-tolerant genotypes. Wheat genetic resources with high grain yield, combining both abiotic and biotic stress tolerance are continuously being developed by international organizations such as the International Maize and Wheat Improvement Centre (CIMMYT) and International Centre for Agricultural Research in Dry Areas (ICARDA), as well as by private seed companies and national research institutes such as the Agricultural Research Council (ARC) of South Africa. Some of these genotypes have excellent quality attributes for cultivation in diverse agroecologies (Hernandez-Espinosa et al., 2018). However, it is very important to evaluate such genotypes in multi-environment trials so as to identify areas in which they can be recommended for production. Wheat genotypes that are evaluated in this study were sourced from some of the above-named organizations. In this study, the genotypes were evaluated at two sites over two consecutive winter seasons, so as to facilitate a comprehensive investigation of the interactions.

Bruckner, and Frohberg (1987), reported that, due to the dynamic nature of abiotic stresses, the experimental approach to selecting for drought tolerance emphasizes screening under both optimal and stressed conditions to observe yield stability and yield potential. Replicated, multi-location and multi-year variety testing has been adopted by plant breeders to identify varieties that perform best across a wide range of environments as part of practical breeding-based programs. To breed a crop to tolerate a specific environment, direct selection under such environment results in higher stability and durability of the crop yield (Ceccarelli, 1987; Ceccarelli et al., 1998). Johnson and Frey (1967) supported this observation by confirming that, varieties selected directly from stressed conditions exhibit a low genotype by environment (G×E) interaction compared to those selected under optimal conditions.

Ali et al., (1999) reported that the phenotypic performance of a genotype differs significantly under diverse agroecological conditions. Some genotypes may perform well in certain environments but fail in several other environments (Hebert et al., 1995) due to the interaction of numerous factors. Therefore, the investigation of genotype by environment interaction (GxE) is useful in the evaluation of plant varieties as it affects the stability of genotypes and complicates the selection of desirable plants for targeted regions (Hebert et al., 1995). Crop performance will be determined by the genotype (G), environment (E), and genotype-by-environment interaction (GxE) (Yan et al., 2007).

Notably, grain yield is difficult to select under drought stress because it has low heritability under such conditions (Calhoun et al., 1994). To overcome this challenge, indirect selection for yield is practiced by selecting traits that are highly correlated with yield under drought stress. A number of agronomic and physiological traits (Sher et al., 2017; Mwadzingeni et al., 2018; Ram et al., 2017), together with drought tolerance selection indices (Shah et al., 2020) have been identified, and successfully utilised in selecting for drought tolerance genotypes under field conditions. However, the utility of these indices also appears to be influenced by genotype x environment interaction (Shah et al., 2020). This study will also seek to identify traits and selection indices that can be utilized for the indirect selection of grain yield under local conditions using available wheat genotypes.

The screening of wheat genotypes for drought tolerance is mostly conducted under field conditions where phenotyping remains a crucial criterion for breeding materials based on drought-adaptive and constitutive morpho-physiological traits including yield and yield-related traits (Monneveux et al., 2012; Passioura, 2012). Yield-related traits such as spikelet per spike (SPS), kennels per spike (KPS), and thousand kernel weight (TKW) are relevant for drought screening. Important traits when breeding for terminal drought stress include reduced duration to heading (DTH) and duration to maturity (DTM) as they allow for drought escape (Lopes et al., 2012). When considering genotypes with improved adaption to varying climatic conditions, selection should target genotypes with relatively high yields under both stressed and optimum conditions. This is made possible by the evaluation of a number of drought tolerance indices including stress tolerance index (STI) of test genotypes (Tardieu, 2012).

1.4 Objectives

The purpose of this study is to identify high-yielding wheat genotypes under drought and optimum conditions using field trials in two different sites in the Eastern Cape Province, South Africa. The specific objectives are:

- To evaluate the response of wheat genotypes under optimum and drought-stressed field conditions.
- To determine the effect of terminal drought stress on wheat grain quality composition.
- To identify appropriate agro-physiological secondary traits and drought tolerance indices that can be used as selection tools under field conditions.

1.5 Hypotheses

- Drought stress has no effect on available wheat genotypes as they exhibit similar performance under optimum and drought-stressed field conditions.
- Drought stress has no effect on wheat grain quality parameters.
- There are no agro-physiological traits and drought tolerance indices that can be used as secondary selection tools under field conditions.

1.6 Structure of the Dissertation

This dissertation consists of six chapters. Chapter one introduces the study, and Chapter two discusses the literature review. Chapter three describes the materials and methodology and presents results from the first objective. Chapters four and five present results from the second and third objectives respectively. Chapter six is an overall discussion, followed by the conclusion and recommendation. Tables of appendix are put on the very last pages of this document, after references.

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Chapter 2

Literature review

2.1 The origin and classification of wheat

Wheat is believed to have been cultivated about 1000 decades ago, as part of the Neolithic Revolution (first Agricultural revolution) whose aim was to move from hunting and gathering food to settled agriculture (Heun et al., 1997). The earliest cultivated forms were diploid (genome AA) and tetraploid (genome AABB) wheat (Nesbitt, 1998), and their genetic relationships revealed that they originated from the south-eastern part of Turkey (Dubcovsky and Dvorak, 2007). Hexaploid wheat made its first appearance when the cultivation of wheat spread to the Near East about 900 decades ago (Feldman, 2001).

Landraces were the earliest cultivated forms of wheat, and they were selected by farmers from wild populations. The domestication of wheat was guided by some important traits which contributed to yield gain. These traits included loss of shattering of the spike at maturity, and the change from hulled forms, in which the glumes adhere tightly to the grain, to free-threshing naked forms. Shuttering is an important trait that ensures seed dispersal in natural populations and the non-shattering trait is determined by mutations at the Br (brittle rachis) locus (Nalam et al., 2006). Figure 2.1 illustrates the evolution of modern wheats and variations in spike and grain types.

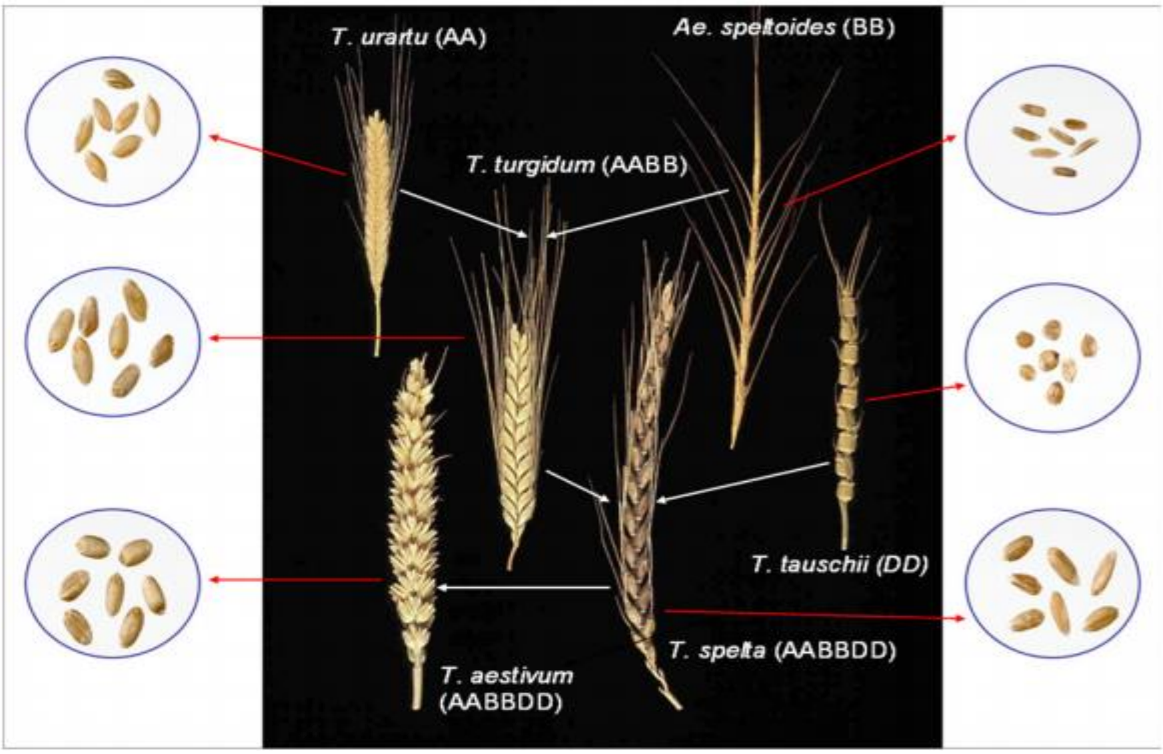

Figure 2. 1: The evolution of cultivated wheat (Snape and Pa'nkova, 2006).

Wheat is classified as either winter wheat or spring wheat, depending on the season during which the crop is grown. For winter wheat, plants undergo a vernalization period of cold winter temperatures (0-5°C) before heading takes place. Winter wheat is normally planted in autumn and is expected to germinate and develop into young plants that remain in the vegetative phase during the winter and resume growth in early spring. This planting time provides moisture for germination and makes effective use of early sunshine, warmth, and rainfall in spring. Spring wheat does not require vernalization, and is normally planted in spring, grows, develops, and matures in late summer (Anderson and Garlinge, 2000).

South African commercial wheat production (bread and durum wheat) was initiated in the early 1910s with varieties brought by Dutch traders to Cape Town. DAFF, (2018) noted that the main uses of wheat in South Africa include human consumption (especially for making flour for the bread industry), industrial (important sources of grain for alcoholic beverages, starch, and straw), and animal feed (bran from flour milling as an important source of livestock feed, grain as animal feed). These two basic types of commercially cultivated wheat differ in genetic complexity, adaptation, and specific uses. Durum wheat (*Triticum turgidum*) resulted from the fusion of two grass species, whereas bread wheat (*Triticum aestivum*) resulted from a cross between durum wheat and a third grass species (Trethowan et al., 2005). Bread wheat is processed into leavened and unleavened bread, biscuits, cookies, and noodles; and durum wheat is used to make pasta. In South Africa, durum wheat represents a very small percentage of the annual wheat production compared to bread wheat (Trethowan et al., 2005).

2.2 South African wheat production

The first wheat production in South Africa occurred in the winter of 1652 when Jan van Riebeeck planted the first winter wheat (Du Plessis, 1933). Today, South African wheat cultivars are adapted to different production regions, namely, Summer Rainfall Areas (SRAs), Winter Rainfall Areas (WRAs), and Irrigated Areas (IRAs). From 36 of South Africa's crop production regions, different types of wheat (winter or intermediate wheat; dryland spring wheat; irrigated spring wheat) are produced in 32 regions as shown in Figure 2.2. Western Cape (winter rainfall), Free State (summer rainfall), and Northern Cape (irrigation) are the main wheat-producing provinces in South Africa (Southern African Grain Laboratory, 2012). The other wheat-producing provinces in South Africa include Mpumalanga, North West, KwaZulu Natal, Limpopo, and Gauteng (Southern African Grain Laboratory, 2012).

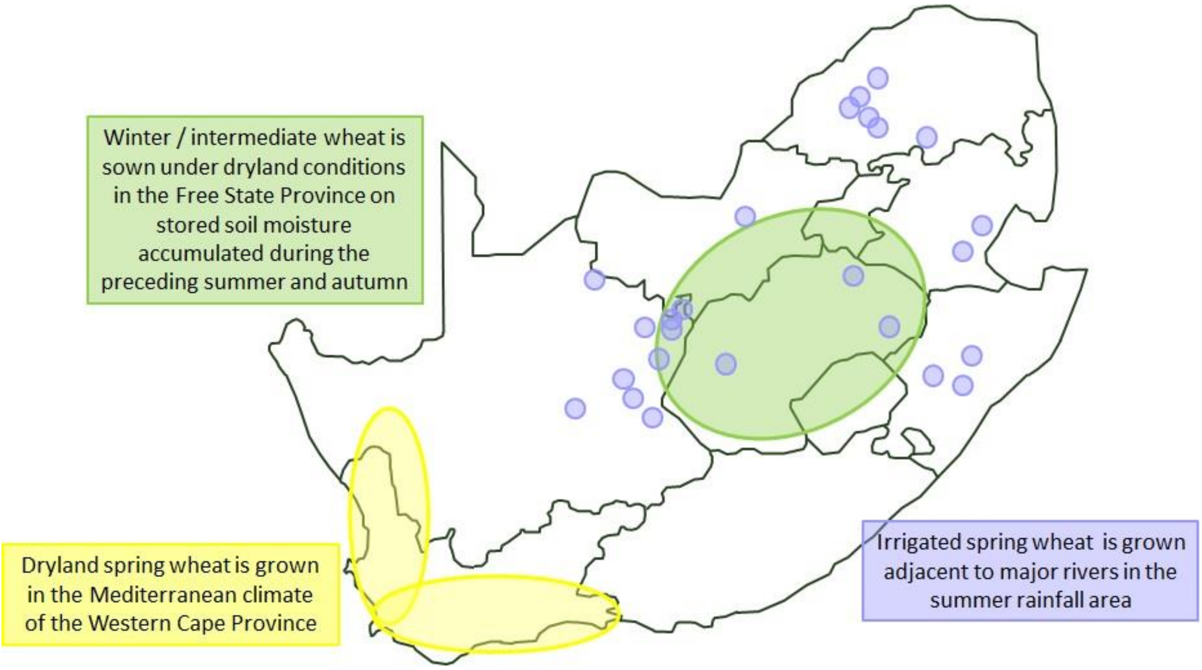

Figure 2. 2: Three distinct wheat production areas of South Africa with their distinct wheat types (Sihlobo, 2019).

The Eastern Cape Province has recently initiated dryland wheat production in a small number of localities (Eastern Cape Agri Report, 2019). South Africa’s annual wheat production ranges from 1.5 to 3 million tonnes, with 2 to 2.5 tonnes/ha productivity rates under dryland and at least 5 tonnes/ha under irrigation. The Western Cape contributes more to the local production (about 650 000 tonnes), Free State (580 000 tonnes), Northern Cape (300 000 tonnes), North-West (162 000 tonnes), and Mpumalanga (92 000 tonnes) (DAFF, 2018). Most of the production (at least 50%) happens under dryland conditions in both summer and winter rainfall regions. In the summer rainfall region, at least 30% of the total harvest is produced under irrigation (Pannar, 2009). Production under irrigation has a higher yield potential than dryland wheat production. Dryland productivity in South Africa is very low compared to that of the major wheat-producing countries in the world (Pannar, 2009).

Farmers in the region of South Africa are lacking interest in dryland wheat production due to a high risk of yield loss associated with increased rainfall variability coupled with rising temperatures resulting from global warming (Blignaut et al., 2009; Van der Westhuizen and Trapnell, 2015). Low air humidity, hot and dry winds, as well as low soil moisture supply during critical stages of crop growth result in drought in the SRAs. The crop growth stages at which drought conditions occur differ across geographic regions and seasons, and this variability is likely to be enhanced by climate change (Thomas et al., 2007; Wiid and Ziervogel 2012). Plant breeders are faced with the challenges of developing wheat cultivars that give adequate yields in drought seasons, and high yields in good seasons. Different wheat cultivars differ in their response to drought under field conditions.

2.3. Response of wheat to drought stress at different growth stages

2.3.1 Germination and Seedling Stages

Drought stress negatively affects all traits related to germination and early seedling phases. These traits include germination rate, seedling vigor, and lengths of coleoptile, shoot, and/or root (Kizilgeci et al., 2017). Researchers have put less or no focus on yield losses in wheat due to drought at the germination and seedling stages. However, Kandic et al., (2009) and Dodig et al., (2015) reported a positive association of seedling traits with reproductive traits, including grain yield. These findings suggest that seedling drought tolerance could be used to estimate plant performance under drought during later stages of growth. He et al., (2017) noted that sufficient moisture in the soil, along with optimum temperature, is required for uniform germination, and Mukherjee et al., (2019) added that drought-sensitive varieties suffer under water-limited conditions. This is because germination-related traits significantly differ among different wheat varieties under optimum and water-limited conditions (Ahmad et al., 2014).

2.3.2 Tillering and Stem Elongation Stages

The occurrence of drought during tillering and stem elongation in wheat reduces the number of grains per spike and therefore grain yield (Ding et al., 2018; Saeidi et al., 2015). Spikelet initiation begins at the seedling stage and proceeds until the tillering stage, and floret initiation starts at tillering and continues during the stem elongation period. This explains the importance of maintaining the spikelet number per plant and spikes per plant which directly contribute to grain yield. It has been reported that water stress during the vegetative stage reduced grain yield by up to 54 percent (Saeidi et al., 2015). In a similar study, Ding et al., (2018) observed a 72 percent grain yield decline due to the extreme water stress during the stem elongation period, as compared to the reproductive period. A study conducted by Keyvan, (2010) showed that the stem elongation stage is more susceptible to drought as compared to the booting and grain-filling stage, and as a result, the greatest yield decrease was observed when drought was imposed during this stage of growth. Sarto et al., (2017) further added that plant height, stem growth, and the number of productive tillers were also reduced by drought at the stem elongation stage. The overall plant biomass, which is an indicator of grain yield at physiological maturity, is also negatively affected by drought during tillering and stem elongation (Saeidi et al., 2015; Ding et al., 2018). This results in changing source-sink relationships, resulting from an increased fraction of available carbon being allocated to the root system rather than to the shoot when plants are under limited water supply (MacNeill et al., 2017).

Contrary to the above observations, Liu et al., (2016) noted an improvement in canopy structure and the maintenance of photosynthesis at the canopy level that was observed when mild water stress was applied at stem elongation without a reduction in grain yield. As argued by different researchers, mild drought stress during this phase may not be very critical to the final grain yield. Mild drought stress during tillering and stem elongation stages primes wheat plants to become acclimated to tolerate drought during the grain-filling period (Wang et al., 2015). The mechanism involves low accumulation of hydrogen peroxide (H_2O_2) due to increased activity of H_2O_2 scavenging enzymes such as ascorbate peroxidase (APX) and guaiacol peroxidase (POX) (Khanna-Chopra and Selote, 2007). Despite some reports, the above evidence suggests that drought at tillering and stem elongation stages negatively affects grain yield (Wang et al., 2015; Liu et al., 2016).

2.3.3 Heading, Anthesis, and Grain Filling Stages

The occurrence of drought at the heading and anthesis stage reduces pollen viability leading to failures in fertilization, and hence, spikelet sterility (Ji et al., 2010; Su et al., 2013). Maximum evapotranspiration, which aggravates the impact of drought and leads to severe crop failure, is known to occur at the heading and anthesis stages. Liu et al., (2016) and Ding et al., (2018) noted that some genotypes may promote the translocation of assimilates under moderate stress at this stage of growth. A number of studies reported that drought occurring at flowering and anthesis, also known as terminal drought (TD), severely reduces grain yields (Ji et al., 2010; Fahad et al., 2017; Sarto et al., 2017). Water stress at the heading and anthesis stages results in multiple impacts, but among these, Varga et al., (2015) noted a decrease in the number of grains per head and grain weight.

Drought occurring after anthesis results in about 28 percent grain yield decline as revealed by Gevrek and Atasoy, (2012). These authors reported that kernel weight and kernel numbers decreased by 5.2 percent and 20.7 percent respectively. Ding et al., (2018) reported that during the grain-filling stage, mild drought does not appear to cause a significant reduction in final grain yield. Though water availability becomes critical for translocating photosynthates to the grain during the grain filling stage, pre-anthesis storage reserves such as those in the stem can play crucial roles in preventing yield loss, to mitigate the negative impact of moisture stress on photosynthate assimilation (Liu et al., 2016). On the other hand, Wang et al., (2015) reported that moderate drought during vegetative growth stages may prime plants to acclimate to drought during grain filling. The mechanism involves reduced photo-inhibition in the flag leaves at this later stage associated with increased accumulation of abscisic acid (ABA). Furthermore, the accumulation of dehydrins may result in drought tolerance during the grain-filling stage (Lopez et al., 2002).

2.4 Effects of Terminal drought stress on grain development

Drought after anthesis affects the duration and rate of grain filling and changes the size and composition of grain (Dupont and Altenbach, 2003). A substantial yield reduction in wheat during reproductive and grain-filling phases is fundamentally due to several factors as reported by a number of scientists (Tyagi and Pandey, 2022). These factors include leaf senescence; oxidative damage to photo-assimilatory machinery; reduced rates of carbon fixation and assimilate translocation; pollen sterility; reduced grain set and development; and reduced sink capacity (Farooq et al., 2009; Asada, 2006; Cattivelli et al., 2008; Nawaz et al., 2013; Liang et al., 2001).

2.4.1 Leaf Senescence

Leaf senescence, as defined by Hafsi et al., (2000) and Chandler, (2001), is a gradual deterioration of leaf functions with changes in colour due to chlorophyll and membrane breakdown, along with decreased water content with age. Gregersen and Holm, (2007) reported that the primary symptom of leaf senescence is chlorosis, which leads to a decline in photosynthesis. In wheat, the flag leaf is the major source of assimilates (30-50 percent of the total) during grain development (Sattar et al., 2020). Leaf senescence is severely accelerated by drought, which in turn is largely responsible for grain yield reductions in wheat during the reproductive stage (Yang et al., 2001; Mi et al., 2002; Nawaz et al., 2013). Better yields are obtained in wheat genotypes sustaining flag-leaf photosynthesis for longer periods (Larbi and Mekliche, 2004). Therefore, the start and rate of senescence of flag leaves in wheat are important factors for determining resistance to drought stress. On the other hand, the whole wheat plant senescence is stimulated by terminal drought; and thus, this may increase the remobilization of pre-anthesis stored carbohydrates from the stem and leaves to developing grains, which may compensate for senescence-induced losses in grain yield (Yang et al., 2001, 2003; Plaut et al., 2004).

2.4.2 Grain Development

Photosynthesis and redistribution of assimilates from reserve pools in vegetative tissues determine grain filling in wheat (Farooq et al., 2011). The life cycle and duration of grain filling in wheat are shortened by terminal drought. Madani et al., (2010) and Wei et al., (2010) reported that the grain-filling rate under drought decreases due to reduced photosynthesis, accelerated leaf senescence, and sink limitations. Therefore, water scarcity during early grain development determines potential grain size due to the reduced rate and duration of grain filling (Saini and Westgate, 2000). Terminal drought has more influence on grain number, rather than grain size, which largely accounts for the decline in wheat grain yields under drought (Dolferus et al., 2011). Meiosis and anthesis are extremely susceptible to drought, and their failure directly affects grain number, thus causing a substantial reduction in grain yield (Cattivelli et al., 2008).

Plaut et al., (2004) noted that drought after anthesis has no effect on grain number in wheat, and little effect on the rate of grain filling, but grain filling duration is shortened, which causes substantial decreases in grain dry weight (Wardlaw and Willenbrink, 2000; Altenbach et al., 2003). Grain filling is a process of starch biosynthesis and accumulation. Four enzymes including adenosine diphosphate-glucose-pyrophosphorylase, sucrose synthase, starch branching enzyme, and starch synthase play a key role during grain filling (Hurkman et al., 2003). On the contrary, drought during grain filling may accelerate the rate of grain filling. This increment in grain-filling rate is mainly attributed to an increase in sink activity through the regulation of key enzymes involved in the change of sucrose to starch, especially sucrose synthase, soluble starch synthase, and the starch branching enzyme (Yang et al., 2004).

2.5 Effects of Terminal drought stress on grain quality composition

The nutritional and economic value of wheat depends on wheat grain quality. Li et al., (2013) noted that these values vary according to genotype, environmental factors, and the interaction between genotype and environment. As drought stress limits the amount of assimilates through the acceleration in leaf senescence, and reduction in the area and period of photosynthesis, therefore increases grain protein content as it lowers grain yield (Balla et al., 2011). Panozzo and Eagles, (2000) also noted that drought alters the carbohydrate and nitrogen assimilation rates, which gives rise to significant changes in the grain chemical composition, and Balla et al, (2011) further noted some changes in protein composition, and starch granule size. Zhao et al., (2009) reported that mild water stress during the grain-filling period positively affected bread quality. This correlation was supported by an increase in the protein, gluten, gliadin, globulin, phosphorus, and zinc content in grain. On the other hand, Gooding et al., (2003) stated that severe drought in the first 14 days of grain development reduced sedimentation volume. Furthermore, Guler, (2001) reported that severe drought decreases the protein ratio, gluten rate, and sedimentation volume, and Tsenov et al., (2015) added that hectolitre weight, sedimentation volume, and gluten rate were also decreased.

Water stress that is occurring a few days after anthesis negatively affects grain weight by both delaying endosperm cell division and quality, and decreasing the protein content of gliadin, gluten, and avenin (Begcy and Walia, 2015). Although drought after anthesis increases the rate of grain protein, it may reduce the synthesis of high molecular weight protein subunits and the ratio of glutenin macromolecules (Jiang, 2009). While drought improves quality characteristics in some varieties, it decreases the protein ratio, gluten rate, and sedimentation volume in varieties in which the photosynthesis rate is reduced due to stress (Ali et al., 2011). Li et al., (2013) determined that drought increased sedimentation volume, alveograph tenacity, and glutenin index, and decreased alveograph extensibility and water retention capacity. The effect of environment on wheat quality may be higher than that of genetic factors (Panozzo and Eagles, 2000), and varieties may respond differently to drought stress in terms of protein composition (Ali et al., 2011).

2.6 Screening of wheat genotypes for drought tolerance under different growing conditions.

Wheat genotypes can be assessed for drought tolerance at different levels of growth depending on different screening environments such as laboratory, glasshouse, or field environments. Under laboratory conditions, effective high-throughput methods such as osmotic stress culture are used to screen large numbers of early-generation lines for their potential drought tolerance (Munns et al., 2010). Assessment of plant response can be achieved by manual evaluation of seedling traits or by using advanced phenotyping technologies and platforms (Araus and Cairns, 2014).

Screening for drought tolerance under glasshouse conditions is predictive of drought tolerance under field environments (Passioura, 2006). Under glasshouse conditions, the selection of promising genotypes is based on vigorous seedlings, which are a vital index in describing the yield of a plant in a short period (Noorka et al., 2013). Zhang, and Wang (2012) reported that a genotype with drought stress tolerance has more impermeable rooting abilities to boost the preoccupation of soil moisture and to lessen the distinct effects of drought during development and growth. Screening of wheat genotypes for drought tolerance at the seedling stage used various assessment criteria such as root-to-shoot ratio and relative water content (Bilal et al., 2015). Relative water content was recommended by Almeselmani et al., (2011) as a good criterion for drought tolerance in wheat. The advantageous part of RWC is that it shows the balance between water absorbed by the plant and disbursed through transpiration (Arjenaki et al., 2012).

The best option is to test genotypes under field conditions in order to breed crops for improved grain yield under drought conditions. This requires the selection of genotypes with improved yield performance under field conditions (Rebetzke et al., 2013). Field-based drought experiments are very important as they span the entire crop cycle, are resource-intensive, and subject crops to seasonal variability. Dudley, (2008) reported that phenotyping offers an easy way of visualization and selecting of favorable traits. Reduction in phenological traits like duration to heading and duration to maturity is an avoidance mechanism in plants against drought stress (Lopes et al., 2012).

Under field evaluation of genotypes for drought tolerance, numerous types of environmental factors such as high temperatures, high irradiance, and nutrient toxicities or deficiencies, can challenge crop plants simultaneously. Grain yield remains the best criterion for the selection of drought-tolerant genotypes under such conditions. These environmental effects are being addressed by some statistical analysis (Brancourt-Hulmel and Lecomte, 2003; Yan and Kang, 2003), to minimise their confounding effects. The selection of wheat varieties with the best performance under water-stress environments could, therefore, increase production in rainfed areas (Ahmad et al., 2014; Ahmad et al., 2017; Noorka et al., 2013). Field screening also allows plant breeders to understand the responses of wheat to drought stress at all stages of growth.

2.7 Agronomic and physiological traits associated with drought tolerance under field conditions.

Agronomic traits are highly heritable and can be correlated with grain yield, therefore, they can be used as indirect selection criteria for breeding and cultivar development (Chen et al., 2012; Abdolshahi et al., 2015; Liu et al., 2015; Gao et al., 2017). Lopes et al., (2012), suggested that genetic progress in yield can be achieved if several traits conferring better agronomic and physiological performance with biotic and abiotic stress tolerance are simultaneously selected and introgressed in a single variety. To aid cultivar development and increase grain yield potential and genetic gains in wheat, some important agronomic traits that have been successfully used before, include early flowering and maturity, high biomass production, and kernel weight.

The understanding of physiological traits associated with genetic gains in yield is crucial for breeding (Beche et al., 2014; Aisawi et al., 2015; Zhang et al., 2016). Reynolds et al., (2012) reported that approximately 50 percent of grain yield genetic gains can be improved by breeding wheat genotypes incorporating physiological traits. Physiological traits that have contributed to grain yield improvement in wheat include canopy temperature; chlorophyll content; photosynthetic capacity; water-soluble carbohydrates (Reynolds et al., 2012).

2.8 Drought tolerance indices

The major work of breeders is to improve grain yield productivity and drought tolerance through the study of genotypes under stress conditions (Benmahammed et al., 2010). Good selection measures are needed to identify the drought-tolerant wheat genotypes for the effective breeding of drought-tolerant wheat varieties. Grain yield is always considered the basis of genotype selection for improving drought tolerance (Talebi et al., 2009; Farshadfar et al., 2012). High yield potential under optimum conditions does not necessarily result in improved yield under stress conditions, and genotypes with high yield may not be drought-stress tolerant under critical conditions (Sio-Se Mardeh et al., 2006).

A number of researchers have considered selecting yield under both non-stress and stress conditions, as this idea is more efficient, mostly under regions with unreliable rainfall with various yearly drought scenarios (Mohammadi et al., 2010; Farshadfar et al., 2012a, b, 2014). Therefore, many drought indices have been proposed for screening drought-tolerant genotypes based on yield performance under stressed (Ys) and optimum (Yp) conditions (Talebi et al., 2009; Mohammadi et al., 2010; Nouri et al., 2011).

Various yield-based drought tolerance indices such as tolerance index (TOL), mean productivity (MP), stress susceptibility index (SSI), modified stress severity index (MSTI), geometric mean productivity (GMP), stress tolerance index (STI), harmonic mean of yield (HM), and sensitivity drought index (SDI) have been used over decades (Dadbakhch et al., 2011; Farshadfar and Javadinia, 2011; Farshadfar and Elyasi, 2012; Singh et al., 2011). Boussen et al., (2010) further confirmed that the best indices are those which a have high correlation with grain yield in both conditions and would be able to identify higher-yielding and drought-tolerant genotypes. The following mathematical equations aid in calculating the above-mentioned indices.

$TOL = Yp - Ys$ (Rosielle and Hamblin, 1981)

$MP = (Yp + Ys)/2$ (Rosielle and Hamblin, 1981)

$SSI = (1 - Ys/Yp)/SI$ (Fischer and Maurer, 1978)

$SI = 1 - \bar{Y}s/\bar{Y}p$ (Fischer and Maurer, 1978)

$MSTI = k_i * STI$ while $k_1 = (Yp^2)/(\hat{Y}p^2)$ and $k_2 = (Ys^2) / (\hat{Y}s^2)$ where k_i is the correction coefficient (Dadbakhch et al., 2011).

$GMP = \sqrt{Yp \ x \ Ys}$ (Fernandez, 1992)

$STI = Yp \ x \ Ys/(Yp)^2$ (Fernandez, 1992)

$HM = 2(Yp * Ys)/(Yp + Ys)$ (Farshadfar and Elyasi, 2012)

$SDI = (Yp - Ys)/Yp$ (Singh et al., 2011)

An index of tolerance (TOL) is defined as grain yield difference in optimum (Yp) and drought (Ys) conditions, and therefore, drought-sensitive genotypes show low values of this index. The average yield under drought stress (Ys) and optimum conditions (Yp) define the index of mean productivity (MP). The stress susceptible index (SSI) explains that genotypes having values less than one are tolerant. These drought-tolerant indices, together with other statistical parameters, could be used by plant breeders to compare the changes in grain yield in optimum and drought conditions (Yadav and Bhatnagar, 2001). Mitra, (2001) noted that this comparison aids in the assortment of genotypes into high yields and drought tolerance as selecting genotypes having tolerant genes is challenging.

Many researchers have used different selection approaches to identify genotypes with drought tolerance using tolerance and/or susceptibility indices (Farshadfar et al., 2013). In this regard, indices such as STI, SSI, and TOL have been widely used to characterize genotypes resistant to water deficit in wheat (El-Rawy and Hassan, 2014) and other crops like maize (Naghavi et al., 2013), sorghum (Menezes et al., 2014), and sunflower (Gholinezhad et al., 2014)

2.9 Environmental effect in cultivar selection under field conditions

The environmental effect is the cause of significant yield variations among test genotypes, especially under drought-stressed environments. Genotype-by-environment interaction (GxE) refers to varying responses of given genotypes across a range of production environments. GxE is commonly encountered when different genotypes are being evaluated in multi-environmental trials under field conditions (Brancourt-Hulmel and Lecomte, 2003; Yan and Kang, 2003). Both genotype and genotype by environment interaction should be simultaneously considered for genotype evaluation (Yan et al., 2000; Yan and Kang, 2003). The main objectives in the analysis of GxE data are genotype evaluation and environment identification. There are several statistical methods in which GxE can be analyzed, these include genotype plus genotype-by-environment interaction (GGE biplot) analysis (Yan and Tinker, 2006), and additive main effect and multiplicative interaction analysis (AMMI) (Gauch, 1992).

The AMMI aids in the quantification of genotypic, environmental, and GxE effects on traits of interest (Yan et al., 2001). Furthermore, AMMI analysis is an approach, which combines both the ANOVA (with additive parameters) and Principal Component Analysis (with multiplicative parameters) into a single analysis (Zobel et al., 1988; Gauch et al., 1992; Gauch et al., 1996). It is also an effective tool to diagnose genotype-environment interaction patterns graphically. As the interaction plays a significant role in the expression of different genotypes in different environments, GxE studies are important for grain yield trials. Shah et al., (2009) reported that interactions were highly significant for all the studied traits under genotype-environment, genotype-year, and genotype-environment-year in wheat genotypes. Akcura et al., (2011) also successfully studied the stability of wheat genotypes under different environments using the AMMI analysis.

2.10 Summary of literature review

To date, wheat has existed for more than thousands of decades. Due to the industrial revolution, numerous biotic and abiotic constraints have occurred and hampered wheat production from a global scale to a national level, with drought being one of the major constraints. The steadily increasing demand for wheat necessitates different disciplines, from scientists and researchers in the laboratories to farmer's perspectives in the field, to unite and come up with concrete strategies to maximize grain yield under such trajectory environments. Drought stress has been observed to challenge all wheat growth stages from germination to physiological maturity. As wheat is normally produced under different production regions, from dryland to winter rainfall, to irrigation regions, drought occurrence is spreading widely and also limiting available fresh water for irrigation. However, the breeding companies are working very hard to develop wheat germplasm that will survive under critical conditions and give adequate grain yield under optimum conditions. To screen and condone the promising genotypes under drought stress, research, and academic institutes are doing the utmost important task of evaluating wheat genotypes under different environments such as laboratories, glasshouse, up to the field with the aid of different statistical and drought tolerance indices. Drought severity differs in different growing seasons and different geographic regions. Therefore, multi-environmental coupled with consecutive seasons aid in identifying superior genotypes for production so as to maintain high grain yields.

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Chapter 3

Response of wheat genotypes under optimum and drought-stressed field conditions in the Eastern Cape, South Africa.

Abstract

The decline in wheat yields due to diminished soil moisture is exacerbated by poorly distributed rainfall in wheat-producing regions of South Africa. Herein, a field experiment was conducted at two different sites namely University of Fort Hare Research Farm (UFH), and Zanyokwe irrigation scheme (ZAN), over two seasons (2020 and 2021). The objective was to evaluate the response of wheat genotypes under optimum and drought-stressed conditions. Forty diverse wheat genotypes from different companies were evaluated using a 5x8 alpha lattice design and replicated twice under two water regimes (optimum and drought). Under drought conditions, irrigation was terminated from 50 percent flowering up to physiological maturity. The agro-physiological traits examined included chlorophyll content (CC); canopy temperature (CT); duration to heading (DTH); duration to flowering (DTF); duration to maturity (DTM); plant height (HT); spike length (SL); number of spikelets per spike (SPS); number of kernels per spike (KPS); and grain yield per plot (GY). The combination of two sites and two water regimes gave four environments (E) namely UFH-drought (ufhD); UFH-optimum (ufhW); ZAN-drought (zanD); and ZAN-optimum (zanW). Average data (2020 and 2021) from the above-mentioned agro-physiological traits was subjected to the analysis of variance. Combined ANOVA revealed significant differences among environments (E), genotypes (G), water regimes (WR), and genotype by environment interaction (GxE) for all studied traits. These significant differences led to further assessment of the genotype by environment interaction through the aid of AMMI analysis. The study identified genotypes G21 (3117kg/ha) and G33 (3143kg/ha) as high yielding, with above grand mean (2088kg/ha) performance, and they were also stable across sites. UFH site appeared to be more productive than ZAN site as it had the highest grand mean.

Keywords: drought stress; wheat genotypes; AMMI; GxE

3.1 Introduction

Wheat production in arid and semi-arid regions is limited by low and poorly distributed rainfall, resulting in yield loss by drought-susceptible genotypes (Chen et al., 2012). Arid and semi-arid regions often experience 'terminal' drought stress, which occurs during the last phase of the wheat growth cycle, from the anthesis to the grain filling stage. This results in reduced grain yield (Mohammadi et al., 2010; Hristov et al., 2010; Mohammadi and Amri, 2013; Rozbicki et al., 2015). However, Farooq et al., (2014) noted that dryland wheat production is the major contribution to reduced grain yield. In South Africa, agricultural lands devoted to wheat production are continuously replaced with alternative crops due to drought, among other factors. Dube et al., (2016) reported that dryland wheat is mostly cultivated under water-limited environments and often subjected to drought stress resulting in marked yield losses in South Africa. Farooq et al., (2014) noted that yield reductions can be up to 92 percent due to drought stress. Smit et al., (2010) further indicated that drought stress has resulted in decreased local production and heavy reliance on wheat imports to meet local demands.

In the Eastern Cape Province, South Africa, 70 percent of the rains normally come during the summer months from November to April. Nonetheless, the stored soil moisture is unable to sustain wheat production up to physiological maturity. Developing drought-tolerant wheat genotypes is a key strategy for improving yields under water-limited environments (Lopes et al., 2012; Wu et al., 2014; Aisawi et al., 2015; Mwadzingeni et al., 2016). Key agronomic traits that can improve grain yield (GY) and drought tolerance in wheat include the duration to heading (DTH), duration to maturity (DTM), plant height (HT), number of productive tillers per plant (TN), and number of kernels per spike (KPS) (Sher et al., 2017; Mwadzingeni et al., 2018). Similarly, important physiological traits such as chlorophyll content (CC), canopy temperature (CT), and stomatal conductance (SC) have been reported to be well-correlated with GY and drought tolerance in wheat (Ram et al., 2017). Thus far, there has been limited progress in developing drought-tolerant wheat germplasm that can increase yield under water-limited environments. Therefore, the selection of promising genotypes with drought tolerance is critical to boost wheat yields in water-limited environments of South Africa.

The International Maize and Wheat Improvement Centre (CIMMYT) develops and releases novel heat and drought-tolerant genetic resources for improving grain yields in water-limited environments (Sharma et al., 2012). CIMMYT's drought and heat-tolerant wheat germplasm is genetically divergent given that the genetic pool is developed using diverse sources of drought tolerance through gene introgressions from landrace varieties, elite varieties, and synthetic hexaploid wheat lines (Mwadzingeni et al., 2016). This allowed the development of drought-tolerant wheat germplasm serving as useful genetic resources for breeding (Crespo-Herrera et al., 2018). To hasten the development and deployment of high-yielding wheat genotypes appropriate for drought-prone areas in South Africa, diverse wheat genotypes were sourced from CIMMYT's pre-breeding drought and heat tolerance nurseries, as well as from local companies. Evaluation of these wheat genetic resources for drought tolerance is useful to identify genotypes with stable performance across numerous water-limited environments in South Africa. Breeding wheat genotypes with desired characteristics such as high yield, enhanced water-use efficiency, and drought tolerance has become a critical strategic objective for boosting yields in dry regions (Merchuk-Ovnat et al., 2016).

As rainfall is usually poorly distributed under arid and semi-arid regions, the performance of genotypes also differs across different environments and seasons (Mohammadi et al., 2010, 2011). Selection of superior genotypes is therefore complicated, resulting in the delay in cultivar release and recommendation (Mohammadi and Amri, 2013). Genotype-by-environment interaction (GxE) analysis of data from multi-environment trials is mostly used for the identification, selection, and recommendation of suitable genotypes to produce in target environments (Mohammadi et al., 2010). Additive main effect and multiplicative interaction (AMMI) (Gauch, 1992) and genotype plus genotype-by-environment interaction (GGE) biplot (Yan and Tinker, 2006) analyses are some of the statistical methods generally used to assess GxE. The AMMI aids in the quantification of genotypic, environmental, and GxE effect on grain yield performance of genotypes of interest (Yan et al., 2001). On the other hand, the GGE biplot aids in the identification of the best-performing genotypes in suitable production environments (Yan et al., 2000; Yan and Kang, 2003; Yan and Tinker, 2006). In wheat, AMMI and GGE analyses have been successfully applied to aid the identification of suitable wheat genotypes and production environments (Mohammadi et al., 2010, 2013; Subira et al., 2015; Yabwalo et al., 2018).

3.2 Objectives

i) To evaluate the response of wheat genotypes under optimum and drought-stressed field conditions in the Eastern Cape Province, South Africa.
ii) To identify wheat genotypes with stable performance across different environments.

3.3 Hypotheses

i) Drought stress has no effect on available wheat genotypes as they exhibit similar grain yield performance under optimum and drought-stressed field conditions.
ii) There are no wheat genotypes that display stable performance across different environments.

3.4 Materials and methods

3.4.1 Experimental sites

The experiment was conducted in the field at two sites namely University of Fort Hare Research Farm (UFH) in Alice, Raymond Mhlaba Local Municipality in Amathole District (Latitude: -32.79’06’’; Longitude: 26.84’55’’), and Zanyokwe irrigation scheme (ZAN) in Middeldrift, Raymond Mhlaba Municipality in Amathole District (Latitude: -32.74’50’’; Longitude: 27.02’45’’). The experiment was done over two consecutive winter seasons, that is during the winters of 2020 and 2021.

3.4.2 Plant materials

Forty genetically diverse wheat genotypes were obtained from five different sources. Ten wheat genotypes were obtained from SENSAKO (PTY) LTD; two from Stellenbosch University; fifteen from ARC-grain crops research Institute; ten from Corteva Agriscience; and three from the International Centre for Agriculture Research in the Dry Areas (ICARDA). These genotypes are adapted to different production regions, namely, Summer Rainfall Areas (SRAs), Winter Rainfall Areas (WRAs), and Irrigated Areas (IRAs). **Table 3.1** presents the list of genotypes, their sources, and some basic descriptive information available on each genotype.

Table 3. 1: Descriptive information of wheat genotypes

Genotype Name	Code	Source	Production Region	Drought response
PAN 3497	G1	Corteva	Irrigation	Unknown
PAN 3111	G2	Corteva	Summer rainfall	Tolerant
PAN 3368	G3	Corteva	Summer rainfall	Tolerant
PAN 3133	G4	Corteva	Summer rainfall	Tolerant
PAN 3471	G5	Corteva	Winter rainfall	Unknown
PAN 3161	G6	Corteva	Summer rainfall	Tolerant
PAN 3541	G7	Corteva	Irrigation	Unknown
PAN 3555	G8	Corteva	Irrigation	Unknown
PAN 3400	G9	Corteva	Irrigation	Unknown
PAN 3252	G10	Corteva	Summer rainfall	Unknown
SST 8156	G11	SENSAKO	Irrigation	Unknown
MMF19-159	G12	SENSAKO	Unknown	Unknown
MMF19-162	G13	SENSAKO	Unknown	Unknown
SST 806	G14	SENSAKO	Irrigation	Unknown
SST 843	G15	SENSAKO	Irrigation	Tolerant
SST 347	G16	SENSAKO	Summer rainfall	Tolerant
SST 356	G17	SENSAKO	Summer rainfall	Tolerant
SST 374	G18	SENSAKO	Summer rainfall	Unknown
SST 387	G19	SENSAKO	Summer rainfall	Tolerant
SST 398	G20	SENSAKO	Summer rainfall	Tolerant
Utique96/Flag-3	G21	ICARDA	Summer rainfall	Unknown
20 USP 10	G22	Stellenbosch	Unknown	Unknown
Elands	G23	ARC	Summer rainfall	Unknown
Matlabas	G24	ARC	Summer rainfall	Tolerant
Koonap	G25	ARC	Summer rainfall	Unknown
Senqu	G26	ARC	Summer rainfall	Unknown
Gariep	G27	ARC	Summer rainfall	Unknown
Ratel	G28	ARC	Irrigation	Unknown
Steenbok	G29	ARC	Summer rainfall	Unknown
Tredou	G30	ARC	Unknown	Unknown
Tankwa	G31	ARC	Irrigation	Unknown
Kariega	G32	ARC	Irrigation	Unknown
Renoster	G33	ARC	Irrigation	Unknown

Koedoes	G34	ARC	Irrigation	Unknown
Krokodil	G35	ARC	Irrigation	Unknown
Duzi	G36	ARC	Irrigation	Unknown
Umzumbi	G37	ARC	Irrigation	Unknown
Gonglase-4	G38	ICARDA	Summer rainfall	Unknown
20 USP 11	G39	Stellenbosch	Unknown	Unknown
126-M7HPYT-H18	G40	ICARDA	Irrigation	Unknown

3.4.3 Experimental design and trial establishment

This multi-site experiment was laid out in a 5x8 alpha lattice design (**Figure 3.1**) with two replications (Rep 1 and Rep2) and two water regimes (optimum and drought conditions), with plots being numbered from 1 to 40 for the first replicate, and 41 to 80 for the second replicate. Land preparation was done by ploughing and disking to produce a seedbed with a fine soil tilth.

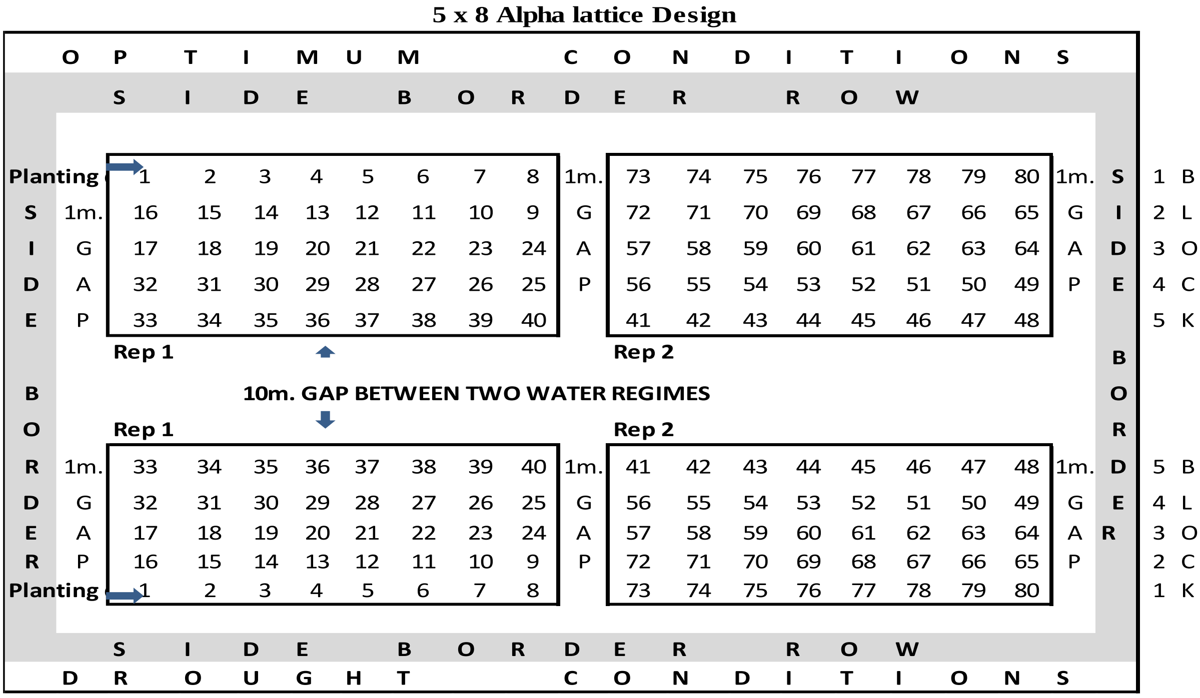

Figure 3. 1: Experimental field layout.

The seeds were placed about 3-5 cm deep in the soil using an automated Wintersteiger planter. Each plot consisted of eight rows that are 4 meters long, with an inter-row spacing of 0.2 meters. The intra-row spacing was neglected as plants were planted at a high density per row. Thousand kernel weight was used to compute the seed density per genotype for each plot.

The formula below was used to determine the seed density per genotype per plot:

$$\frac{1000\ \text{seed weight}}{1000}\ x\ seed\ density\ x\ plot\ area$$

In wheat, seed density ranges from 220 to 250 seeds per square meter.

The treatments were arranged in a split-plot manner, where the watering regime was the main plot, and the genotypes were in the sub-plots. To simulate terminal drought stress, irrigation was terminated from 50% flowering up to maturity (Farooq et al., 2009), while the optimum environment was irrigated once a week throughout the experiment.

3.4.4 Crop fertilization and crop protection

The fertilizer application rate was 130 kg/N for a yield target of 5 to 6 t/ha, adopted from the South African wheat production guideline (DALRRD, 2019). The nitrogen fertilizer was obtained from a compound NPK 2:3:4 (30) fertilizer and was applied at planting at a rate of 1000kg/ha. To improve fertilizer uptake, phosphorus fertilizer, which does not easily move down the soil profile, was applied as single superphosphate fertilizer (SSP) at a rate of 600 kg/ha. Topdressing with LAN was split during vegetative growth at tillering and jointing stage, which was about 4 and 8 weeks respectively, after planting at a rate of 22 kg/ha per split application.

Weed control was done using several herbicides as follows: Logran 750 WG at a rate of 15 g/ha was applied as a pre-emergence herbicide. Derby 175 SC at a rate of 50 ml/ha; Monitor 75 WG at a rate of 40 g/ha, and MCPA 400 SL at a rate of 3 L/ha, were applied alternatively as post-emergence herbicides at or before the four-leaf stage of weed growth. Pest and disease control was done using Bumper 250 EC (fungicide) at a rate of 400 ml/ha; Duett Ultra (fungicide) at a rate of 550 ml/ha; and Mulan 20 SP (insecticide) at a rate of 50 g/ha.

3.4.5 Data collection

Data were collected on various phenotypic traits. Duration to heading (DTH), and duration to flowering (DTF) were counted as the number of days between the sowing date and the date when 50 percent of all the shoots in a plot had fully emerged spikes, and flowers, respectively. Plant height (HT) was measured in centimeters (cm) from the ground to the tip of the awn at physiological maturity when plants could no longer grow taller. Plant height (HT) was measured from four randomly selected plants in each plot before harvesting. Duration to maturity (DTM) was counted from the sowing date to 50 percent senescence of the spikes. Spike length (SL) was measured in cm. The number of spikelets per spike (SPS) and the number of kernels per spike (KPS) were recorded after harvesting from the main tillers of four randomly selected plants. Grain yield per plot (GY) was determined as the weight (grams) of the grain from a plot and was converted to (Kg/ha) using the formula below:

$$\text{Yield (Kg/ha)} = \frac{\boldsymbol{10\ x\ (100\% - moisture\%) x\ Net\ Yield\ (g/plot)}}{\boldsymbol{Net\ Plot\ (m^2) x\ 87{,}5}}$$

*Where : **Nett plot** is usually harvested plot (4m long x 8 rows x 0.2m apart) = 6.4m*^{2}

***: Nett yield** differs across different plots or genotypes and is given in grams per plot.*

***: 10** and **87.5** are the constants of the formula.*

3.4.6 Statistical Data Analysis

Average data from two seasons was subjected to the analysis of variance. Combined ANOVA was done for all variables collected using Genstat 18th edition software, to determine the variation attributable to the environment (E), genotype (G), and genotype by environment interaction (GxE). In this case, environment E was the combination indicating how sites interacted with each water regime, resulting in four environments (ufhD; ufhW; zanD; and zanW). Mean separation was done using Tukey's HSD test at a 5% level of significance. The AMMI model, which combines ANOVA with principal component analysis (PCA), was used to study the agronomic nature of genotype-environment interactions using Proc IML procedures of SAS 9.3. The yield-stability (YSi) statistic was generated as well as the yield-regression (Ybi) statistic and yield-distance (Ydi) statistic was also generated. Stability in Ybi refers to the regression coefficient, bi (Eberhart and Russell, 1966), where the genotypes with regression coefficients (bi) equal to 1 were regarded as stable, and those genotypes with regression coefficients greater or less than 1 would be unstable.

Stability in the Ydi statistic refers to the AMMI statistic coefficient (Di), which was calculated as follows:

$$Di = \sqrt{\sum_{r-1}^{N} Y^2 is}\ (i = 1, 2, .., n)$$

Where Di is the distance of interaction principal component (IPC) point with origin in space, N is the number of significant IPCs, and Yis is the score of genotype i in an IPC. The statistic Di gives the GE interaction estimate of a particular genotype for a group of environments. The greater the Di value of a genotype, the greater the distance of the genotype from the origin of IPC axes. The genotype with the lowest value of the statistic Di would be more stable (Zhang *et al.,* 1998).

3.5 Results

3.5.1 Combined ANOVA of wheat genotypes exposed to optimum and drought-stressed field conditions at two sites over two seasons.

The outputs of the combined analysis of variance are shown in **Table 3.2**. In this case, environment E (p<0.001) is the combination of seasons and sites to make four environments namely UFH-drought; UFH-optimum; ZAN-drought; and ZAN-optimum. The ANOVA table shows that all the interactions were significant for grain yield and other agronomic traits (p<0.001). This implies that the performance of wheat genotypes over two sites and seasons was different. Therefore, **Tables 3.3 and 3.6** show the ANOVA tables for UFH and ZAN respectively, with a more detailed assessment of the response of the 40 genotypes for all traits that were recorded.

3.5.2 Analysis of variance for agronomic and physiological traits under optimum and drought stress at University of Fort Hare Research Farm over two seasons.

Table 3.3 shows highly significant differences (p<0.001) among genotypes for all studied agronomic and physiological traits, except canopy temperature (CT). Significant differences in water regimes (p<0.001) were also observed for all agro-physiological traits except spike length (SL); number of spikelets per spike (SPS); and number kernels per spike (KPS). The effects of season showed significant differences (p<0.001) for all traits except spike length. The genotype by water regime interaction (G*WR) showed non-significant differences for all physiological traits, and two agronomic traits, (SL and SPS), with all other agronomic traits being highly significant (p<0.0001)

Table 3.2: Combined Analysis of variance showing mean squares for 40 wheat genotypes exposed to drought and optimum field conditions over two seasons and sites.

SV	DF	CC	CT	DTH	DTF	DTM	KPS	HT	SL	SPS	GY
E	3	3744,27**	1088,1**	10321,2**	13001,6**	35199,1**	1699,8**	16216,1**	37,2**	37,9**	103584803**
E_R	4	5,5NS	21,6**	60,8**	114,4**	92,5**	157,4*	337*	1,9^{ns}	7,4**	1151624^{ns}
E_R_B	32	33,18**	10,6**	444,7**	417**	226,4**	234,6**	684,9**	4,2**	9,5**	3722516**
G	39	67,11**	3,6^{ns}	2581,1**	2497,9**	832,8**	222,7**	2242,6**	19**	22,9**	6053638**
WR	1	482,1**	1242,9**	72,2*	108,1**	11314,8**	616,7**	3540,8**	24,5**	32,8**	3752403**
G*WR	39	13,32^{ns}	2,2^{ns}	15,5NS	31,9**	48,4**	55,5^{ns}	122,7^{ns}	1,1^{ns}	2,9^{ns}	824218*
G*E	117	38,21**	4,1^{ns}	126,2**	135,5**	111,8**	126,9**	231,7**	2,4**	5,3**	1315351**
G*E*WR	120	15,29NS	24,3**	14,4^{ns}	23,5**	77,1**	68,4*	101,6^{ns}	1,1^{ns}	4,1**	1527306**
CV %		8,08	6,7	3,76	3,71	3,41	18,22	10,59	10,11	8,38	34,99

Where SV: source of variance; E: environment; E_R: Environment by replicate for each site; E_R_B: Environment by rep by block for each site; G: genotype; WR: water regime; CV%: coefficient of variance; DF: degrees of freedom; CC: chlorophyll content; CT: canopy temperature; DTH, DTF, and DTM: duration to heading, flowering, and maturity respectively; KPS: kernels per spike; HT: plant height; SL: spike length; SPS: spikelet per spike; GY: grain yield per plot; NS: non-significant; *: significant; **: highly significant; ***: highly significant.

Table 3.3: Analysis of variance showing sum of squares for 40 wheat genotypes exposed to drought and optimum field conditions over two seasons at UFH site.

SV	DF	CC	CT	DTH	DTF	DTM	HT	SL	SPS	KPS	GY
G	39	1364,96^{ns}	108,53^{ns}	81797,26***	81874,8***	34097,43***	81568,35***	739,06***	932,2***	7556,74***	287889397***
R	1	7,98^{ns}	202,24***	50,79^{ns}	39,75**	63,9^{ns}	638,6^{ns}	3,57*	5,99^{ns}	247,48^{ns}	2124736^{ns}
B	4	55,99^{ns}	6,65^{ns}	7,69^{ns}	7,63^{ns}	146,09*	243,69NS	3,75^{ns}	13,65^{ns}	125,91^{ns}	2338964^{ns}
S	1	96,18**	112,01***	15600,9***	16176,21***	40590,41***	55600,58***	0,69^{ns}	14,74**	4109,63***	185120743***
WR	1	177,13***	1892,79***	82,92**	150,19***	9496,3***	4434,33***	3,99**	0,009^{ns}	54,84^{ns}	75767162***
G*WR	39	672,98^{ns}	64,25^{ns}	1270,06***	2105,37***	3154,15***	8675,78**	18,55^{ns}	69,06^{ns}	4280,95**	36441952**
G*S	39	1051,09***	112,52^{ns}	6940,38***	6500,02***	3216,73***	13935,62***	103,83***	312,05***	7357,49***	84337352***
S*WR	1	46,12^{ns}	3,33^{ns}	551,48***	1120,88***	5474,25***	127,03^{ns}	21,4***	19,15**	254,84*	35749563***
G*WR*S	39	649,59^{ns}	82,13^{ns}	1125,82***	1345,72***	1943,65***	5525,47^{ns}	23,69^{ns}	51,77^{ns}	2960,53^{ns}	18868927^{ns}
CV%		8,18	5,6	2,3	2,38	3,4	12,63	7,11	7,95	19,55	30,61

Where SV: source of variance; G: genotype; R; replicate; B: block; S: season; WR: water regime; G*WR: genotype by water regime interaction; G*S: genotype by season interaction; S*WR: season by water regime interaction; G*WR*S: genotype by water regime by season interaction; CV%: coefficient of variance; DF: degrees of freedom; CC: chlorophyll content; CT: canopy temperature; DTH, DTF, and DTM: duration to heading, flowering, and maturity respectively; KPS: kernels per spike; HT: plant height; SL: spike length; SPS: spikelet per spike; GY: grain yield per plot; NS: non-significant; *: significant; **: highly significant; GM: grand mean.

3.5.2.1 Response of different agro-physiological traits under optimum and drought stress at UFH.

3.5.2.1.1. Spike length

Table 3.4 below shows the ranking of the top five and bottom five genotypes at the UFH site. The top five genotypes had their means below the grand mean of 10.07 with G34 having the shortest spike; and bottom five genotypes had their means above the grand mean with G2 having the largest spike.

Table 3.4: Response of wheat genotypes to spike length at the UFH site.

Top five genotypes		**Bottom five genotypes**	
Genotype	**LSM**	**Genotype**	**LSM**
G34	7.84^{a}	G23	11.68^{mno}
G36	8.13^{ab}	G27	11.82^{no}
G33	8.30^{abc}	G30	11.95^{no}
G11	8.43^{abcd}	G6	12.26^{o}
G8	8.62^{abcde}	G2	12.27^{o}
GM **10.07**			

Table 3.4 shows the mean separation and least square mean (LSM) of the top five and bottom five genotypes with respect to spike length at UFH site. Genotypes followed by the same letters are not significantly, while those followed by different letters are significantly different.

3.5.2.2 Number of spikelets per spike

Table 3.5 below shows the ranking of the top five and bottom five genotypes at the UFH site. The top five genotypes had their means above the grand mean of 17.195 with G2 having many spikelets per spike; and the bottom five genotypes had their means below the grand mean with G13 having few spikelets per spike.

Table 3.5: Response of wheat genotypes to the number of spikelets per spike at the UFH site.

Top five genotypes		**Bottom five genotypes**	
Genotype	**LSM**	**Genotype**	**LSM**
G2	20.03^{a}	G33	15.66^{fghij}
G6	20.03^{a}	G32	15.37^{ghij}
G17	19.81^{ab}	G40	15.03^{hij}
G19	19.53^{abc}	G29	14.91^{ij}
G16	18.72^{abcd}	G13	13.97^{j}
GM **17.195**			

Table 3.5 shows the mean separation and least square mean (LSM) of the top five and bottom five genotypes with respect to the number of spikelets per spike at UFH site. Genotypes followed by the same letters are not significantly, while those followed by different letters are significantly different.

3.5.2.5 Duration to heading.

Figure 3.2 shows the response of genotypes to DTH across water regimes in the UFH site. Genotypes under optimum conditions (99.4) headed earlier than drought stress (100.06) as differences in grand means depict. Five genotypes that headed early under drought stress include: G13; G33; G34; G15; and G38, with the earliest genotype (G13) having a mean of 83.6 under drought stress and was below the grand mean. On the other hand, the latest genotype (G2) under drought stress had a mean of 123.4 which was above the grand mean of 99.73.

3.5.2.6 Duration to flowering.

Figure 3.3 shows the response of genotypes to DTF across water regimes in the UFH site. Genotypes flowered earlier under optimum conditions (104.8) than under drought stress (106). Five genotypes that flowered early under drought include: G13; G33; G15; G34; and G38, with the earliest genotype (G13) having a mean of 90 under drought and was below the grand mean. On the other hand, the latest genotype (G4) under drought had a mean of 134.2 which was above the grand mean of 105.4.

3.5.2.7 Duration to maturity

Figure 3.4 shows the response of genotypes to DTM across water regimes in the UFH site. Genotypes matured earlier under drought stress (137.3) than under optimum conditions (145.8). Five genotypes that matured early under drought include: G15; G34; G33; G12; and G38, with the earliest genotype (G15) having a mean of 125.05 under drought. On the other hand, the latest genotype (G20) under drought had a mean of 153.08 which was above the grand mean of 141.55.

3.5.2.8 Plant height

Figure 3.5 shows the response of genotypes to plant height across water regimes in the UFH site. Genotypes under optimum conditions were taller (101.61) than genotypes under drought stress (95.56). Five genotypes that were the taller under drought include: G18; G25; G27; G19; and G24, with the tallest genotype (G18) having a mean of 144.43 under drought. On the other hand, the shortest genotype (G33) under drought had a mean of 69.8 which was below the grand mean of 98.585.

3.5.2.9 Kernels per spike

Figure 3.6 shows the response of genotypes to KPS across water regimes in the UFH site. Genotypes under optimum conditions (39.64) had more kernels per spike than genotypes under drought stress (38.95). Five genotypes that had a larger number of kernels under drought include: G14; G12; G37; G1; and G5, with genotype (G14) having many kernels per spike and a mean of 50.56 under drought. On the other hand, genotype (G2) had fewer kernels under drought with a mean of 24.31 which was below the grand mean of 39.295.

3.5.2.10 Grain yield

Figure 3.7 shows the response of genotypes to GY (kg/ha) across water regimes in the UFH site. Genotypes under optimum conditions (2693) yielded more than genotypes under drought stress (1907). The five most yielding genotypes at UFH under drought include: G9; G5; G21; G22; and G36, with the most yielding genotype (G9) under drought, having a mean of 2875.05. Genotype (G24) was the lowest yielding genotype under drought with a mean of 562.67 which was below the grand mean of 2300.

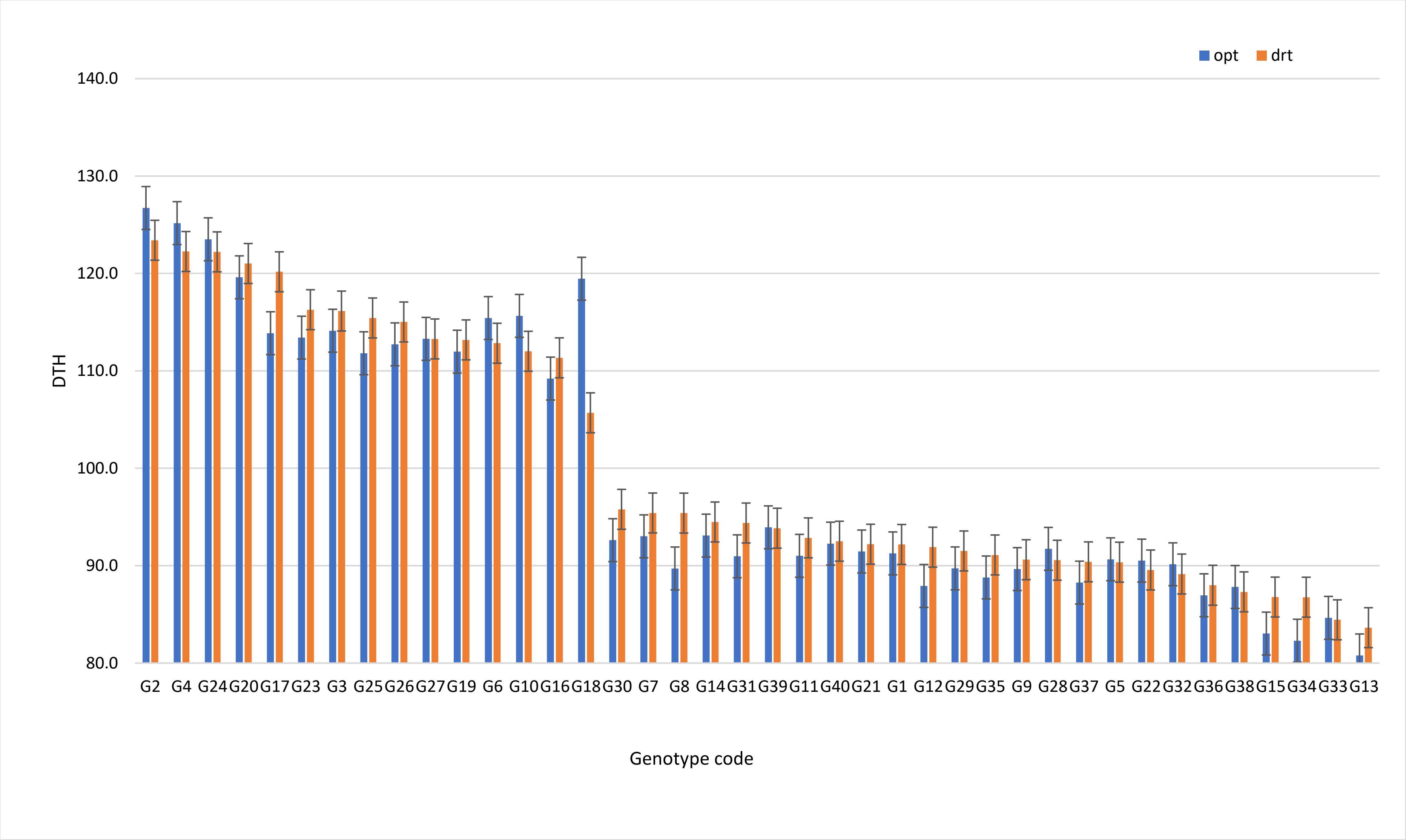

Figure 3. 2: Response of wheat genotypes to the duration of heading under drought (drt) and optimum (opt) conditions at the UFH site.

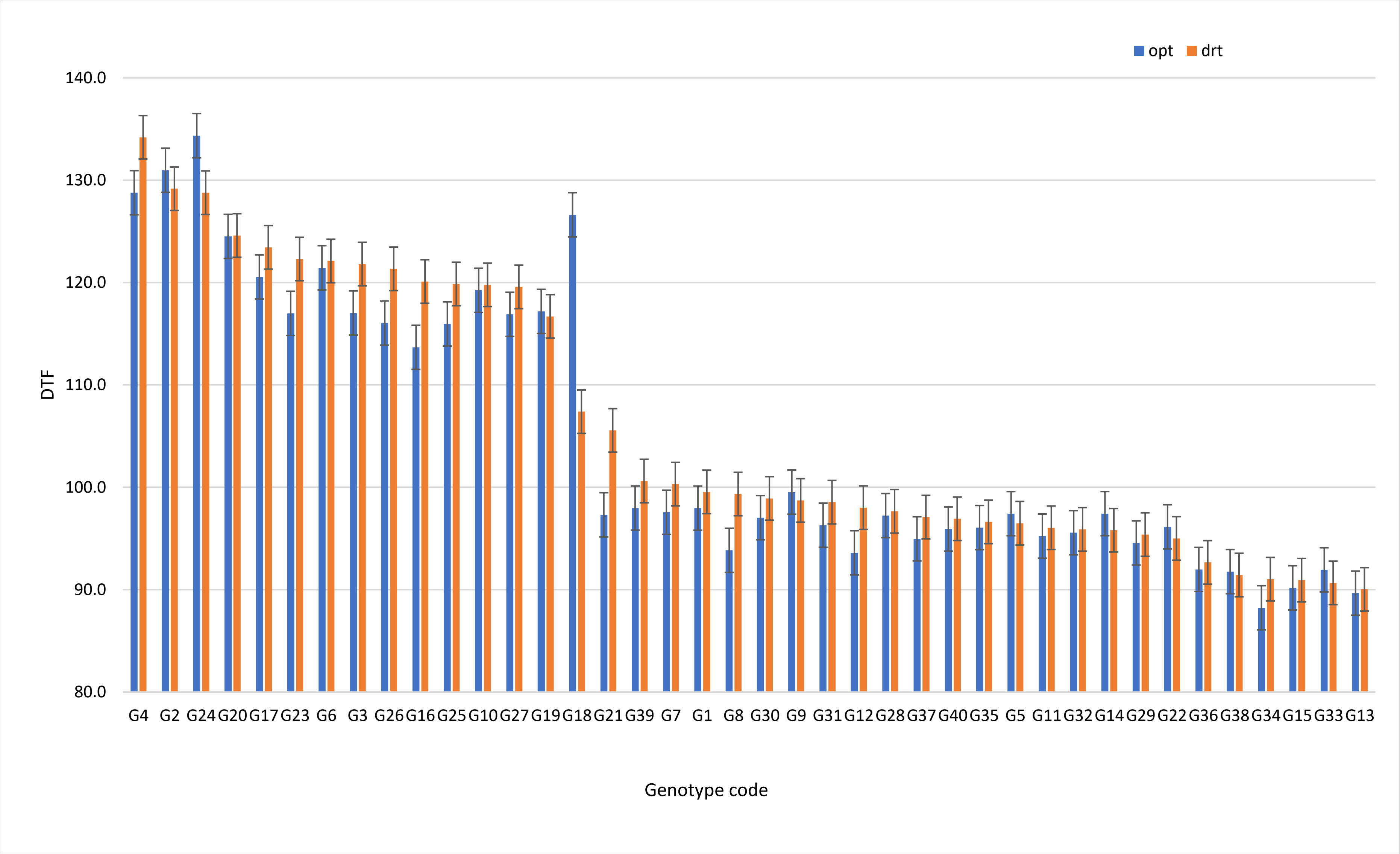

Figure 3. 3: Response of wheat genotypes to the duration of flowering under drought (drt) and optimum (opt) conditions at the UFH site.

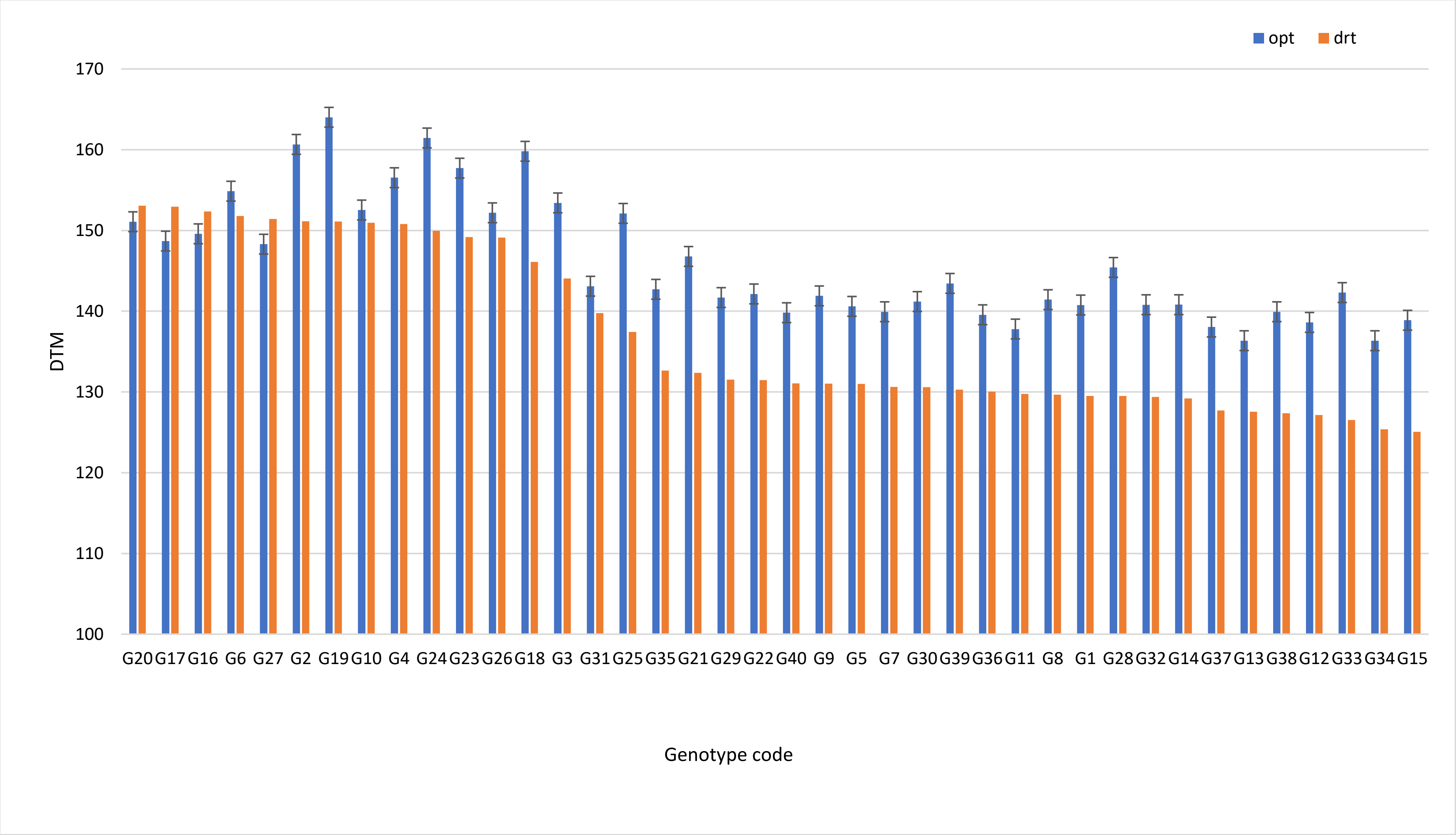

Figure 3. 4: Response of wheat genotypes to the duration of maturity under drought (drt) and optimum (opt) conditions at the UFH site.

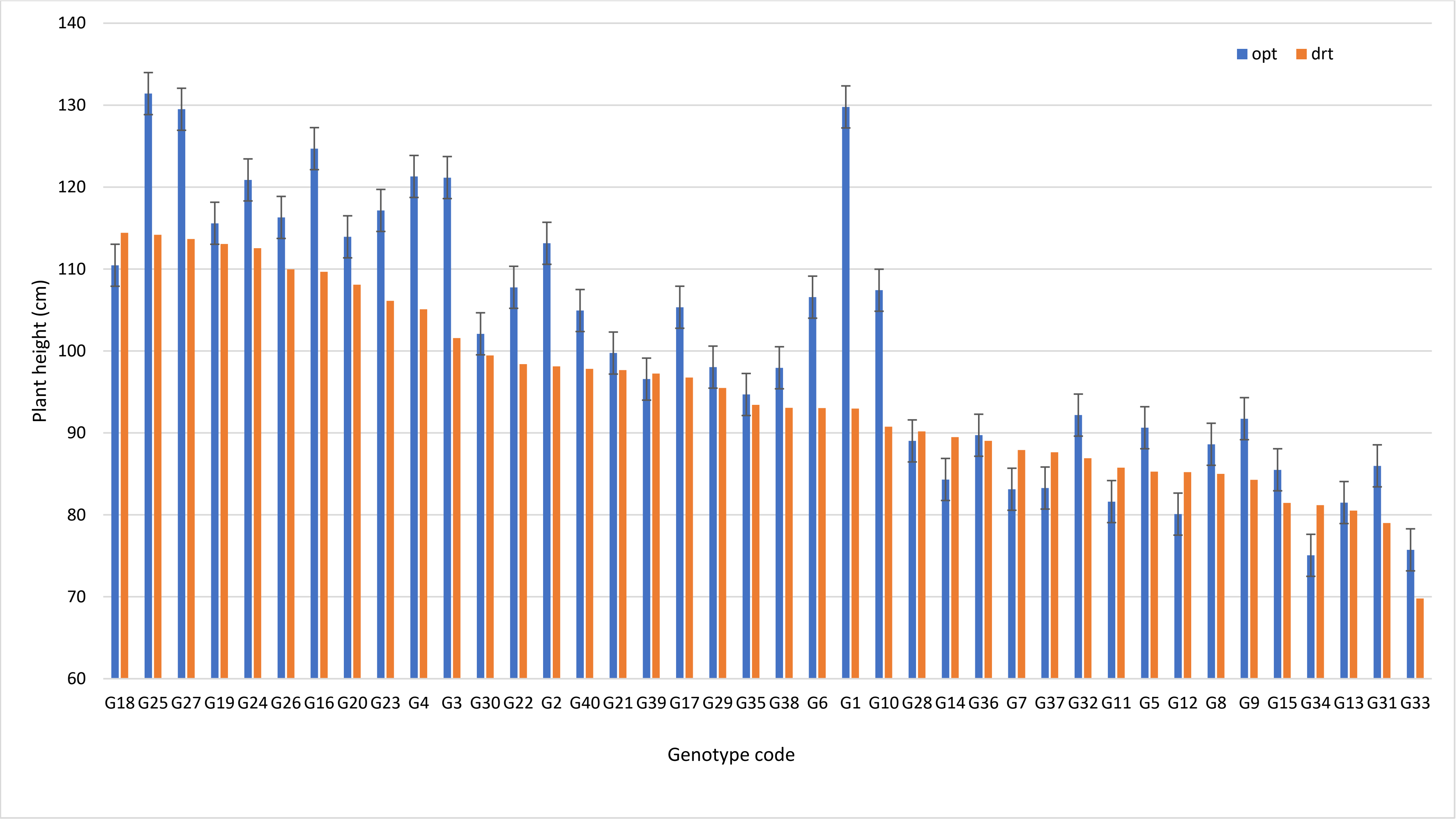

Figure 3. 5: Response of wheat genotypes to plant height (cm) under drought (drt) and optimum (opt) conditions at the UFH site.

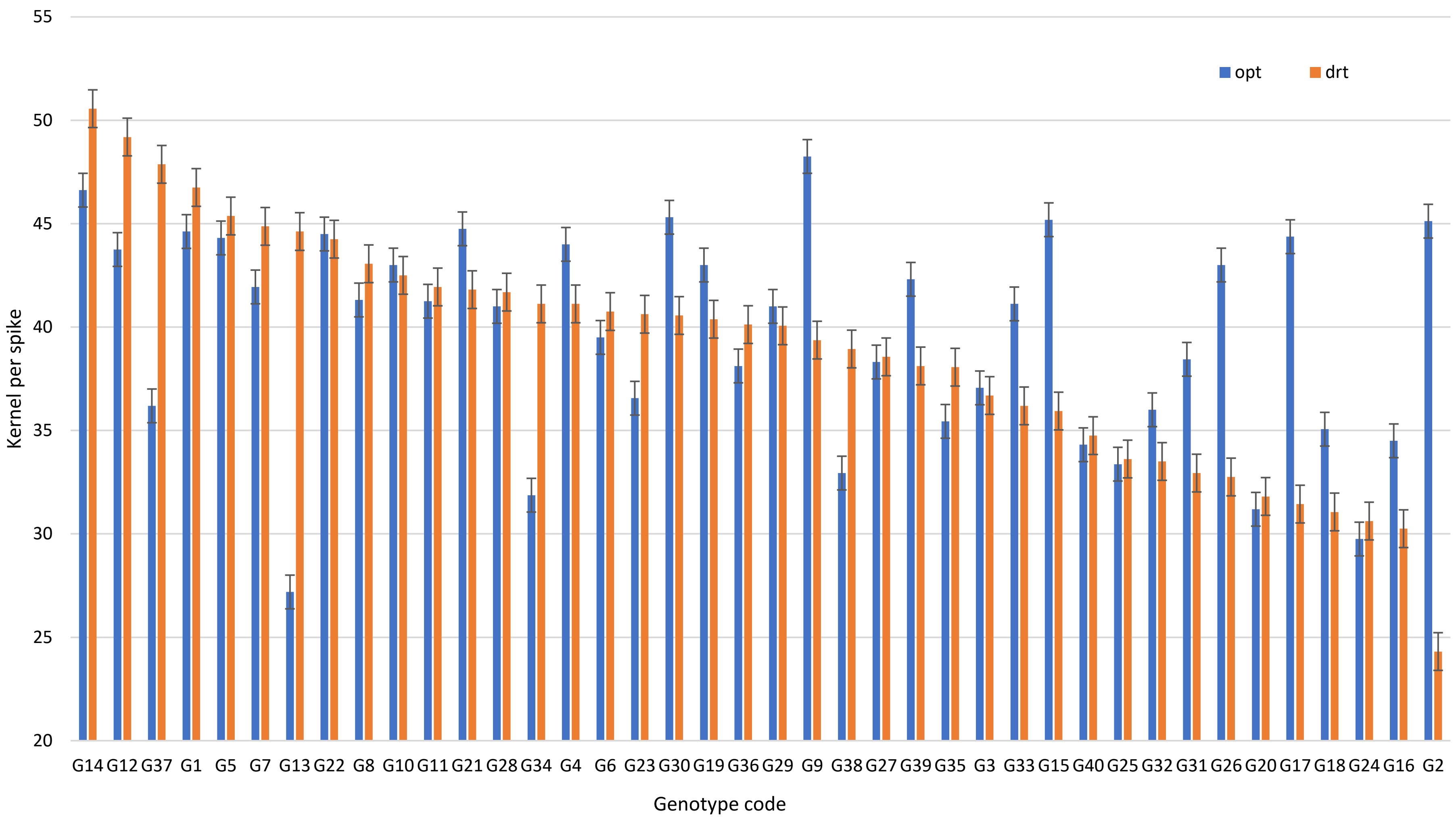

Figure 3. 6: Response of wheat genotypes to kernel per spike under drought (drt) and optimum (opt) conditions at the UFH site.

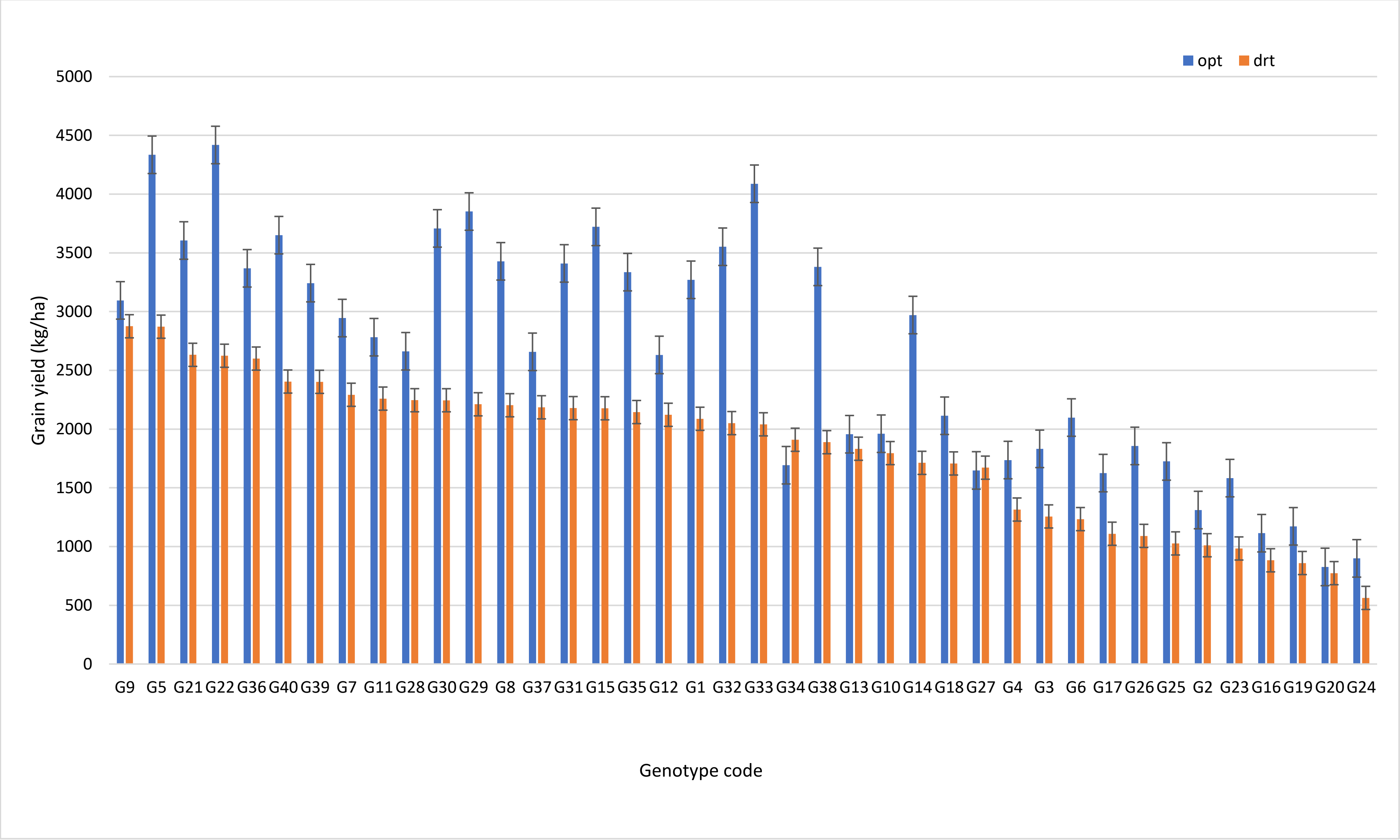

Figure 3. 7: Response of wheat genotypes to grain yield (kg/ha) under drought (drt) and optimum (opt) conditions at the UFH site.

Table 3.6: Analysis of variance showing sum of squares for 40 wheat genotypes exposed to drought and optimum field conditions over two seasons at the ZAN site.

SV	DF	CC	CT	DTH	DTF	DTM	HT	SL	SPS	KPS	GY
G	39	4078,3***	577,6***	76963,6***	76415,5***	22477,6***	74772,08***	518,67***	648,11***	12249,38***	212233476***
R	1	1,1^{ns}	100,9***	157,1*	237,0**	596,96***	292,75^{ns}	5,898^{ns}	2,78^{ns}	539,41**	2981071*
B	4	118,8*	28,2^{ns}	79,7^{ns}	49,8^{ns}	85,36^{ns}	380,12^{ns}	4,67^{ns}	32,35**	823,95**	1768934NS
S	1	6094,3***	2371,9***	27195,4***	38511,9***	108105,03***	21009,57***	168,39***	67,45***	2429,44***	218961731***
WR	1	370,3***	1,0^{ns}	5,6^{ns}	0,063^{ns}	9706,5***	1312,99***	44,77***	144,99***	352,99**	36584940***
G*WR	39	545,1^{ns}	106,7^{ns}	391,1^{ns}	709,6^{ns}	1103,54^{ns}	1354,85^{ns}	48,75^{ns}	236,32***	2029,46^{ns}	33121481***
G*S	39	3328,8***	395,5***	14277,9***	16430,1***	14820,97***	13460,73***	165,78***	588,67***	10687,32***	91228185***
S*WR	1	22,4^{ns}	2117,4***	14,0^{ns}	3,3^{ns}	953,44***	300,64*	0,06^{ns}	265,24***	1005,22***	48266059***
G*WR*S	39	487,5^{ns}	256,6*	357,3^{ns}	585,3^{ns}	2520,3***	1870,89^{ns}	42,92^{ns}	159,58***	1901,53^{ns}	30338733**
CV%		8,23	8	5,04	5,08	3,52	9,12	12,95	9,07	18,31	42,72

Where SV: source of variance; G: genotype; R; replicate; B: block; S: season; WR: water regime; CV%: coefficient of variance; DF: degrees of freedom; CC: chlorophyll content; CT: canopy temperature; DTH, DTF, and DTM: duration to heading, flowering, and maturity respectively; KPS: kernels per spike; HT: plant height; SL: spike length; SPS: spikelet per spike; GY: grain yield per plot; ns: non-significant; *: significant; **: highly significant; ***: highly significant.

3.5.2.2 Response of agro-physiological traits under optimum and drought stress at ZAN.

3.5.2.2.1 Chlorophyll content

Table 3.7 shows the ranking of the top five and bottom five genotypes at the ZAN site. The top five genotypes had their means above the grand mean of 41.92 with G2 having the highest chlorophyll content; and the bottom five genotypes had their means below the grand mean with G34 having the lowest chlorophyll content.

Table 3.7: Response of wheat genotypes to chlorophyll content at the ZAN site.

Top five genotypes		**Bottom five genotypes**	
Genotype	**LSM**	**Genotype**	**LSM**
G2	46.76^{a}	G18	37.68^{efghi}
G31	46.67^{a}	G29	37.22^{fghi}
G40	46.63^{a}	G9	36.95^{ghi}
G4	46.29^{ab}	G1	36.94^{hi}
G10	46.26^{ab}	G34	35.48^{i}
GM	**41.92**		

Table 3.7 shows the mean separation and least square mean (LSM) of the top five and bottom five genotypes with respect to chlorophyll content at ZAN site. Genotypes followed by the same letters are not significantly, while those followed by different letters are significantly different.

3.5.2.2.2 Canopy temperature

Table 3.8 shows the ranking of the top five and bottom five genotypes at the ZAN site. The top five genotypes had their means below the grand mean of 28.915 with G18 having the highest canopy temperature; and the bottom five genotypes had their means above the grand mean with G4 having the lowest canopy temperature.

Table 3.8: Response of wheat genotypes to canopy temperature at the ZAN site.

Top five genotypes		**Bottom five genotypes**	
Genotype	**LSM**	**Genotype**	**LSM**
G18	26.76^{a}	G11	30.11^{ab}
G20	26.83^{a}	G22	30.12^{ab}
G17	27.12^{ab}	G23	30.12^{ab}
G10	27.18^{ab}	G16	30.56^{ab}
G2	27.37^{ab}	G4	31.76^{b}
GM	**28.915**		

Table 3.8 shows the mean separation and least square mean (LSM) of the top five and bottom five genotypes in response to canopy temperature at ZAN site. Genotypes followed by the same letters are not significantly, while those followed by different letters are significantly different.

3.5.2.2.3 Duration to heading.

Table 3.9 shows the ranking of the top five and bottom five genotypes at the ZAN site. The top five genotypes had their means above the grand mean of 98.015 with G2 having the longest duration; and the bottom five genotypes had their means below the grand mean with G15 with the shortest duration.

Table 3.9: Response of wheat genotypes to the duration of heading at the ZAN site.

Top five genotypes		**Bottom five genotypes**	
Genotype	**LSM**	**Genotype**	**LSM**
G2	$124,69^{a}$	G34	$84,69^{hij}$
G24	$123,28^{ab}$	G33	$84,63^{hij}$
G10	$117,20^{bc}$	G38	$84,26^{hij}$
G6	$117,20^{bc}$	G13	$84,19^{ij}$
G4	$117,11^{bc}$	G15	$82,01^{j}$
GM **98.015**			

Table 3.9 shows the mean separation and least square mean (LSM) of the top five and bottom five genotypes in response to the duration of heading at ZAN site. Genotypes followed by the same letters are not significantly, while those followed by different letters are significantly different.

3.5.2.2.4 Duration to flowering.

Table 3.10 shows the ranking of the top five and bottom five genotypes for the ZAN site. The top five genotypes had their means above the grand mean of 104.6 with G2 having the longest duration; and the bottom five genotypes had their means below the grand mean with G15 having the shortest duration.

Table 3.10: Response of wheat genotypes to the duration to flowering at the ZAN site.

Top five genotypes		**Bottom five genotypes**	
Genotype	**LSM**	**Genotype**	**LSM**
G2	130,21^{a}	G34	90,73^{hij}
G24	129,19^{ab}	G33	90,18^{ij}
G6	125,29^{abc}	G13	90,05^{ij}
G23	123,72^{abcd}	G38	89,58^{ij}
G4	123,04^{abcd}	G15	88,69^{j}
GM **104.6**			

Table 3.10 above shows the mean separation and least square mean (LSM) of the top five and bottom five genotypes in response to the duration of flowering at ZAN site. Genotypes followed by the same letters are not significantly, while those followed by different letters are significantly different.

3.5.2.2.5 Duration to maturity

Table 3.11 shows the ranking of the top five and bottom five genotypes for the duration to maturity at the ZAN site. The top five genotypes had their means above the grand mean of 138.2 with G19 having the longest duration; and the bottom five genotypes had their means below the grand mean with G36 having the shortest duration.

Table 3.11: Response of wheat genotypes to the duration to maturity at ZAN site.

Top five genotypes		**Bottom five genotypes**	
Genotype code	**LSM**	**Genotype code**	**LSM**
G19	152,4322^{a}	G12	130,2466^{lmn}
G4	152,4219^{a}	G13	129,1224^{mn}
G24	149,7526^{ab}	G29	129,0381^{mn}
G26	148,9675^{abc}	G15	128,8461^{mn}
G16	147,8293^{abcd}	G36	128,0974^{n}
GM	**138.2**		

Table 3.11 shows the mean separation and least square mean (LSM) of the top five and bottom five genotypes in response to the duration to maturity at ZAN site. Genotypes followed by the same letters are not significantly, while those followed by different letters are significantly different.

3.5.2.2.6 Plant height

Table 3.12 shows the ranking of the top five and bottom five genotypes for plant height at the ZAN site. The top five genotypes had their means below the grand mean of 100.5 with G33 being the shortest; and the bottom five genotypes had their means above the grand mean with G18 being the tallest.

Table 3.12: Response of wheat genotypes to plant height at ZAN site.

Top five genotypes		**Bottom five genotypes**	
Genotype	**LSM**	**Genotype**	**LSM**
G33	77.4^{a}	G16	118.2^{ij}
G13	82.2^{ab}	G3	119.1^{ij}
G9	83.3^{ab}	G19	121.5^{ij}
G34	84.0^{ab}	G24	122.5^{ij}
G11	85.9^{abc}	G18	127.9^{j}
GM	**100.5**		

Table 3.12 shows the mean separation and least square mean (LSM) of the top five and bottom five genotypes in response to plant height at ZAN site. Genotypes followed by the same letters are not significantly, while those followed by different letters are significantly different.

3.5.2.2.7 Spike length

Table 3.13 shows the ranking of the top five and bottom five genotypes for spike length at the ZAN site. The top five genotypes had their means above the grand mean of 9,9465 with G20 having the largest spike; and the bottom five genotypes had their means below the grand mean with G33 having the shortest spike.

Table 3.13: Response of wheat genotypes to spike length at ZAN site.

Top five genotypes		**Bottom five genotypes**	
Genotype	**LSM**	**Genotype**	**LSM**
G20	12.537^{a}	G1	8.828^{defgh}
G30	12.087^{ab}	G5	8.666^{efgh}
G6	11.841^{abc}	G36	8.472^{fgh}
G10	11.453^{abcd}	G34	8.275^{gh}
G27	11.309^{abcde}	G33	7.859^{h}
GM **9.9465**			

Table 3.13 shows the mean separation and least square mean (LSM) of the top five and bottom five genotypes in response to spike length at ZAN site. Genotypes followed by the same letters are not significantly, while those followed by different letters are significantly different.

3.5.2.2.8 Kernels per spike

Table 3.14 shows the ranking of the top five and bottom five genotypes for the number of kernels per spike at the ZAN site. The top five genotypes had means above the grand mean of 38.77 with G12 having many kernels per spike; and the bottom five genotypes had their means below the grand mean with G4 having few kernels per spike.

Table 3.14: Response of wheat genotypes to kernels per spike at ZAN site.

Top five genotypes			**Bottom five genotypes**	
Genotype		**LSM**	**Genotype**	**LSM**
G12		$50,40^{a}$	G5	$32,55^{ghi}$
G9		$49,43^{ab}$	G18	$32,27^{fghi}$
G11		$47,86^{abc}$	G40	$31,76^{ghi}$
G14		$45,83^{abcd}$	G24	$29,13^{hi}$
G21		$44,39^{abcde}$	G4	$24,63^{i}$
GM	**38.77**			

Table 3.14 shows the mean separation and least square mean (LSM) of the top five and bottom five genotypes in response to kernels per spike at ZAN site. Genotypes followed by the same letters are not significantly, while those followed by different letters are significantly different.

3.5.2.2.8 Number of spikelets per spike

Figure 3.8 shows the response of genotypes to the number of spikelets per spike across water regimes in the ZAN site. Genotypes under optimum conditions (17.31) had fewer spikelets per spike than the genotypes under drought stress (18.22). Five genotypes with many spikelets at ZAN under drought included: G6; G10; G27; G16; and G25, with genotype G6 having the most number of spikelets per spike under drought, with a mean of 21 which is larger than the grand mean of 17.765. Genotype G32 had the least number of spikelets per spike with a mean of 15.31 which is less than the grand mean.

3.5.2.2.9 Grain yield

Figure 3.9 shows the response of genotypes to GY (kg/ha) across water regimes in the ZAN site. Genotypes yielded more under drought stress (2140) than under optimum conditions (1643). The five most yielding genotypes at ZAN under drought included: G33; G35; G7; G12; and G22, with genotype G33 having a mean of 3810.6736 under drought and it is above the grand mean of 1891.5. Genotype G4 was the lowest-yielding genotype with a mean of 729.999 which was below the grand mean.

3.5.3 Correlation Analysis for agro-physiological traits across sites

Tables 3.15; 3.16; 3.17; and **3.18** present the correlation analysis for UFH and ZAN sites separately. Due to significant differences observed in the performance of genotypes under the two different water regimes, the correlation was done for each water regime for each site. The correlation analysis summarizes correlation coefficients describing the degree of correlations among measured agronomic traits and grain yield. A strong significant and positive correlation was only observed between the duration of heading and flowering for all water regimes and all sites. There were generally weak negative correlation coefficients between agro-physiological traits and grain yield.

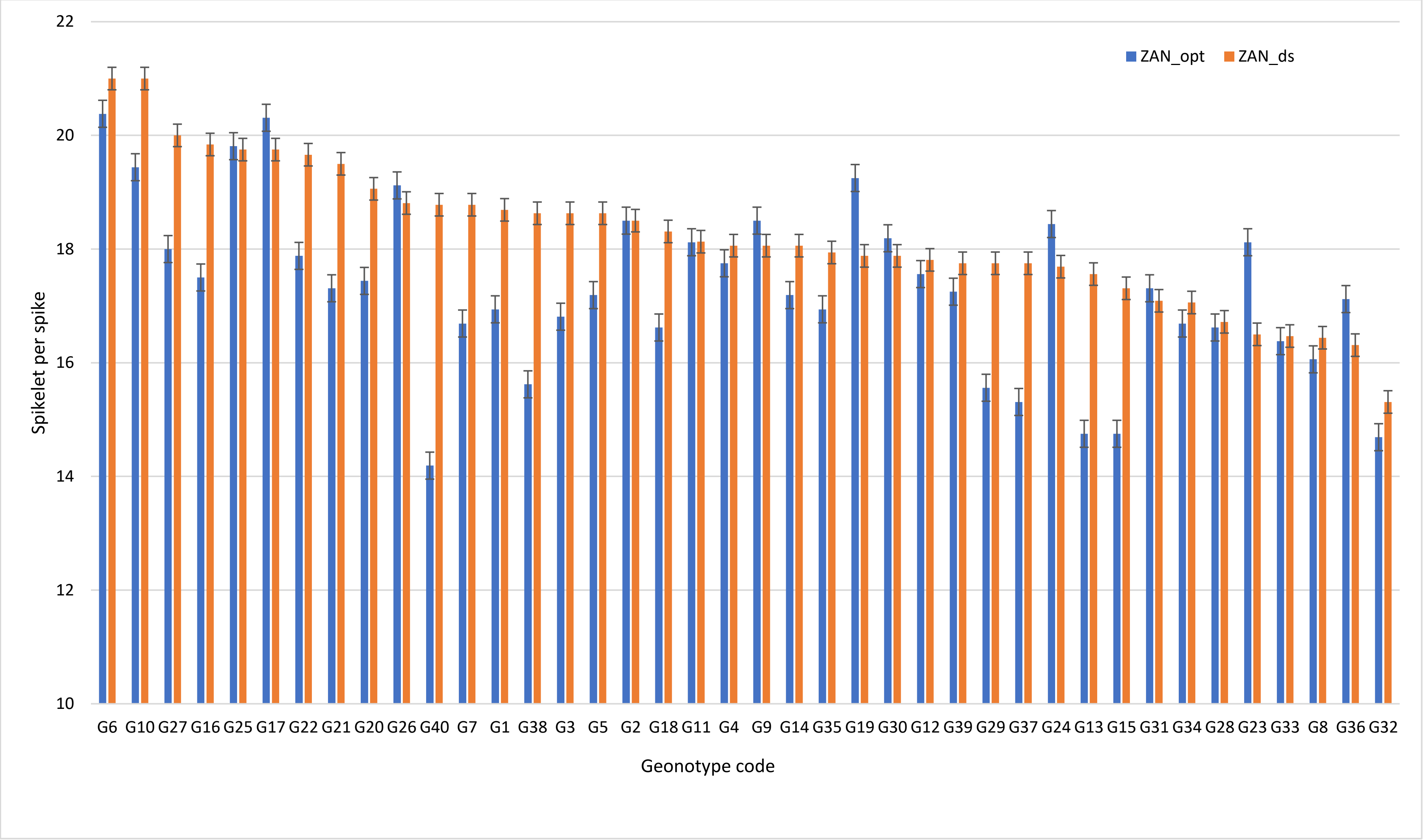

Figure 3. 8: Response of wheat genotypes to spikelet per spike under drought (drt) and optimum (opt) conditions at ZAN site.

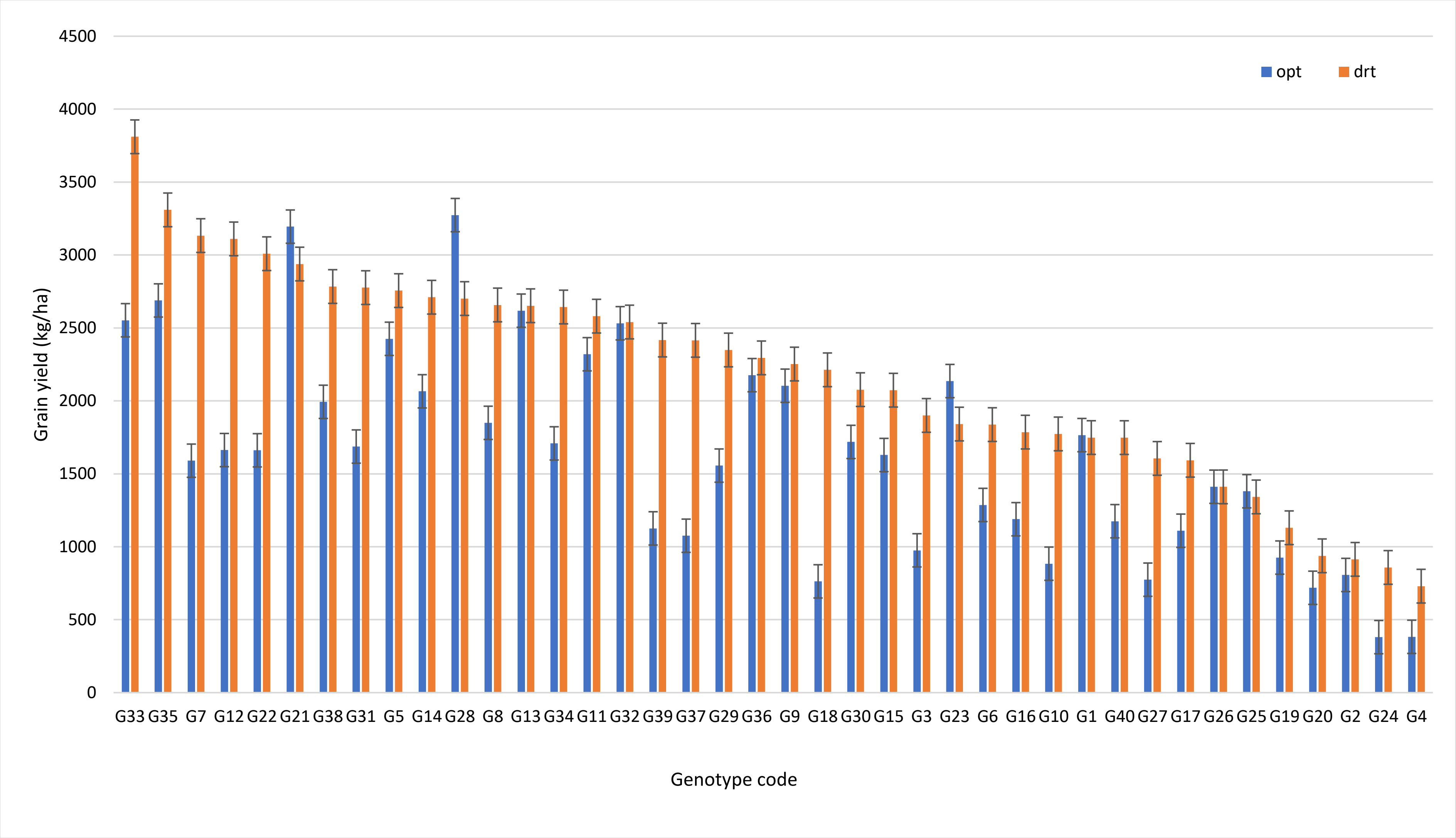

Figure 3. 9: Response of wheat genotypes to grain yield (kg/ha) under drought (drt) and optimum (opt) conditions at the ZAN site.

Table 3.15: Correlation analysis for agro-physiological traits under optimum conditions at UFH site.

	CC	**CT**	**DTH**	**DTF**	**DTM**	**HT**	**SL**	**SPS**	**KPS**	**GY**
CC										
CT	0,08ns									
DTH	-0,16*	0,20*								
DTF	-0,15ns	-0,18*	0,99***							
DTM	-0,12ns	-0,23**	0,84***	0,85***						
PH	-0,08ns	-0,08ns	0,29***	0,30***	0,08ns					
SL	-0,13ns	-0,12ns	0,56***	0,54***	0,43***	0,44***				
SPS	-0,10ns	-0,06ns	0,51***	0,51***	0,33***	0,42***	0,72***			
KPS	-0,01ns	0,02ns	-0,16*	-0,16*	-0,21**	0,15ns	0,28***	0,46***		
GY	-0,03ns	-0,21**	-0,28***	-0,29***	-0,05ns	-0,42***	-0,41***	-0,36***	0,11ns	

Where CC: chlorophyll content; CT: canopy temperature; DTH, DTF, DTM: duration to heading, flowering, maturity; HT: plant height; SL: spike length; SPS: spikelet per spike; KPS: kernels per spike; GY: grain yield.

Table 3.16: Correlation analysis for agro-physiological traits under drought conditions at UFH site.

	CC	**CT**	**DTH**	**DTF**	**DTM**	**HT**	**SL**	**SPS**	**KPS**	**GY**
CC										
CT	0,05ns									
DTH	0,10ns	0,14ns								
DTF	0,09ns	0,13ns	0,98***							
DTM	0,04ns	0,22**	0,81***	0,82***						
PH	0,06ns	-0,41***	0,13ns	0,08ns	0,16*					
SL	0,07ns	0,04ns	0,64***	0,60***	0,51***	0,27***				
SPS	0,07ns	0,01ns	0,49***	0,46***	0,32***	0,29***	0,60***			
KPS	-0,08ns	-0,21**	-0,41***	-0,40***	-0,49***	0,14ns	-0,12ns	0,02ns		
GY	-0,05ns	0,02ns	-0,32***	-0,27***	0,06ns	-0,34***	-0,31***	-0,32***	0,14ns	

Where CC: chlorophyll content; CT: canopy temperature; DTH, DTF, DTM: duration to heading, flowering, maturity; HT: plant height; SL: spike length; SPS: spikelet per spike; KPS: kernels per spike; GY: grain yield.

Table 3.17: Correlation analysis for agro-physiological traits under optimum conditions at ZAN site.

	CC	**CT**	**DTH**	**DTF**	**DTM**	**HT**	**SL**	**SPS**	**KPS**	**GY**
CC										
CT	0,52***									
DTH	0,00ns	-0,41***								
DTF	-0,06ns	-0,47***	0,98***							
DTM	-0,32***	-0,67***	0,71***	0,78***						
PH	-0,08ns	-0,42***	0,68***	0,68***	0,53***					
SL	0,02ns	-0,35***	0,64***	0,64***	0,49***	0,52***				
SPS	-0,04ns	-0,40***	0,63***	0,65***	0,59***	0,38***	0,63***			
KPS	-0,13ns	-0,12ns	-0,03ns	-0,01ns	0,06ns	-0,14ns	0,19*	0,39***		
GY	-0,23**	-0,27***	-0,26**	-0,22**	0,00ns	-0,27***	-0,06ns	0,05ns	0,40***	

Where CC: chlorophyll content; CT: canopy temperature; DTH, DTF, DTM: duration to heading, flowering, maturity; HT: plant height; SL: spike length; SPS: spikelet per spike; KPS: kernels per spike; GY: grain yield.

Table 3.18: Correlation analysis for agro-physiological traits under drought conditions at ZAN site.

	CC	**CT**	**DTH**	**DTF**	**DTM**	**HT**	**SL**	**SPS**	**KPS**	**GY**
CC										
CT	-0,04ns									
DTH	-0,07ns	-0,06ns								
DTF	-0,09ns	-0,07ns	0,98***							
DTM	-0,35***	-0,04ns	0,74***	0,78***						
PH	-0,12ns	-0,21**	0,75***	0,74***	0,62***					
SL	-0,03ns	-0,02ns	0,47***	0,45***	0,35***	0,41***				
SPS	0,24**	-0,01ns	0,15ns	0,14ns	-0,02ns	0,15ns	0,17*			
KPS	-0,08ns	-0,03ns	0,14ns	0,17*	0,23**	-0,06ns	0,09ns	0,23**		
GY	-0,35***	-0,12ns	0,03ns	0,09ns	0,41***	-0,01ns	-0,02ns	-0,19*	0,41***	

Where CC: chlorophyll content; CT: canopy temperature; DTH, DTF, DTM: duration to heading, flowering, maturity; HT: plant height; SL: spike length; SPS: spikelet per spike; KPS: kernels per spike; GY: grain yield.

3.5.4 Additive main effect and multiplicative interaction (AMMI) analysis

The AMMI ANOVA table (**Table 3.19**) revealed that the genotype, environment, and genotype-by-environment interaction (GxE) effects were highly significant (P<0.001). **Table 3.20** presents the top four stable genotypes in each of the four environments. G5 ranked number 1 under both optimum and drought stress at UFH, followed by G22. G33 ranked number 1 under drought at ZAN, and number 3 under optimum conditions. G33 also ranked number 3 at UFH under drought conditions. G21 ranked number 2 under optimum conditions and number 4 under drought conditions at ZAN. G21 also ranked number 4 under drought conditions at UFH. G21 and G33 were generally the most stable genotypes across environments. **Table 3.21** further indicated that the top five genotypes G5; G33; G21; G22; G32 had their means above the grand mean.

Table 3.19: AMMI analysis of variance for grain yield across two sites.

SV	DF	SS	MS
G	39	71059366	1822035***
E	3	24297342	8099114***
I	117	31146771	266212***
IPCA 1	41	18987178	463102***
IPCA 2	39	7484635	191914^{ns}
Residuals	37	4674957	126350

SV: source of variance; G: genotype; E: environments (ufhD; ufhW; zanD; zanW); I: G*E interaction; IPCA: interaction principal component axis; df: degrees of freedom; s.s.: sum of squares; m.s.: mean squares; ***: highly significant; ns: non-significant.

Table 3.20: IPCA scores, AMMI stability values and mean yield (kg/ha) across sites.

Environment	Mean	Score	1	2	3	4
zanW	1633	32.91	G28	G21	G33	G35
zanD	2122	27.87	G33	G35	G12	G21
ufhD	1906	-12.36	G5	G22	G33	G21
ufhW	2693	-48.42	G5	G22	G29	G30

zanD: Zanyokwe drought; zanW: Zanyokwe optimum; ufhD: UFH drought; ufhW: UFH optimum.

Table 3.21: AMMI scores of the top five and bottom five genotypes based on mean grain yield.

Top five genotypes				**Bottom five genotypes**			
G-Code	**Mean**	**IPCAg1**	**IPCAg2**	**G-Code**	**Mean**	**IPCAg1**	**IPCAg2**
G5	3324	-18.85	7.49	G19	1102	5.23	2.04
G33	3129	5.95	1.88	G4	1044	-5.23	-3.25
G21	3100	1.46	10.37	G2	1006	5.46	1.24
G22	2925	-22.25	-2.07	G20	794	8.33	-1.27
G32	2550	0.002	14.04	G24	712	4.81	-0.74
Grand mean	**2088,33**						

G: genotype; G-Code: genotype code; IPCAg1: interaction principal component axes for genotypes one and two respectively.

3.5.5 AMMI BIPlot

The first two principal components, IPCA1 and IPCA2, explained 84.99% of the total GEI variation (**Figure 3.10**). The length of the vector of an environment from the biplot origin is proportional to the amount of genotype by environment interaction. The environments with longer vectors indicate strong interactive forces, while those with shorter vectors indicate weak interactive forces. zanW had the strongest interactive forces followed by ufhW. The environment ufhD and zanD had almost similar, and weaker interactive forces. Hence, genotypes G36; G24; G17; G2; G38; G20; G31; and G6 were generally stable and had almost similar yield performances, as they were very close to the centre of the origin. A genotype and an environment with markers in the same direction from the origin had a positive GEI, in opposite directions a negative interaction, and at right angles a small interaction. Therefore, genotypes G28; G5; and G12 had positive GEI with environments zanW, ufhW, and zanD respectively. Likewise, genotypes G23 and G30 had negative GEI with zanD and ufhD, respectively.

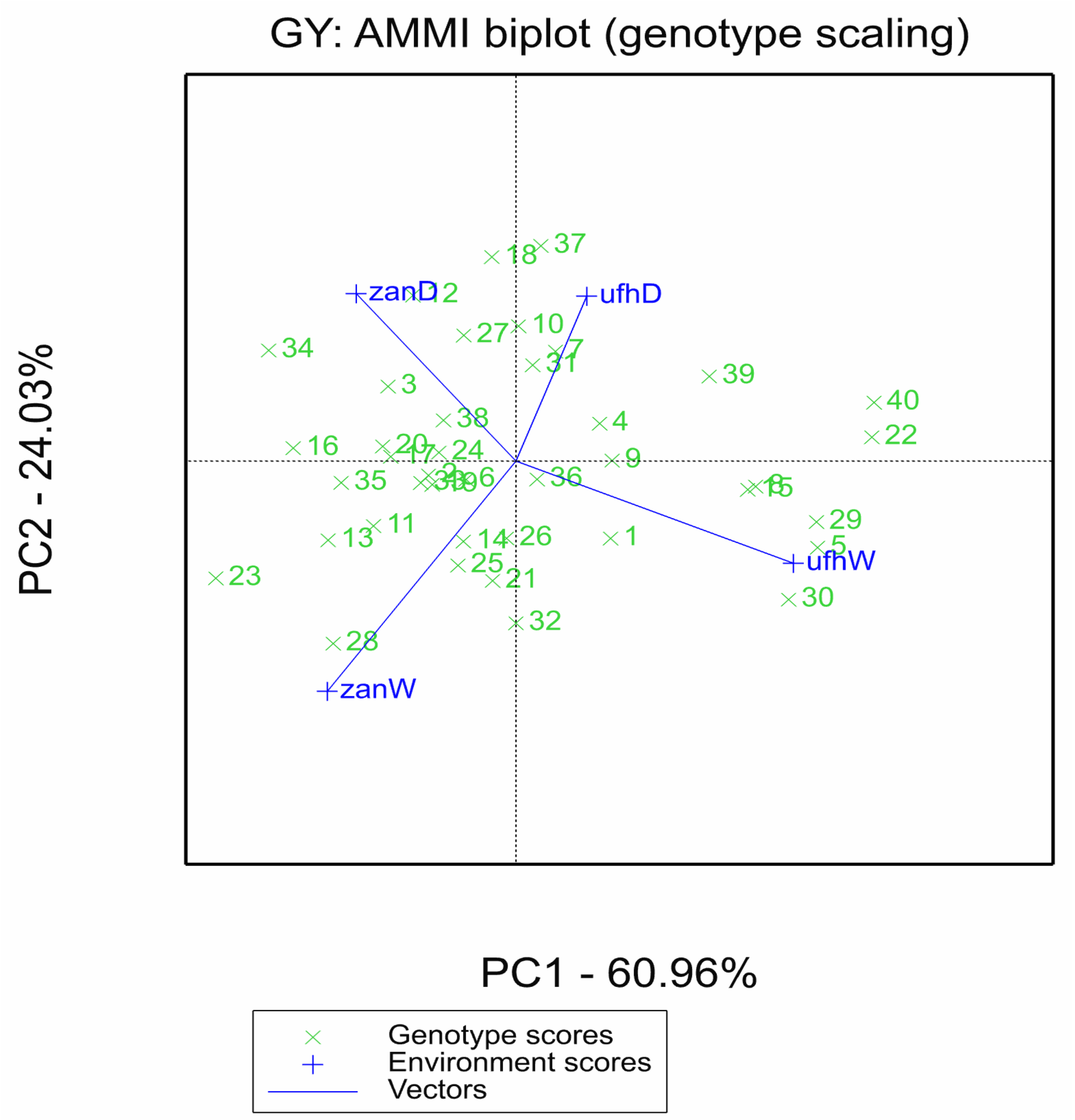

Figure 3. 10: AMMI biplot analysis of GEI. ufhD: University of Fort Hare drought; ufhW: University of Fort Hare optimum; zanD: Zanyokwe drought; zanW: Zanyokwe optimum; numbers (x1 to x40): genotype code from G1 to G40.

3.6 Discussion

Agro-physiological traits

Agro-physiological traits generally had their means below the grand mean as presented in **Table 3.2**. Also, as shown in **Table 2**, agro-physiological traits had different mean values under two different watering regimes. Under drought conditions, mean values were generally reduced compared to optimum conditions except for canopy temperature, duration of heading, flowering, and maturity. Chlorophyll content, plant height, spike length, spikelet per spike, kernels per spike, and grain yield were reduced under drought conditions. A significant reduction in these traits due to drought stress has also been reported in previous studies (Liu et al., 2015; Qaseem et al., 2019; Etminan et al., 2019). The combined ANOVA (**Table 3.2**) revealed highly significant differences between genotype G, environment E, and the interaction of genotype-by-environment GxE, indicating that the genotypes had significantly different mean performances in each of the test environments.

The grain filling period is an important phenological stage that is associated with current photosynthesis that depends on the plant's chloroplast, and relocation of assimilates from reserve pools in vegetative tissues. Importantly, drought reduces the life cycle and duration of grain filling. Under drought conditions, the grain filling period decreases due to accelerated leaf senescence due to water stress and high temperature, reduced photosynthesis, and sink limitations. Furthermore, the reduced grain filling period directly influences grain number and grain size, which largely accounts for the decrease in wheat yields (Dolferus and Richards, 2011). The number of kernels per spike which largely depend on the number of spikelets per spike and spike length is another important aspect that was reported to improve grain yield in wheat (Liu et al., 2018).

An increase in the leaf temperature limits the activity of photosynthetic enzymes (such as Rubisco), leading to a reduction of carbon dioxide fixation (Shahenshah and Isoda, 2010). Smirno, (1993) further indicated that the limitation of carbon dioxide fixation increases the rate of active oxygen formation in chloroplasts, which in turn would either manifest as oxidative damage to the plant or result in the activation of defense systems that could prevent such damage from occurring. In this situation, different genotypes can adapt through the protection of their photosystem apparatus II from photodamaging effects by dissipating the excess excitation energy thermally through down-regulating photosystem apparatus (Ullah and Isoda, 2005). Drought stress increased canopy temperature when compared to optimum conditions. However, some of the genotypes may have a strong ability to protect themselves through the maintenance of high levels of photosystem apparatus II. Chlorophyll content on the other hand was observed to be increased in modern wheat cultivars and was positively and moderately correlated with grain yield (Beche et al., 2014). This was made possible by the stay-green trait that allowed for more light interception and utilization, and therefore, improved wheat yield (Cossani and Reynolds, 2012).

The efficiency of breeding programs in diverse environments can be improved by gaining an understanding of the associations between grain yield and different agro-physiological traits. Although many agronomic, morphological, and physiological traits have been studied for their use in breeding programs for drought tolerance, only a few traits are currently recommended for application in practical programs. For instance, early heading, flowering, and maturing genotypes were reported to be high yielding under dryland wheat production due to adaptive mechanisms (Mondal et al., 2016). Some studies argued that genotypes with early heading-to-maturity duration reduced the time available for assimilate partitioning required for the development of high grain yield (Royo et al., 2007). Genotypes with height-reducing genes are reported to increase genetic gains in wheat and significantly contribute to increased yield (Zhang et al., 2016). There was a moderate negative and significant correlation between plant height and grain yield. However, in the present study, the significant differences among GxE justified the need for genotype assessment and ranking in each site, and therefore **Tables 3.3** and **3.6** showed the ANOVA for UFH and ZAN respectively.

The interaction of genotypes by water regime was not significant for all studied physiological traits across sites. However, significant differences were observed among genotypes for physiological traits across sites, except canopy temperature at UFH site. Therefore, this necessitated the ranking of genotypes as presented in **Tables 4.1** and **4.2**. The highest and lowest genotypes with respect to chlorophyll content in UFH site are G19 and G12 respectively; G4 and G15 for canopy temperature. For the ZAN site, the highest and lowest genotypes with respect to chlorophyll content are G2 and G34; and in respect to canopy temperature, they were G18 and G4 respectively.

AMMI Analysis

Additive main effect and multiplicative interaction AMMI ANOVA presented in **Table 3.19** showed significant differences between genotype G, environment E, interaction I, and IPCA1. There were no significant differences for the IPCA2. Due to the significant differences observed in the genotype by environment interaction (GEI) as shown in **Table 3.6**, Yan and Tinker, (2006) reported the need for stability analysis where the interaction of genotype by environment is significant. **Table 3.20** presents the stability analysis where G5 ranked number 1 under both optimum and drought stress at UFH, followed by G22. G33 ranked number 1 under drought and number 3 under optimum conditions at ZAN. G33 also ranked number 3 at UFH under drought conditions. G21 ranked number 2 under optimum conditions and number 4 under drought conditions at ZAN. G21 also ranked number 4 under drought conditions at UFH. A stable and widely adapted genotype as defined by Gurmu et al., (2009), and Annicchiarico, (2002), is the one with the ability to perform consistently and produce a mean performance that is above the grand mean in all test sites. According to this scenario, the most stable genotypes were G21 and G33 and could be utilized in further breeding programs, as they also exhibit similarities (6-day difference) in their maturity dates. **Table 3.24** further indicated that the top five genotypes with regard to the AMMI score include G5; G33; G21; G22; G32, and their means were above the grand mean.

The AMMI Biplot presented in **Figure 3.10** shows the first two principal components, IPCA1 and IPCA2, which explained 84.99% of the total GEI variation. The length of the vector of an environment from the biplot origin is proportional to the amount of genotype by environment interaction. The environments with longer vectors indicate strong interactive forces, while those with shorter vectors indicate weak interactive forces. Environment zanW had the strongest interactive forces followed by ufhW. Environment ufhD and zanD had almost similar, and weaker interactive forces. Hence, genotypes G36; G24; G17; G2; G38; G20; G31; and G6 were generally stable and had almost similar yield performances, as they were very close to the centre of the origin. A genotype and an environment with markers in the same direction from the origin have a positive GEI, in opposite directions a negative interaction, and at right angles a small interaction. Therefore, genotypes G28; G5; and G12 had the positive GEI with environments zanW, ufhW, and zanD respectively. Likewise, genotypes G23 and G30 had negative GEI with zanD and ufhD, respectively.

Environmental differences in terms of productivity were also revealed in **Table 3.20**, where ufhW had a mean of 2693kg/ha, above the grand mean of 2088.33kg/ha; followed by zanD with a mean of 2122kg/ha, also above the grand mean. The environments ufhD with a mean of 1906kg/ha, and zanW with a mean of 1633kg/ha both had their means below the grand mean. The performance per site (ufhW + ufhD) which is UFH and (zanW + zanD) which is ZAN, was determined by means of combining two treatment means for each site and ranking them. Therefore, UFH site had the overall mean of 4599kg/ha which was greater than an overall mean of 2122kg/ha at ZAN site. From **Figure 3.10**, different winning genotypes in different test environments indicated (those very close to their respective vectors) the presence of crossover of GEI. The presence of crossover GEI could be attributed to the differences among the genotypes and the environmental conditions of test environments (Tukamuhabwa et al., 2012; Mushoriwa, 2013). The differences in altitudes, soil types, weather conditions, and rainfall distributions characterize and differentiate the test environments. The selection and recommendation of genotypes to environments are complicated by the presence of GEI crossover. GEI can be further categorized by identifying genotypes that are best suited to specific environments; across environments (wide adaptation) and/or partitioning the environments into mega-environments and identifying genotypes that are adapted to the mega-environments. Yan and Tinker, (2006) reported that the mean performance data of genotypes for at least two or more years/seasons can be used to assess the repeatability of crossover GEI across years/seasons. Therefore, the analysis of the current study as shown in **Table 3.19** was able to respond to all categories of GEI crossover. For example, G5 performed well at UFH season 1 under both drought and optimum conditions and season 2 under optimum conditions. The superiority of UFH site over ZAN site could be attributed to the combination of suitable soils, and favourable environmental conditions that favour wheat productivity throughout the growing season. On the other hand, G33 (3143kg/ha) and G21 (3117kg/ha) could appeal to both farmers and breeders because they were both stable and high-yielding genotypes, ranked second and third respectively after G5 (3457kg/ha).

3.7 Conclusion

This chapter examined yield responses of 40 genetically diverse wheat genotypes sourced from different companies. Agro-physiological traits were assessed under both optimum and drought conditions over two seasons and two sites in the Eastern Cape Province, South Africa, with more focus on grain yield data. Genotypes G21 and G33 were high-yielding and stable across two sites, as they yielded above average. These genotypes can therefore be recommended as useful genetic resources for breeding for drought tolerance and further wheat production in the province or similar environments.

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Chapter 4

The effect of drought stress on wheat grain quality composition of different wheat genotypes.

Abstract

Drought or moisture stress severely limits wheat crop production and adversely affects grain quality. The effect is more exacerbated when the grain-filling stage of the crop coincides with drought. The nutritional complement of stressed grains is significantly decreased, which will contribute to malnutrition for consumers who mainly rely on wheat as a source of food. The present study aims to determine the effect of drought stress on the wheat grain quality composition of different wheat genotypes. The experiment took place over two seasons (2020, and 2021) and two sites (UFH, and ZAN) using a 5x8 alpha lattice design with two replications and two water regimes (optimum and drought). Grain yield of forty different wheat genotypes was subjected to the near-infrared spectrum (NIR) machine to examine grain quality traits such as wet gluten (WG); fixed protein (PF); hectolitre mass (HLM); and thousand kernel weight (TKW). Average data on the abovementioned quality traits was subjected to analysis of variance. A combined ANOVA revealed significant differences (p<0.001) among the interaction of genotypes by environments (G*E) except PF. This implied that the performance of wheat genotypes across sites was different, therefore, necessitated separate analysis of variance for each site. Significant differences (p<0.001) among genotypes (G), water regimes (WR), and the interaction of genotypes by water regime (G*WR) were observed for all studied quality traits except PF in both sites. G*WR showed no significant differences for TKW in the ZAN site. However, the idea was to identify genotypes with stable-quality parameters across water regimes. Therefore, G38 was stable for wet gluten; G31 and G26 were stable for PF; G36 was stable for HLM; and G11, G15, and G29 were stable for TKW at the UFH site. G6 was stable for both WG and PF; G13 and G15 were stable for HLM; and G35, G21, and G40 were stable for TKW at the ZAN site. These results suggest that the quality of wheat grains was affected under drought stress conditions except PF. The observed genetic variability for quality parameters allows improvement for desired traits to facilitate stability of quality under optimum and drought-stressed conditions.

Keywords: drought stress; ANOVA; wheat grain quality

4.1 Introduction

Wheat (*Triticum aestivum* L.) is one of the important staple grain crops globally. In South Africa, the importance of wheat in terms of production ranks number two after maize. Wheat's nutritional benefits include vitamins B and E, micro-nutrients including zinc, iron, and macronutrients such as manganese, calcium, and magnesium (Amiri et al., 2015; Migliorini et al., 2016; Velu et al., 2017). Wheat grain is rich in protein, estimated to be up to 14 percent, and suitable for improving human diets in a number of end-use food products such as bread, biscuits, and pasta (Migliorini et al., 2016; Guzman et al., 2017; Giunta et al., 2019). Wheat flour quality and end-user products are determined by grain protein content and the ratio between the different protein fractions in processing industries (Xue et al., 2016; Luo et al., 2019). For instance, flour blending capacity and dough properties are influenced by gluten proteins. However, Godfrey et al., (2010) further indicated that dough extensibility is determined by gliadin proteins, whereas dough elasticity and strength are influenced by glutenin. The balance between dough, viscosity, and elasticity is determined by the gliadin to glutenin ratio, which then measures molecular weight distribution or protein size in wheat.

Just like grain yield, grain quality is affected by genotype-by-environment (G×E) interaction, necessitating the selection of promising genotypes to develop market-preferred varieties (Flagella et al., 2010; Malik et al., 2013; Rozbicki et al., 2015; Migliorini et al., 2016; Hernandez-Espinosa et al., 2018). Yan and Kang, (2003) reported that differences in genotypic performance for test traits across a diverse range of environments determine genotype by environment interaction (GxE). This may delay the effective selection of promising genotypes possessing important attributes for grain quality improvement. Wheat is cultivated in arid and semi-arid environments where recurrent drought spells affect nutritional and industrial processing quality (Li et al., 2013; Hernandez Espinosa et al., 2018). At present, minimal genetic gains are reported for grain protein content and protein fractions (Subira et al. 2014; Sanchez-Garcia et al. 2015). Therefore, improving grain quality attributes is crucial for developing high-quality and market-preferred end-user products.

High-yielding wheat genotypes are developed by different wheat breeding programs in and outside South Africa. These genotypes possess novel grain quality traits suitable for various food processing industries, and for the production of quality end-products for human consumption (Reynolds et al., 2017; Guzman et al. 2017). The South African wheat industry places immense value on grain quality, and quality is always prioritized over grain yield. Emphasis on grain quality is thus considered to have worked against the development of high-yielding wheat genotypes. To widen the wheat genetic pool for production and breeding activities in South Africa, genetically diverse wheat germplasms were acquired from different companies such as Corteva AgriScience PTY LTD; Sensako PTY LTD; ARC grain crops institute; Stellenbosch University, and ICARDA. Understanding the magnitude of GxE interaction under target production environments on quality traits for nutritional purposes is crucial for selecting well-adapted genotypes for recommendation to processing industries and improving genetic gains for grain quality traits. Therefore, the objective of this chapter is to determine the effect of drought stress on the grain quality composition of forty genetically diverse wheat genotypes sourced from different companies.

4.2 Objective

To determine the effect of drought stress on wheat grain quality composition of 40 different wheat genotypes.

4.3 Hypothesis

Drought stress has no effect on grain quality and protein content of available wheat genotypes.

4.4 Materials and methods

The materials and methods were the same as described in **Chapter 3.2.** Wheat grain yield from drought and optimum conditions was first cleaned using a Haldrup blower where chaff was separated from the clean healthy seeds, then subjected to the seed Data Count S25$^+$ for thousand kernel weight (TKW). The data count has a vibrator and a slight-slopy surface that discharges seeds gently to a container put on top of the scale. Any number that a data count would do will automatically be converted to TKW. The near-infrared-spectrum (NIR) IM9500 (perten instruments) was used for the determination of grain moisture content (MC), fixed protein (PF), wet gluten (WG), and hectolitre mass (HLM). The machine would be calibrated to work on a specific type of crop, which was wheat. A maximum of 500 grams of grain would be poured on the opening top, the machine would run and analyse for up to one minute and display the reading with their specific units on a machine's small screen. Data from these pieces of equipment was subjected to statistical analysis as described in **Chapter 3.2.**

4.5 Results

4.5.1 Grain quality response of wheat genotypes under optimum and drought-stressed field conditions at UFH and ZAN.

The effect of drought stress generally affected the grain quality of genotypes when compared to the optimum conditions. Quality traits had mean values reduced under drought for the fixed protein and thousand kernel weight at both sites. Hectolitre mass and wet gluten were reduced under optimum conditions and higher under drought conditions. The interaction of genotype by water regime was significant for all studied quality traits across sites except fixed protein. The interaction of genotype by water regime was also not significant for thousand kernel weight at ZAN. Highly significant differences were observed on all other traits such as hectolitre mass and wet gluten across sites, and also thousand kernel weight in UFH site. Considering the correlation analysis as presented in **Tables 4.5** and **4.6**, there were generally weak but significant correlations between grain quality traits under both sites. Fixed protein and other quality traits had non-significant correlations except wet gluten.

The combined analysis of variance (**Table 4.1**) presented the environment (E); replication in each environment (E_R); replication and blocking in each environment (E_R_B); genotype (G);

water regime (WR); genotype by water regime interaction over two sites and seasons (G*WR); genotype by environment interaction over two sites and season (G*E); and genotype by environment by water regime interaction (G*E*WR) as sources of variance. Environment E ($p<0.001$) was the combination of water regimes and sites to make four environments namely UFH-drought (ufhD); UFH-optimum (ufhW); ZAN-drought (zanD); and ZAN-optimum (zanW). ANOVA table shows that the genotype by environment interaction was significant ($p<0.001$) for all quality traits except fixed protein content (PF). This indicated that the performance of wheat genotypes over two sites differed and necessitated the assessment of wheat genotypes under separate sites. **Table 4.2** and **4.4** shows the ANOVA tables for UFH and ZAN respectively.

4.5.2. Grain quality traits of 40 wheat genotypes under optimum and drought stress at UFH site.

Table 4.1 shows significant differences ($p<0.001$) among genotypes for all studied quality traits, except fixed protein content. The genotype by environment interaction also showed significant differences for all quality traits, except fixed protein content.

Table 4. 1: Combined ANOVA table for quality traits over two seasons and sites.

SV	DF	TKW	HLM	WG	PF
E	3	477,1**	158**	10710,5**	731,5**
E_R	4	25,7*	21,3**	10,4^{ns}	78,4^{ns}
E_R_B	32	35,7**	34,3**	40**	84,5^{ns}
G	39	220,9**	78,5**	43,7**	80,7^{ns}
WR	1	272,6**	17,4*	53,7**	45,1^{ns}
G*WR	39	10,2^{ns}	15,4**	15,9**	68,6^{ns}
G*E	117	67,1**	40,7**	12,7**	69,3^{ns}
G*E*WR	120	28,9**	9,4**	17,9**	71,5^{ns}
CV %		7,83	2,14	7,99	57,28

Where SV: source of variance; E: environment; E_R: Environment by replicate for each site; E_R_B: Environment by rep by block for each site; G: genotype; WR: water regime; CV%: coefficient of variance; DF: degrees of freedom; TKW: thousand kernel weight; HLM: hectolitre mass (specific weight); WG: wet gluten; PF: fixed protein; GY: grain yield per plot; ns: non-significant; *: significant; **: highly significant.

Table 4. 2: ANOVA table for 40 wheat genotypes exposed to drought and optimum field conditions over two seasons at the UFH site.

SV	DF	TKW	HLM	WG	PF
G	39	5374,8***	1781,9***	1416,1***	4741^{ns}
R	1	45,2*	6,5^{ns}	12,7^{ns}	92,7^{ns}
B	4	31,7^{ns}	12,4^{ns}	105,5**	934,3^{ns}
S	1	1321,1***	424.8**	31955.1**	245.1^{ns}
WR	1	1494,7***	54**	149.3**	0.24^{ns}
G*WR	39	887,6***	333,7***	390,4**	4825,8^{ns}
G*S	39	2150,5***	3065,8***	603,1***	4712,9^{ns}
S*WR	1	20,6^{ns}	88.6**	0.4^{ns}	167.1^{ns}
G*WR*S	39	545,8***	216,5***	257,1^{ns}	4407,4^{ns}
CV%		7,44	2,3	8,76	60,18

Where SV: source of variance; G: genotype; R; replicate; B: block; S: season; E: environment; CV%: coefficient of variance; DF: degrees of freedom; TKW: thousand kernel weight; HLM: hectolitre mass; WG: wet gluten; PF: protein fixed; GY: grain yield per plot; ns: non-significant; *: significant; **: highly significant; ***: highly significant.

4.5.2.1 Response of wheat genotypes to grain quality traits under optimum and drought stress at UFH site.

4.5.2.1.1 Thousand kernel weight.

Figure 4.1 shows the response of genotypes to TKW across water regimes at the UFH site. The five best genotypes at UFH under drought included: G29; G10; G11; G1; and G15, with the genotype G29 having a mean of 42.45 under drought which was above the grand mean of 33.485. Genotype G18 had the lowest thousand kernel weight under drought with a mean value of 25.43 which was lower than the grand mean. Genotypes under optimum conditions (35.46) had more thousand kernel weight than genotypes under drought stress (31.51).

4.5.2.1.2 Hectolitre mass

Figure 4.2 shows the response of genotypes to HLM across environments at the UFH site. Genotypes under drought stress (75.72) had more hectolitre mass than genotypes under optimum conditions (75.42). The five best genotypes at UFH under drought included: G24; G7; G36; G9; and G12, with the genotype G24 having a mean of 80.55 under drought which was above the grand mean of 75.57. Genotype G3 had the lowest hectolitre mass under drought with a mean value of 70.49 which was lower than the grand mean.

4.5.2.1.3 Wet gluten

Figure 4.3 shows the response of genotypes to WG across environments at the UFH site. Genotypes under drought stress (28.46) had more wet gluten than genotypes under optimum conditions (26.92). The five best genotypes at UFH under drought included: G26; G14; G40; G15; and G38, with genotype G26 having a mean of 33.09 under drought which was above the grand mean of 27.69. Genotype G18 had a low wet gluten under drought with a mean value of 24.07 which was below the grand mean.

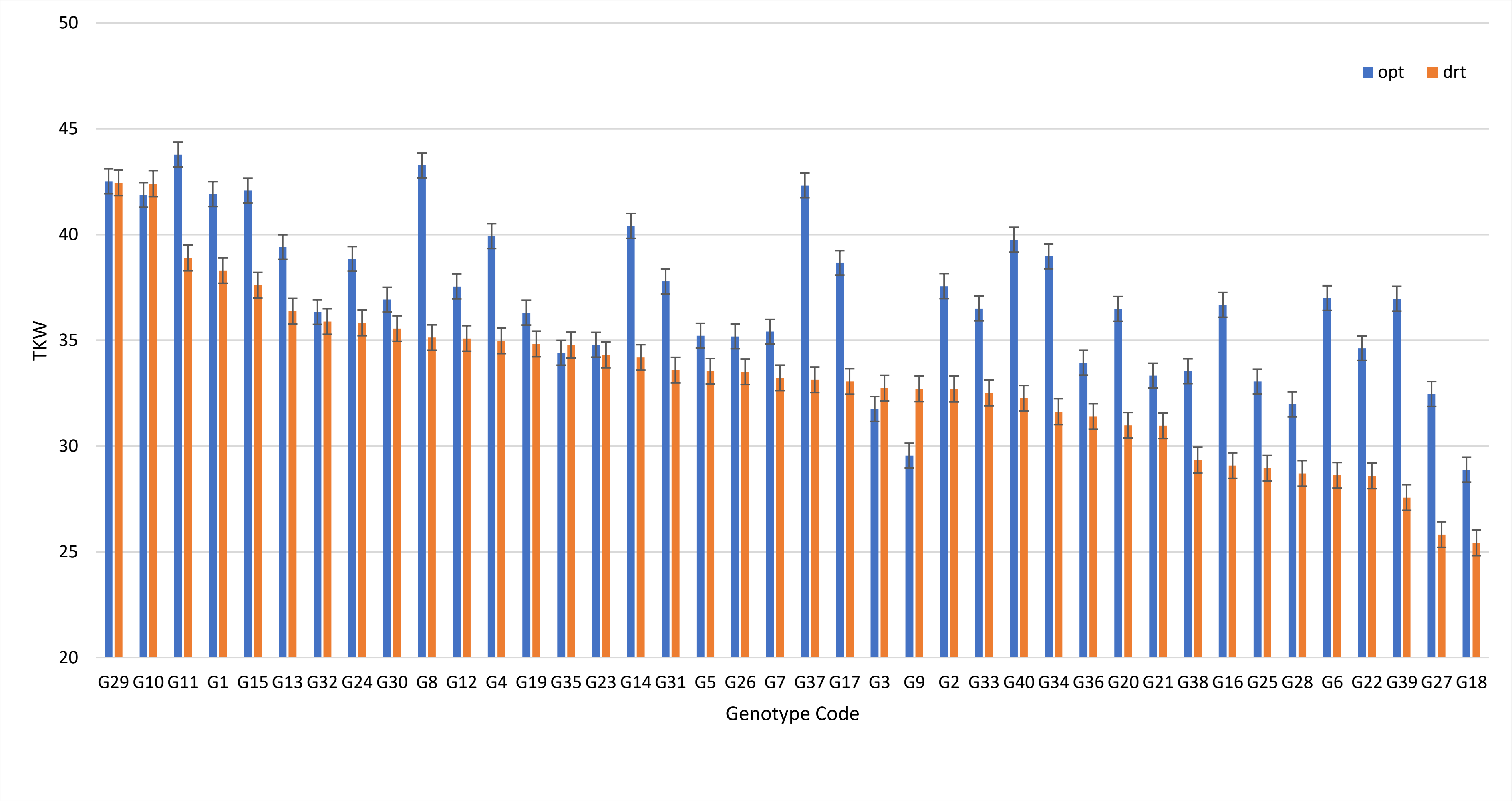

Figure 4. 1: Response of wheat genotypes to thousand kernel weight under drought (drt) and optimum (opt) conditions at UFH site.

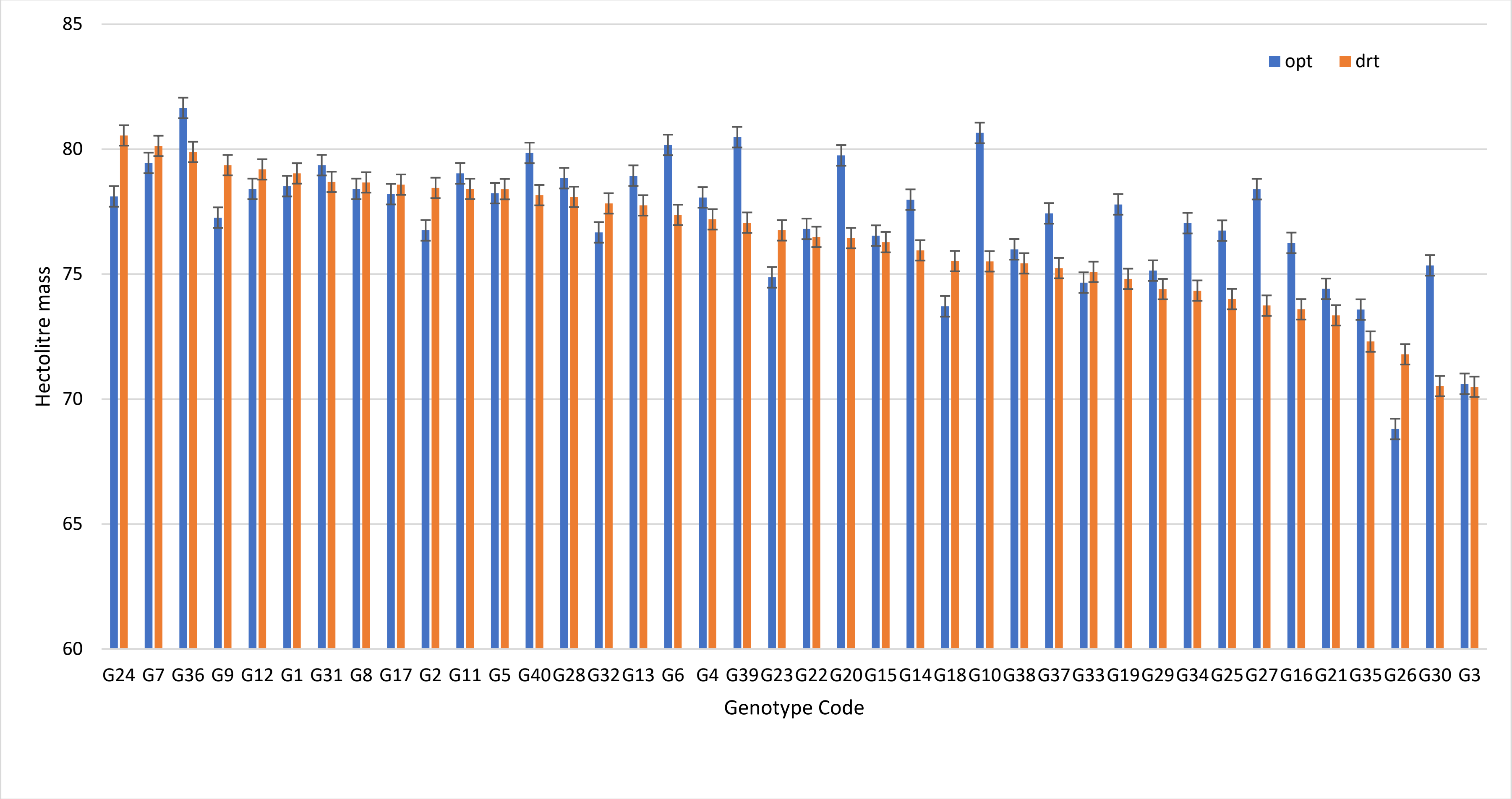

Figure 4. 2: Response of wheat genotypes to hectolitre mass under drought (drt) and optimum (opt) conditions at UFH.

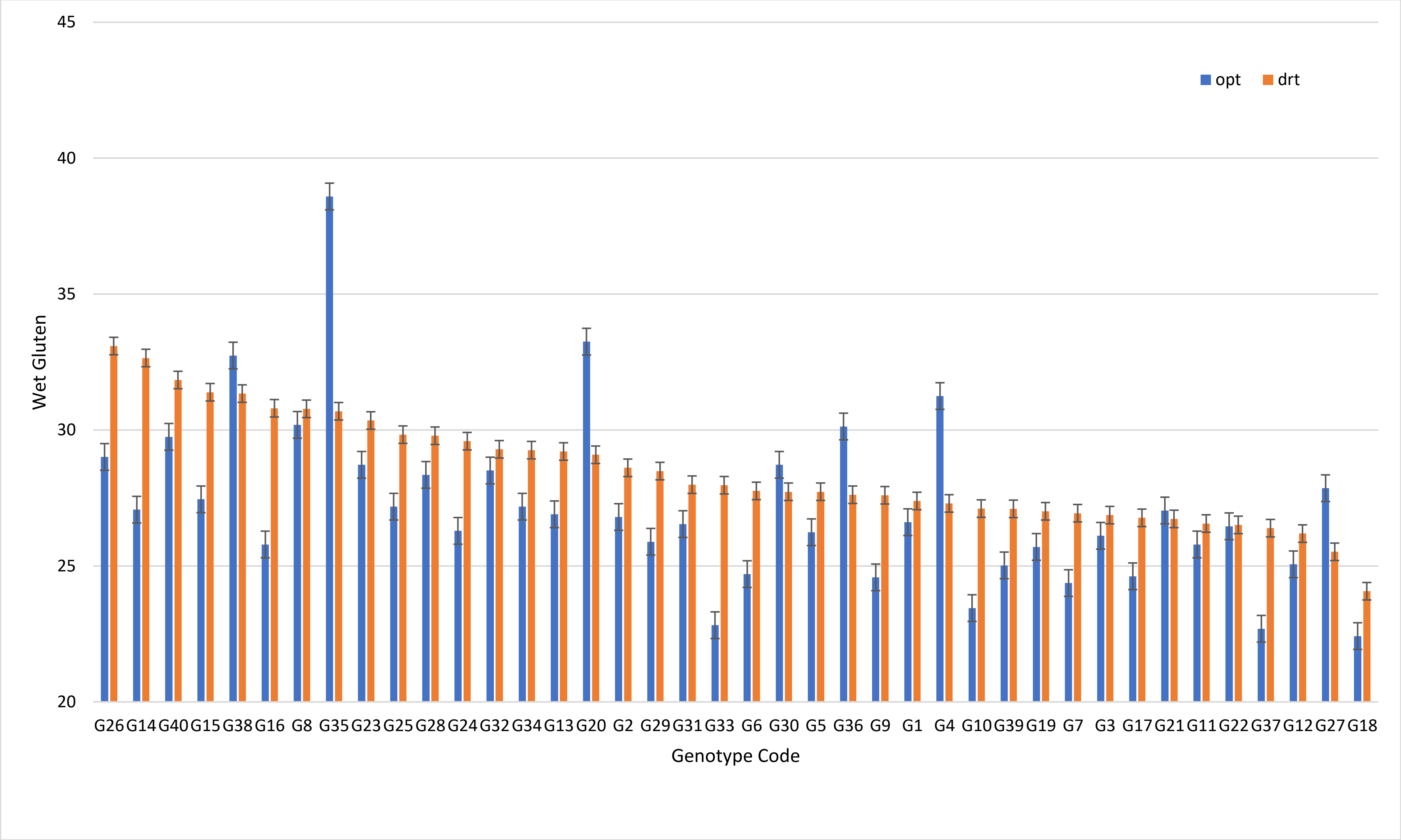

Figure 4. 3: Response of wheat genotypes to wet gluten under drought (drt) and optimum (opt) conditions at UFH.

4.5.3 Grain quality traits of 40 wheat genotypes under optimum and drought stress at ZAN site.

Table 4.3 showed significant differences (p<0.001) among genotypes for all studied quality traits, except fixed protein. The genotype by environment interaction also showed significant differences for all quality traits, except fixed protein.

Table 4. 3: ANOVA table for 40 wheat genotypes exposed to drought and optimum field conditions over two seasons at ZAN.

SV	DF	TKW	HLM	WG	PF
G	39	11329,5***	1499,9***	2303,7***	1286,1^{ns}
R	1	52*	19,1*	9,8^{ns}	60,97^{ns}
B	4	73,2*	58,2***	27,9^{ns}	46,9^{ns}
S	1	709,3***	0,5^{ns}	0,4^{ns}	42^{ns}
WR	1	236,9***	208**	26,2*	79,1^{ns}
G*WR	39	371^{ns}	280,4***	918,7***	1164,1^{ns}
G*S	39	5576,2***	1473,9***	849,6***	1328,4^{ns}
S*WR	1	1768,9***	1,3^{ns}	618,6**	266,5*
G*WR*S	39	554,9**	108,4*	874,3***	1249,3^{ns}
CV%		8,4	2,19	8,34	51,27

Where SV: source of variance; G: genotype; R; replicate; B: block; S: season; E: environment; CV%: coefficient of variance; DF: degrees of freedom; TKW: thousand kernel weight; HLM: hectolitre mass; WG: wet gluten; PF: protein fixed; GY: grain yield per plot; ns: non-significant; *: significant; **: highly significant; ***: highly significant.

4.5.3.1.1 Thousand kernel weight

Table 4.4 shows the ranking of the top 5 and bottom 5 genotypes for the thousand kernel weight. The absence of genotype by WR interaction implied that the ranking of genotypes did not vary across the water regimes. The top 5 genotypes had their mean values above the grand mean of 36.07, and the bottom five genotypes had their mean value lower than the grand mean.

Table 4. 4: Top and Bottom five genotypes in response to TKW at ZAN.

Top	**five genotypes**	**Bottom**	**five genotypes**
Genotype	**LSM**	**Genotype**	**LSM**
G35	45,02^{a}	G20	30,45^{opqr}
G40	44,12^{ab}	G10	29,09^{pqrs}
G28	43,75^{ab}	G24	27,92^{qrs}
G21	43,58^{ab}	G2	26,35^{rs}
G32	42,64^{abc}	G4	24,54^{s}
Grand Mean	**36.07**		

Table 4.4 above shows the mean separation (letters) and the means of the top five and bottom five genotypes in response to thousand kernel weight at ZAN site. Genotypes followed by the same letter are not significantly different, while those with different letters are significantly different.

4.5.3.1.2 Hectolitre mass

Figure 4.4 shows the response of genotypes to HLM across water regimes at the ZAN site. Genotypes under drought stress (78.46) had more hectolitre mass than genotypes under optimum conditions (76.64). The five best genotypes at ZAN under drought included: G11; G13; G15; G8; and G5, with the genotype G11 having a mean value of 81.98 under drought which was above the grand mean of 77.55. Genotype G24 had a lower hectolitre mass under drought with a mean value of 61.8 which was below the grand mean.

4.5.3.1.3 Wet gluten

Figure 4.5 shows the response of genotypes to WG across water regimes at the ZAN site. Genotypes under optimum conditions (28.28) had more wet glutted than genotypes under drought stress (27.7). The five best genotypes at ZAN under drought included: G30; G19; G5; G6; and G7, with the genotype G30 having higher wet gluten under drought with a mean of 34.85 which was above the grand mean of 27.99. Genotype G2 had a lower wet gluten under drought with a mean value of 22.78 which was lower than the grand mean.

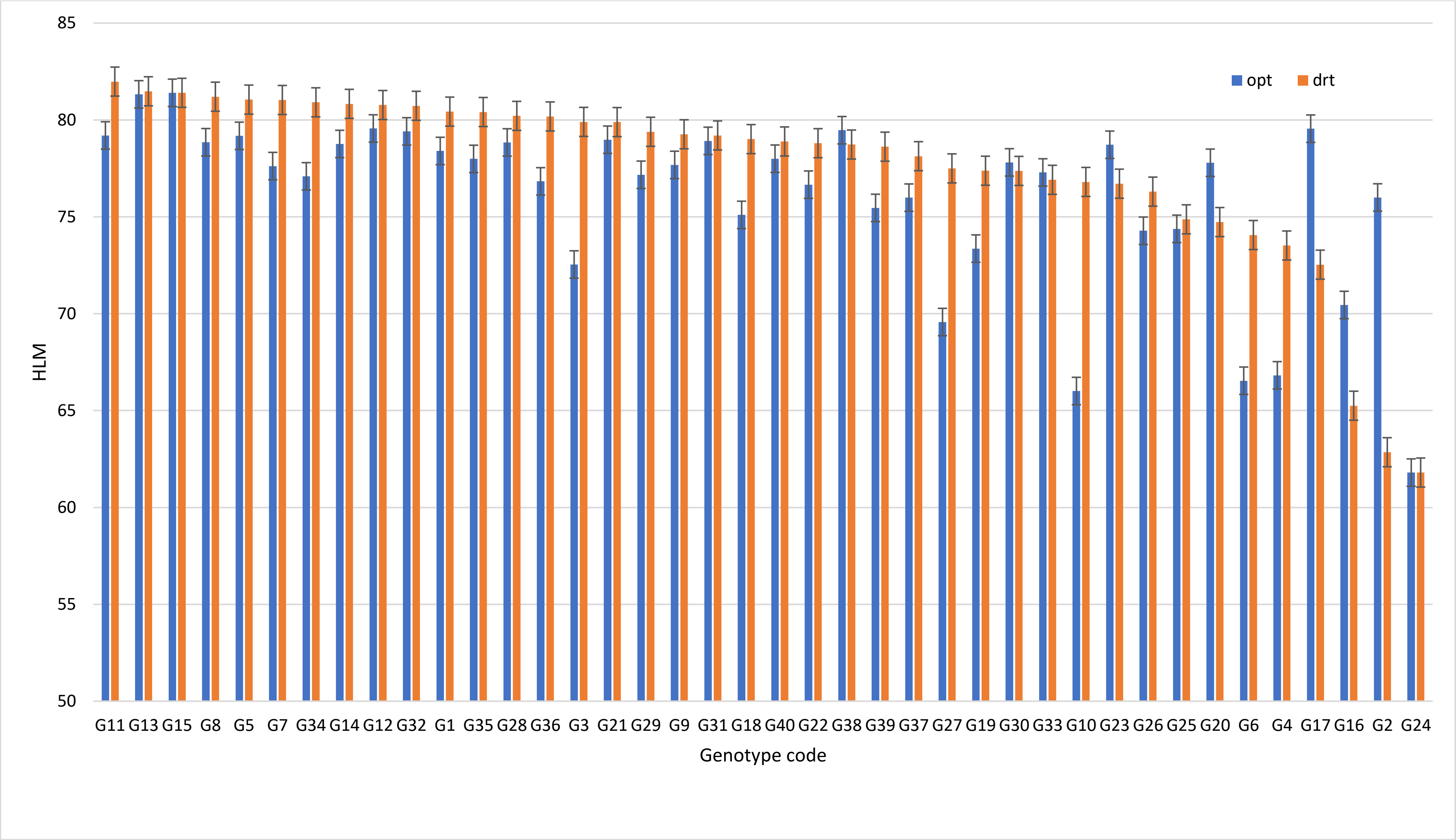

Figure 4. 4: Response of wheat genotypes to hectolitre mass under drought (drt) and optimum (opt) conditions at ZAN.

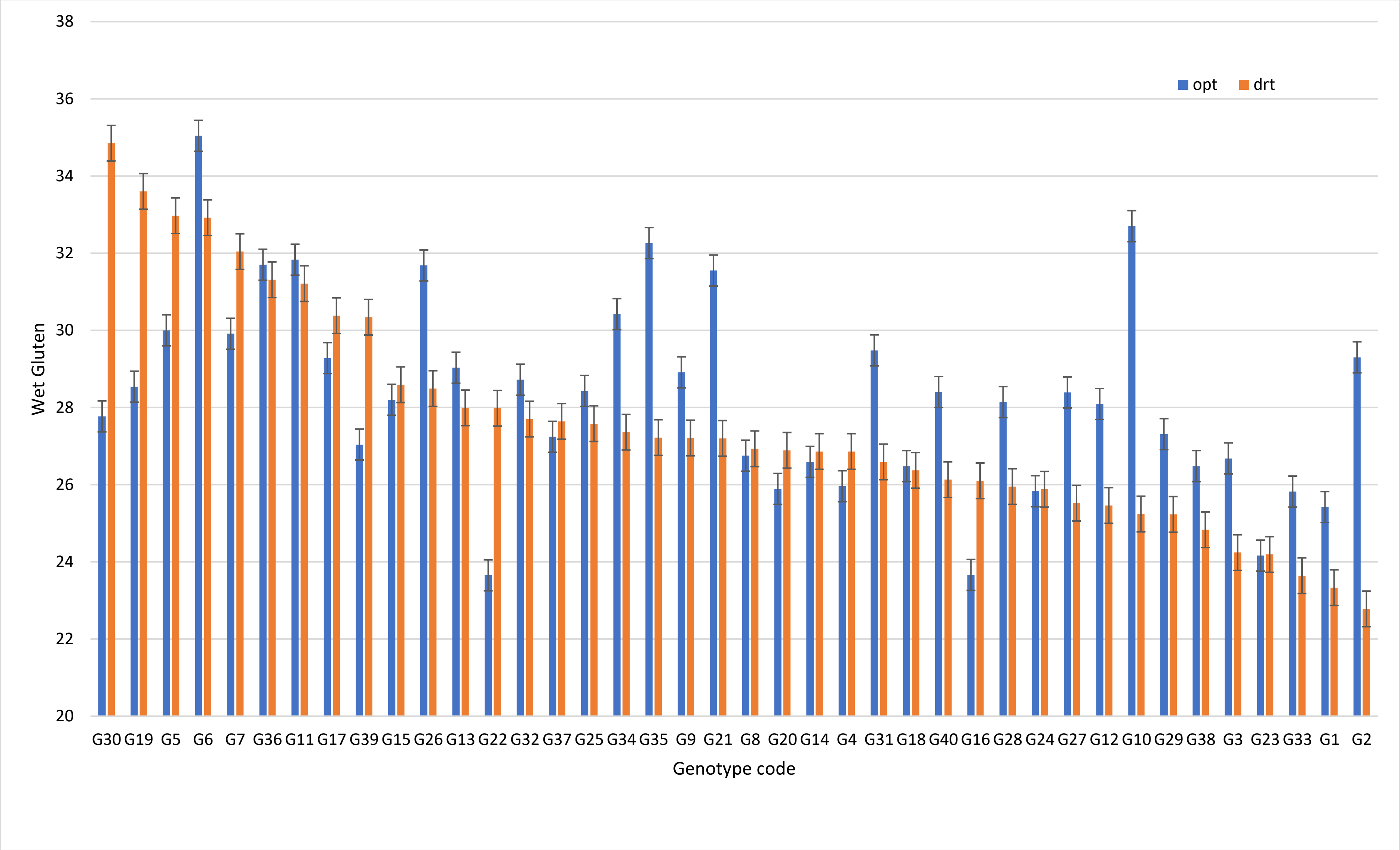

Figure 4. 5: Response of wheat genotypes to wet gluten under drought (drt) and optimum (opt) conditions at ZAN.

4.5.4 Correlation analysis for quality traits across sites

Tables 4.5; 4.6; 4.7; and 4.8 present the correlation analysis for UFH and ZAN sites separately. Due to significant differences observed in the performance of genotypes under the two different water regimes, the correlation was done for each water regime for each site. The correlation analysis summarizes correlation coefficients describing the degree of correlations among measured agronomic traits and grain yield. A strong significant and positive correlation was observed between hectolitre mass and thousand kernel weight; wet gluten and thousand kernel weight, and hectolitre mass at the UFH and ZAN sites under optimum conditions. Thousand kernel weight had a weak significant and positive correlation with both hectolitre mass and wet gluten at the UFH site under drought conditions. Fixed protein content also had a weak positive and significant correlation with hectolitre mass and wet gluten at the UFH site under drought conditions. Hectolitre mass had a weak positive and significant correlation with thousand kernel weight at ZAN site under drought conditions. Fixed protein content also had a weak positive and significant correlation with wet gluten at ZAN site under drought conditions.

Table 4. 5: Table 4.5: Correlation analysis for quality traits at UFH.

UFH OPTIMUM CORRELATION

	TKW	**HLM**	**WG**	**PF**
TKW				
HLM	0,58***			
WG	0,24**	0,33***		
PF	0,03ns	0,04ns	0,19*	

UFH DROUGHT CORRELATION

	TKW	**HLM**	**WG**	**PF**
TKW				
HLM	0,35***			
WG	0,28***	0,05ns		
PF	-0,12ns	-0,35***	0,39***	

Table 4. 6: Correlation analysis for quality traits at ZAN

ZAN OPTIMUM CORRELATION

	TKW	**HLM**	**WG**	**PF**
TKW				
HLM	0,58***			
WG	-0,21**	-0,16ns		
PF	0,09ns	0,08ns	0,17*	

ZAN DROUGHT CORRELATION

	TKW	**HLM**	**WG**	**PF**
TKW				
HLM	0,68***			
WG	-0,03ns	0,11ns		
PF	-0,06ns	0,07ns	0,92***	

Where GY: grain yield; TKW: thousand kernel weight; HLM: hectolitre mass; WG: wet gluten; PF: protein fixed.

Table 4. 7: Summary table presenting the top and bottom five genotypes for quality traits at UFH.

WG				**PF**				**HLM**				**TKW**			
TOP 5 GENOTYPES															
CODE	**OPT**	**CODE**	**DRT**	**CODE**	**OPT**	**CODE**	**DRT**	**CODE**	**OPT**	**CODE**	**DRT**	**CODE**	OPT	**CODE**	DRT
G35	38,59	**G26**	33,09	**G31**	19,4	**G31**	18,43	**G36**	81,65	**G24**	80,55	**G11**	43,78	**G29**	42,45
G20	33,25	**G14**	32,65	**G26**	18,6	**G19**	18	**G10**	80,65	**G7**	80,13	**G8**	43,27	**G10**	42,41
G38	32,74	**G40**	31,84	**G35**	17,9	**G5**	17,95	**G39**	80,48	**G36**	79,89	**G29**	42,52	**G11**	38,9
G4	31,25	**G15**	31,39	**G32**	17,2	**G21**	17,87	**G6**	80,17	**G9**	79,36	**G37**	42,33	**G1**	38,29
G8	30,19	**G38**	31,34	**G33**	17,1	**G26**	17,75	**G40**	79,85	**G12**	79,19	**G15**	42,09	**G15**	37,61
BOTTOM 5 GENOTYPES															
G7	24,37	**G22**	26,51	**G1**	14	**G28**	15,16	**G21**	74,41	**G21**	73,35	**G27**	32,47	**G6**	28,62
G10	23,45	**G37**	26,39	**G13**	13,7	**G2**	14,82	**G18**	73,71	**G35**	72,3	**G28**	31,98	**G22**	28,6
G33	22,82	**G12**	26,19	**G40**	13,4	**G17**	14,74	**G35**	73,58	**G26**	71,79	**G3**	31,75	**G39**	27,57
G37	22,69	**G27**	25,52	**G27**	13,3	**G1**	14,57	**G3**	70,61	**G30**	70,52	**G9**	29,55	**G27**	25,82
G18	22,42	**G18**	24,07	**G24**	13,2	**G24**	14	**G26**	68,8	**G3**	70,49	**G18**	28,88	**G18**	25,43

WG: wet gluten; PF: fixed protein; HLM: hectolitre mass; TKW: thousand kernel weight; G(n): genotypes code with its respective number; OPT: optimum; DRT: drought.

Table 4. 8: Summary table presenting top and bottom five genotypes for quality traits at ZAN.

WG				**PF**				**HLM**				**TKW**			
TOP 5 GENOTYPES															
CODE	**OPT**	**CODE**	**DRT**	**CODE**	**OPT**	**CODE**	**DRT**	**CODE**	**OPT**	**CODE**	**DRT**	**CODE**	**OPT**	**CODE**	**DRT**
G6	35,04	**G30**	34,85	**G6**	15,1	**G30**	14,96	**G15**	81,4	**G11**	81,98	**G35**	43,99	**G40**	46,164
G10	32,7	**G19**	33,6	**G10**	14,2	**G19**	14,38	**G13**	81,32	**G13**	81,48	**G21**	43,28	**G35**	46,055
G35	32,26	**G5**	32,97	**G14**	14,1	**G6**	14,28	**G12**	79,56	**G15**	81,4	**G38**	42,97	**G28**	46,05
G11	31,83	**G6**	32,92	**G35**	14	**G5**	14,26	**G17**	79,55	**G8**	81,2	**G40**	42,08	**G32**	43,887
G36	31,7	**G7**	32,04	**G11**	13,9	**G7**	13,95	**G38**	79,47	**G5**	81,05	**G39**	41,9	**G21**	43,881
BOTTOM 5 GENOTYPES															
G33	25,82	**G3**	24,24	**G20**	11,6	**G3**	11,01	**G27**	69,57	**G4**	73,52	**G10**	28,95	**G25**	31,203
G1	25,42	**G23**	24,19	**G1**	11,4	**G23**	10,94	**G4**	66,82	**G17**	72,53	**G20**	28,5	**G10**	29,216
G23	24,16	**G33**	23,64	**G23**	10,9	**G33**	10,75	**G6**	66,54	**G16**	65,25	**G24**	27,51	**G24**	28,329
G16	23,66	**G1**	23,33	**G22**	10,7	**G2**	10,42	**G10**	66,01	**G2**	62,85	**G2**	25,79	**G2**	26,92
G22	23,65	**G2**	22,78	**G16**	10,7	**G1**	8,34	**G24**	61,8	**G24**	61,8	**G4**	23,42	**G4**	25,662

WG: wet gluten; PF: fixed protein; HLM: hectolitre mass; TKW: thousand kernel weight; G(n): genotypes code with its respective number; OPT: optimum; DRT: drought.

4.7 Discussion

The effect of drought stress during the grain filling stage does not only limit grain quantity but also grain quality. Grain quality is expressed through a complex of indices including its physical condition, chemical composition, and biochemical characteristics (Ivanova et al., 2013). Liu et al., (2003) reported that among grain quality traits, grain hardness, flour colour, polyphenol oxidase, protein content, protein quality, and starch pasting properties have been shown to affect the quality attributes of wheat. However, the overall means indicated that under drought-stress, hectolitre mass, and fixed protein were slightly increased when compared to optimum conditions. An increase in hectolitre mass was previously reported by Muhammad et al., (2019) on wheat genotypes under drought stress. On the hand, thousand kernel weight and wet gluten were slightly reduced under drought stress compared to optimum conditions. The overall moisture deficit-induced alterations in quality traits were primarily due to a reduction in kernel weight under drought stress (Prabha et al., 2009).

Grain protein and gluten quality are two important parameters that are affected by drought stress. Deviation in protein content and composition significantly alter flour quality for bread making (Branlard et al., 2001). Therefore, high grain protein content is privileged since there is a linear relationship between flour protein content and bread-making quality. The findings of the present study indicated that protein content slightly increased under drought-stress conditions. The results are consistent with that of Francois et al., (1986). An increase in grain protein percentage in the present study under drought stress may be attributed to reduced starch accumulation. Similarly, other researchers (Krisztina et al., 2011; Maryam and Ahmad, 2013) reported an increase in protein content under drought stress. Equivalence to the present findings was also reported by Abdul et al., (2020), where they showed an increase in grain protein content under drought stress conditions. In contrast, Pierre, (2008) observed the opposite, where grain protein was decreased in wheat grown under drought stress. Hasan and Tacettin, (2010) also reported that drought stress conditions increased protein content in wheat as compared to optimum conditions.

4.8 Conclusion

Some genotypes were regarded as stable across two water regimes as they appeared in the top five ranking of genotypes. G38 was stable for wet gluten; G31 and G26 were stable for fixed protein; G36 was stable for hectolitre mass; and G11, G15, and G29 were stable for thousand kernel weight at the UFH site. G26 was in the top five ranking for wet gluten and fixed protein under drought stress at the UFH site. Stable genotypes that appeared on the bottom include G37 and G18 for wet gluten; G1 and G24 for fixed protein; G21, G35, G3, and G26 for hectolitre mass; and G27 and G18 for thousand kernel weight at UFH. G6 was stable for both wet gluten and fixed protein; G13 and G15 were stable for hectolitre mass; and G35, G21, and G40 were stable for thousand kernel weight at the ZAN site. For the ZAN site, stable genotypes that appeared on the bottom ranking include G33, G1, and G23 for wet gluten; G23 and G1 for fixed protein; G4 and G24 for hectolitre mass; and G10, G24, G2, and G4 for thousand kernel weight.

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Chapter 5

To identify appropriate drought tolerance indices that can be used as selection tools under field conditions.

Abstract

Drought stress limits crop production in the world as it is prevalent in most areas. Increasing human population and predicted dry spells, as a result of global warming, will lead to increased problems for agricultural production in future. However, use of high-yielding genotypes having drought tolerance may be the proficient approach to cope with detrimental outcomes of drought stress. The current study was established to screen 40 wheat genotypes using different drought tolerance indices under field conditions. The experiment was established during the 2020 and 2021 winter seasons in the field using a 5x8 alpha lattice design with two replicates and two water regimes (optimum and drought conditions). Data on grain yield, which is a basis for computing drought tolerance indices was recorded under both optimum and drought conditions. Drought tolerance indices that were used in this study include mean productivity (MP), geometric mean productivity (GMP), harmonic mean (HM), tolerance index (TOL), stress susceptible index (SSI), sensitive drought index (SDI), and stress tolerance index (STI). The aim of this study was to identify appropriate drought tolerance indices that can be used as selection tools under field conditions. Mean productivity (MP), geometric mean productivity (GMP), and harmonic mean (HM) were identified as the most appropriate drought tolerance indices as they showed a strong positive and significant correlation with grain yield both under drought and optimum conditions. Tolerance index (TOL), stress susceptible index (SSI), sensitive drought index (SDI), and stress tolerance index (STI) showed weak but positive correlations with grain yield under drought and optimum conditions, and therefore, their effects were not significant. The findings depicted that tolerant genotypes could be identified based on high values of MP, GMP, and HM, and such genotypes included G5, G22, G8, and G21. Based on the results, G19, G16, G2, and G20 were more sensitive to drought as they showed low values of MP, GMP, and HM. Strong and positive correlation coefficients were also observed among these three indices. These three indices are recommended for indirect selection for drought-tolerant wheat genotypes under field conditions. Principal component analysis also revealed that MP, GMP, and HM had the positive loadings into the first principal component.

Keywords: wheat genotypes; drought stress; optimum conditions; tolerance indices; correlation analysis.

5.1 Introduction

Cereals are considered as the food crops of most people in the world, and they supply more than 70 percent of the world's food. Wheat (*Triticum aestivum* L.) is one of the most important commodity crops grown in South Africa (Zamalotshwa et al., 2019). In recent years, interest in crop response to environmental stresses has greatly received attention due to substantial yield loss caused by drought. Hussain et al., (2018) reported that drought is the most important abiotic stress restricting wheat production in arid and semi-arid regions. Crop growth stage and severity of drought stress determine the extent of yield loss (Daryanto et al., 2016). A key strategy to cope with the changing climatic conditions is breeding for drought tolerance using novel genetic resources (Mwadzingeni et al., 2016). Unfortunately, progress in breeding drought-tolerant cultivars is not satisfactory due to the limited availability of resistance sources. Drought-tolerance is also a complicated trait, which is controlled by polygenes, and their expression is determined by different environmental conditions. This slows down the process of selection of drought-tolerant genotypes. Monneveux et al., (2012), and Passioura, (2012) noted that selection based on drought adaptive traits along with the yield and its components may improve yield under different target environments.

Use of high-yielding genotypes having drought tolerance is an efficient approach to lessen the extent of drought damage. With declining resources of water and escalating intensity of drought, loss of yield is a dangerous alarm in arid and semi-arid regions. In that case, attaining drought tolerance exclusively depending, yield is complex due to its low heritability under this stress factor. Likewise, Mitra, (2001) noted that choosing genotypes having tolerant genes is a difficult task when selection is based on yield per se. The yield components have been extensively used for screening against drought tolerance (Mwadzingeni et al., 2016). Duration to heading and maturity also play an important role under terminal drought stress (Lopes et al., 2012). On the other hand, Yadav and Bhatnagar, (2001) suggested that drought tolerance indices could be employed to compare changes in grain yield under normal and drought conditions for the identification of genotypes with high yields and drought tolerance.

An index of tolerance index (TOL) was defined and pioneered by Rosielle and Hamblin, (1981) as grain yield difference in normal (Yp) and drought (Ys) conditions, which specified that drought-sensitive genotypes show low values of this index. The index of mean productivity (MP) also defined by Rosielle and Hamblin, (1981) is the average yield under drought stress (Ys) and normal (Yp) conditions. Fischer and Maurer, (1978) proposed the stress susceptibility index (SSI) and suggested that genotypes having SSI values less than one were tolerant.

South Africa annually imports almost half of its wheat requirements. One of the challenges is the limited knowledge about drought tolerance of local wheat cultivars. Thus, the present research was executed to screen forty wheat genotypes under drought and optimum conditions by means of different drought tolerance indices and to select superior indices that can be used to identify different drought-tolerant genotypes. Wheat genotypes with high amounts of geometric mean productivity (GMP) and stress tolerance index (STI) index and low values of stress susceptible index (SSI) were described to be tolerant in previous studies (Mohammadi et al., 2008). Nouraein et al., (2013) reported that STI index had more advantages for the selection of suitable cultivars in both stress and non-stress conditions.

Mohammadijoo et al., (2015) suggested that the mean productivity (MP), geometric mean productivity (GMP), and stress tolerance indices (STI) were the best indices to evaluate genotypes for drought tolerance. In research done on wheat, Karimi and Saeed, (2013) reported that genotypes with high values of MP and GMP would tolerate drought. It is therefore apparent that the most appropriate indices may be influenced by the interaction between genotypes and the environment, which necessitated this investigation.

5.2 Objectives

- To identify appropriate drought tolerance indices that can be used as selection tools under field conditions.
- To identify drought-tolerant genotypes under field conditions using drought tolerance indices.

5.3 Hypotheses

- There are no drought tolerance indices that can be used as selection tools under field conditions.
- There are no drought-tolerant genotypes that can be identified using drought tolerance indices under field conditions.

5.4 Materials and methods

The materials and methods were the same as described in **Chapter 3.2.** Drought tolerance indices were evaluated using average grain yield data from two seasons (2020 and 2021) only for UFH site, to identify drought-tolerant and sensitive wheat genotypes. These are mathematical equations developed by scientists and researchers decades ago. They aid in quantifying the extent and severity of the stress through differences between stressed and optimum conditions using grain yield of a specific crop of interest, and selection of superior genotypes either through grain yield differences or by means of averages, taking correlation of grain yield under stress and optimum conditions into consideration.

5.4.1 Data analysis

Drought tolerance indices were computed using grain yield under two water regimes namely optimum (Yp) and drought (Ys) conditions. Various yield-based drought tolerance indices such as tolerance index (TOL), mean productivity (MP), stress susceptibility index (SSI), geometric mean productivity (GMP), stress tolerance index (STI), harmonic mean of yield (HM), and sensitivity drought index (SDI) were computed and used to screen for stable genotypes across water regimes. The following mathematical equations aided in calculating the above-mentioned indices.

$TOL = Yp - Ys$ (Rosielle and Hamblin, 1981)

$MP = (Yp + Ys)/2$ (Rosielle and Hamblin, 1981)

$SSI = (1-Ys/Yp)/SI$ (Fischer and Maurer, 1978)

$SI = 1 - \bar{Y}s/\bar{Y}p$ (Fischer and Maurer, 1978)

$GMP = \sqrt{Yp \ x \ Ys}$ (Fernandez, 1992)

$STI = Yp \ x \ Ys/(Yp)^2$ (Fernandez, 1992)

$HM = 2(Yp * Ys)/(Yp + Ys)$ (Farshadfar and Elyasi, 2012)

$SDI = (Yp - Ys)/Yp$ (Singh et al., 2011)

An index of tolerance (TOL) is defined as grain yield difference in optimum (Yp) and drought (Ys) conditions, and therefore, drought-sensitive genotypes show low values of this index. The average yield under drought stress (Ys) and optimum conditions (Yp) defines the index of mean productivity (MP). The stress susceptible index (SSI) has been interpreted to suggest that genotypes having values less than one are tolerant.

Correlation analysis was performed among the above-mentioned indices to determine the most appropriate index that can be used to select superior genotypes.

5.5 Results

Table 5.1 summarizes the top five and bottom five genotypes based on mean yield performance across seasons under optimum and drought, and their respective indices. The selection of superior genotypes was through ranking them from high to low values for grain yield under both optimum (Yp), and drought stress (Ys) conditions; mean productivity (MP); geometric mean productivity (GMP); harmonic mean (HM); and sensitive drought index (SDI). On the other hand, as presented in the same table, tolerant and/or superior genotypes were indicated by low values for the index of tolerance (TOL); stress tolerance index (STI); and stress susceptible index (SSI). **Table 5.2** presents the overall performance of 40 genotypes across seasons ranking from 1 to 40 for yield under drought stress and optimum conditions, as well as the indices. **Table 5.3** shows the correlation coefficients between yield under optimum (Yp) and drought (Ys) conditions and drought tolerance indices.

Table 5. 1: Top and bottom five genotypes based on their respective ranking for their indices.

TOP FIVE GENOTYPES

G Code	Ys	G Code	Yp	G Code	TOL	G Code	MP	G Code	GMP	G Code	HM	G Code	STI	G Code	SDI	G Code	SSI
G22	2969	G5	5093	G37	-285	G5	4008	G5	3859	G5	3715	G14	0,47	G14	0,53	G18	-23,2
G9	2930	G22	4625	G10	-129	G22	3797	G22	3706	G22	3617	G33	0,47	G33	0,53	G12	-18,3
G5	2923	G29	4147	G27	-61	G8	3292	G21	3237	G21	3207	G26	0,54	G26	0,46	G9	-6,9
G21	2825	G8	4129	G34	-52	G29	3282	G8	3184	G9	3104	G6	0,55	G6	0,45	G20	-6,7
G37	2672	G30	4003	G18	82	G21	3267	G29	3166	G8	3079	G24	0,55	G24	0,45	G31	-3,9
BOTTOM 5 GENOTYPES																	
G16	941	G19	1413	G8	1673	G19	1178	G19	1154	G19	1131	G18	0,96	G18	0,04	G14	0,1
G23	933	G2	1298	G30	1730	G16	1110	G16	1097	G16	1084	G34	1,03	G34	-0,03	G37	10,4
G2	864	G16	1280	G29	1730	G2	1081	G2	1059	G2	1038	G27	1,04	G27	-0,04	G10	17,1
G20	762	G24	1029	G33	2083	G20	812	G20	810	G20	809	G10	1,07	G10	-0,07	G27	29,7
G24	570	G20	862	G5	2170	G24	800	G24	766	G24	734	G37	1,12	G37	-0,12	G34	36,1

G Code: genotype code; Yp: yield under optimum conditions; Ys: yield under drought stress; TOL: index of tolerance; MP: mean productivity; GMP: geometric mean productivity; HM: harmonic mean; STI: stress tolerance index; SDI: sensitive drought index; SSI: stress susceptible index.

Table 5. 2: Ranking performance of genotypes using average grain yield and drought tolerant indices across seasons.

Genotype Code	Ys	Yp	TOL	MP	GPM	HM	STI	SDI	SSI
G22	1	2	34	2	2	2	15	15	22
G9	2	11	9	8	7	4	34	34	3
G5	3	1	40	1	1	1	8	8	29
G21	4	8	25	5	3	3	26	26	11
G37	5	24	1	19	19	17	40	40	37
G36	6	14	21	12	10	10	28	28	9
G40	7	6	32	6	6	6	13	13	24
G31	8	17	17	14	12	12	32	32	5
G8	9	4	36	3	4	5	11	11	26
G39	10	12	24	11	11	11	25	25	12
G29	11	3	38	4	5	7	9	9	28
G12	12	23	7	22	22	22	35	35	2
G35	13	20	18	18	17	14	30	30	7
G15	14	9	31	9	9	9	14	14	23
G7	15	13	26	13	13	13	23	23	14
G30	16	5	37	7	8	8	6	6	31
G28	17	19	22	17	16	15	27	27	10
G11	18	21	16	21	21	20	29	29	8
G1	19	16	28	16	18	18	19	19	18
G10	20	28	2	26	26	26	39	39	38
G13	21	22	11	24	23	23	31	31	6
G38	22	18	27	20	20	21	18	18	19
G18	23	27	5	25	25	24	36	36	1
G32	24	10	33	15	15	19	7	7	30
G33	25	7	39	10	14	16	2	2	35
G34	26	30	4	27	27	27	37	37	40
G27	27	33	3	29	28	28	38	38	39
G14	28	15	35	23	24	25	1	1	36
G4	29	31	15	31	31	31	22	22	15
G6	30	26	29	30	30	30	4	4	33
G26	31	25	30	28	29	29	3	3	34
G3	32	32	19	33	32	32	21	21	16
G25	33	29	23	32	33	33	10	10	27
G17	34	34	14	34	34	34	20	20	17
G19	35	36	13	36	36	36	17	17	20
G16	36	38	8	37	37	37	24	24	13
G23	37	35	20	35	35	35	12	12	25
G2	38	37	10	38	38	38	16	16	21
G20	39	40	6	39	39	39	33	33	4
G24	40	39	12	40	40	40	5	5	32

Genotypes were coded from G1 to G40; Ys: yield under stress; Yp: yield under optimum; TOL: index of tolerance; MP: mean productivity; GMP: geometric mean productivity; HM: harmonic mean: STI: stress tolerance index; SDI: sensitive drought index; SSI: stress susceptible index.

Table 5. 3: Correlation coefficients between yield under optimum (Yp) and drought (Ys) conditions, and drought tolerance indices.

	Ys	**Yp**	**MP**	**GMP**	**HM**	**TOL**	**STI**	**SDI**	**SI**	**SSI**
Ys										
Yp	0,82***									
MP	0,93***	0,97***								
GMP	0,95***	0,96***	1,00***							
HM	0,96***	0,94***	0,99***	1,00***						
TOL	0,29ns	0,78***	0,62***	0,58***	0,54***					
STI	0,24ns	-0,33*	-0,11ns	-0,07ns	-0,02ns	-0,81***				
SDI	-0,24ns	0,33*	0,11ns	0,07ns	0,02ns	0,81***	-1,00***			
SI	-0,24ns	0,33*	0,11ns	0,07ns	0,02ns	0,81***	-1,00***	1,00***		
SSI	-0,05ns	-0,14ns	-0,11ns	-0,11ns	-0,11ns	-0,18ns	0,28ns	-0,28ns	-0,28ns	

Yp: yield under optimum conditions; Ys: yield under drought stress; TOL: index of tolerance; MP: mean productivity; GMP: geometric mean productivity; HM: harmonic mean; STI: stress tolerance index; SDI: sensitive drought index; SSI: stress susceptible index; ns: non-significant; *: significant; **: highly significant; ***: highly-highly significant.

Table 5. 4: Principal component analysis of the selection indices and mean yield under optimum and stressed conditions.

Index	PC 1	PC 2	PC 3
GMP	0.43695	0.11312	0.234
HM	0.4246	0.21988	0.74497
MP	0.44976	0.00055	-0.33172
SDI	0.00001	-0.00043	0.00117
SI	0.00001	-0.00043	0.00117
SSI	-0.00059	0.00363	-0.14836
STI	-0.00001	0.00043	-0.00117
Yp	0.5545	-0.68459	-0.19365
Ys	0.34501	0.68569	-0.46979
Explained variance. (Eigen values)	9.635	0.363	0.002
Proportion of total variance (%)	96.35	3.63	0.02
Cumulative variance (%)	96.35	99.98	100

PCA 1, 2, 3: Principal component analysis one, two, and three respectively; GMP: geometric mean productivity; HM: harmonic mean; MP: mean productivity; SDI: sensitive drought index; SI: sensitive index; SSI: stress susceptible index; STI: stress tolerance index; Yp: yield under optimum; Ys: yield under drought.

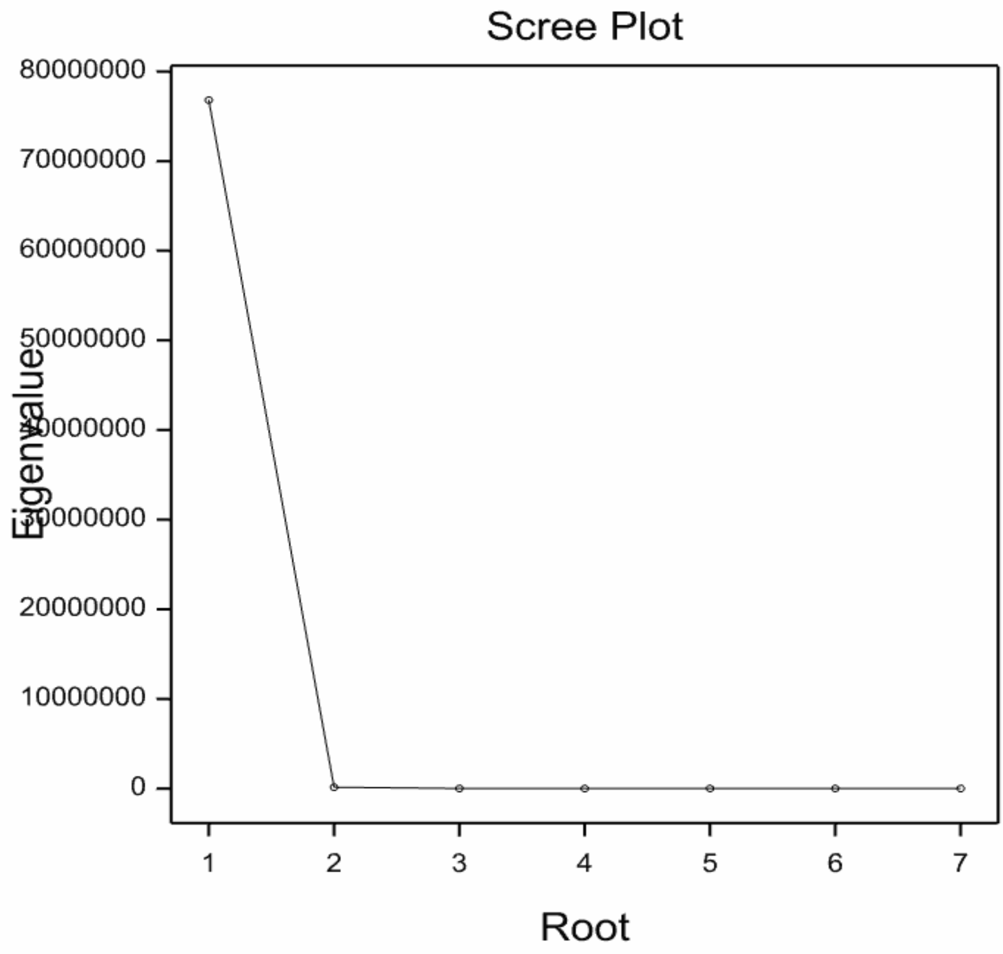

Figure 5.1: A scree plot diagram showing that only two principal components are sufficient to explain the variance accounted by the selection indices and mean yield under optimum and drought conditions.

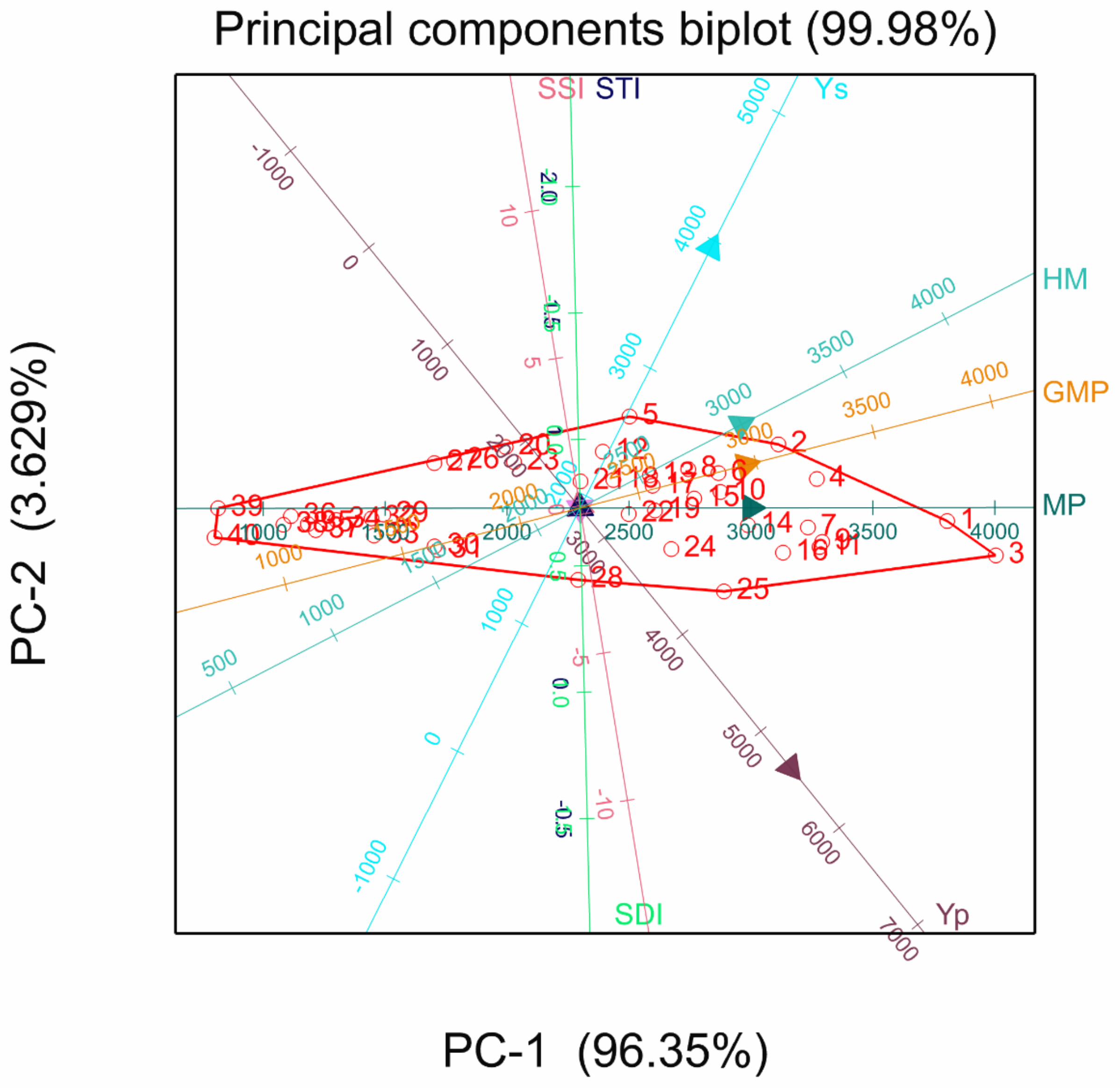

Figure 5.1: Principal component biplot showing genotypic grouping around mean yield under optimum (Yp) and drought stress (Ys) and various yield-based drought tolerance indices which are tolerance index (TOL), mean productivity (MP), stress susceptibility index (SSI), geometric mean productivity (GMP), stress tolerance index (STI), harmonic mean of yield (HM), and sensitivity drought index (SDI).

5.6 Discussion

5.6.1 Drought tolerance indices and principal component analysis

Generally, grain yields under drought conditions were reduced by up to 57 percent when compared to optimum conditions. Results for grain yield across seasons (2020 and 2021) as reflected in **Table 5.1** shows that the highest and lowest yielding genotypes under optimum conditions are G5 (5093 kg/ha) and G20 (862 kg/ha), and under drought-stressed conditions they are G22 (2962 kg/ha) and G24 (570 kg/ha). Ranking the top five genotypes, G22 and G5 appeared both under optimum and drought conditions and therefore were stable and high yielding. G16, G2, G20, and G24 appeared at the bottom five genotypes under drought and optimum conditions and therefore were stable but low yielding.

Some genotypes are high yielding under optimum conditions and low yielding under drought conditions, and therefore, these genotypes are drought sensitive. Tolerance index (TOL), which is the grain yield difference in two different conditions (drought and optimum) is used to determine whether the genotype is tolerant or susceptible to drought. High values of TOL were used to indicate plant susceptibility to stress, and the selection of tolerant genotypes was based on low values of TOL. Based on TOL, the top five tolerant genotypes include G18, G20, G12, G16, and G19, and the bottom five susceptible genotypes include G8, G30, G29, G33, and G5. Some genotypes had their grain yield increased under drought conditions than optimum conditions and were characterized by negative values of TOL. These genotypes include G37 (-284.5 kg/ha), G10 (-129 kg/ha), G27 (-60.5 kg/ha), and G34 (-52 kg/ha). Some genotypes G18 and G20 were the least-to-moderately yielding as they showed low values of TOL. These genotypes appeared to be stable but low yielding. Mean productivity MP is the mean grain yield under drought and optimum conditions. High amounts of mean productivity also indicated more tolerance to drought stress. Genotypes G5, G22, G8, G29, and G21 appeared on the top five tolerant genotypes under MP. These genotypes also appeared on the geometric mean productivity (GMP), and harmonic mean (HM) with slightly different rankings. G29 was replaced by G9 under HM. Genotypes with low values of MP, GMP, and HM were susceptible to drought, and they include G19, G16, G2, G20, and G24. These genotypes had the same ranking across all means. Similar findings on these drought tolerance indices were also reported by Dorostkar et al., (2015).

The concept of stress susceptible index (SSI) is the same as the one for the TOL. Genotypes with high values of SSI generally have high yield under optimum conditions, and critically low yield under drought conditions and therefore these genotypes were recognized as sensitive ones. Drought sensitive genotypes as revealed by SSI include G14, G37, G10, G27, and G34, and drought tolerant genotypes include G18, G12, G9, G20, and G31. The ranking of genotypes based on drought tolerance indices differs, with MP, GMP, and HM ranking the same but differently to SSI and TOL. However, this necessitated correlation analysis between drought tolerance indices and grain yield under optimum and drought conditions.

5.6.2 Correlation Analysis

Table 5.3 presented the correlation coefficients between yield Yp, Ys, and drought tolerance indices. Positive and significant correlations were observed between Yp and all drought tolerance indices except STI and SSI. STI had a negative and significant correlation (-0.33) and SSI had a negative and non-significant correlation (-0.05) with yield under optimum conditions. Yield under optimum conditions (Yp) showed a strong, positive, and significant correlation with MP (0.97), GMP (0.96), HM (0.94), and TOL (0.78). Ys was positively correlated with Yp (0.82), MP (0.93), GMP (0.95), and HM (0.96). Non-significant correlations were observed between Ys, TOL (0.29), and STI (0.24). SDI (-0.24) and SSI (-0.05) showed negative and non-significant correlations with Ys. Interchangeable findings on these drought tolerance indices were reported by Reynolds et al., (2007) and Dorostkar et al., (2015) in wheat. Previous studies inspected the selection criteria effectiveness for evaluating plant drought tolerance and suggested that MP, GMP, and HM are appropriate for screening tolerance as they showed a positive correlation with grain yield under both optimum and drought conditions. TOL was only positively correlated with grain yield under optimum conditions. Strong positive and significant correlation between MP and GMP and HM under both drought and optimum conditions shows that their effects were stronger than those of SSI and TOL and SDI (Sio-Se Mardeh et al., 2006; Geravandi et al., 2010). There was a strong and positive correlation between MP and GMP (1.0). This is probably because GMP is calculated based on MP. Similar findings were attained by Khakwani et al., (2011). All these studies exhibited a positive correlation between grain yield, both under drought and optimum conditions, and MP and GMP which recommend that these indices lead to the selection of high-yielding tolerant genotypes under drought environments.

5.6.3 Principal component analysis of the selection indices

The rotated component matrix (**Table 5.4**) shows the proportion of total variance explained by different principal components and their correlations with selection indices and mean yield under optimum and drought conditions. The scree plot shows that only two principal components were important, contributing 99.44% of the total variation observed. The first principal component was the most influential with a contribution to the total variation of 96.35%. Yield under optimum conditions had the highest positive loading into the first principal component. Selection indices such as mean productivity (MP), geometric mean productivity (GMP), and harmonic mean of yield (HM) had the positive loadings into the first principal component.

The principal component biplots in **Figure 5.2** further illustrate the relationships between the different selection indices and genotypes with respective principal components under both optimum and drought-stressed conditions. Smaller angles between dimension vectors in the same direction indicated a high correlation of index traits in terms of discriminating genotypes. Genotypes excelling in particular indices were plotted closer to the vector line and further in the direction of that particular vector, often on the vertices of the convex hull. Most of the genotypes were scattered around the origin of the plot but more on the positive side of the first principal component along and in the positive directions of indices, MP, GMP, HM, and YP, respectively. Genotypes G3, G1, G4, G9, G11, G16, and G25 excelled in the positive directions of MP, GMP, HM, and YP.

5.7 Conclusion

In the present study, two water regimes (optimum and drought stress conditions) were used for the genotypes evaluation in combination with different drought tolerance indices such as mean productivity (MP), geometric mean productivity (GMP), harmonic mean (HM), tolerance index (TOL), stress susceptibility index (SSI), and sensitive drought index (SDI). Correlation analysis with yield revealed that appropriate drought tolerance indices were MP, GMP, and HM. Based on these indices (MP, GMP, HM) genotypes G5, G22, G8, and G21 were drought tolerant. These genotypes can be exploited to transmit drought-tolerance genes in breeding programs. Genotypes G19, G16, G2, G20, and G24 were sensitive under drought stress. However, these genotypes were reported to be tolerant in Table 3.1, but due to prolonged duration to physiological maturity, drought stress coincided with the grain filling period. The principal component analysis further indicated that MP, GMP, and HM are the most effective selection indices. The observed higher positive loadings for these three selection indices under the first principal component (PC-1) and the fact that they had many genotypes around them as illustrated by the principal component biplot indicating that they have much influence, and therefore, are recommended as selection criteria under optimum and drought conditions. Furthermore, the selection of grain yield under optimum (Yp) conditions was confirmed to be the best when compared to selection under drought conditions.

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Chapter 6

General discussion, conclusion, and recommendations

6.1 General discussion

Drought stress, also referred to as soil moisture stress, is one of the major environmental stresses for the growth and productivity of crops worldwide (Pour-Aboughadareh, 2019). This abiotic stress reduces yield in many dryland wheat production regions, of South Africa as reported in the literature. Effects of drought on wheat are not only based on reduced grain yield but also poor grain quality. Wheat grain yield and quality requirements differ in the whole wheat value chain, depending on whose hands it is passing through from production and harvesting in the field, until being consumed as bread or any other wheat end-use products. While farmers expect a high yield, millers expect a good milling quality and bakers expect flour suitable for the end-product they wish to produce. However, consumers only rely on their senses, what they see, feel, smell, and taste. Different alternative strategies for dealing with the negative effect of drought on wheat production have been proposed. These include proper irrigation facilities and the use of drought-tolerant varieties.

Considering emerging resource-poor farmers who are highly interested in wheat production, irrigation facilities are generally impractical due to their high cost. On the other hand, there is also limited available fresh water for irrigation, and therefore, the use of borehole water may give rise to other unexpected yield-reducing challenges such as soil salinity through dissolved underground salts that would be carried up (Qadir et al., 2014). Use of drought-tolerant varieties is an important, less expensive, and suitable technique for increasing yields in drought-affected regions of the country (Pradhan et al., 2012). Regardless of the genotype and time of stress application, Qaseem et al., (2019) noted that drought stress generally has a negative effect on agronomic and physiological traits in wheat. In this study, an investigation into response of different wheat genotypes to drought and optimum conditions was conducted in the Eastern Cape Province, South Africa, under two different sites over two consecutive winter seasons. The objectives of this study were: i) to evaluate the response of wheat genotypes under optimum and drought-stressed field conditions (Chapter 3); ii) to determine the effect of drought stress on wheat grain quality composition (Chapter 4); and iii) to identify appropriate drought tolerance indices that can be used as selection tools under field conditions (Chapter 5).

Forty genetically diverse wheat genotypes were evaluated under field conditions using a 5x8 alpha lattice design replicated twice and arranged in a split plot manner where water regimes were in a main plot and genotypes were in subplots. To evaluate the response of wheat genotypes under optimum and drought-stressed conditions, agro-physiological traits such as chlorophyll content, and canopy temperature; duration to heading, flowering, and maturity respectively; plant height; spike length; spikelet per spike; kernels per spike, and most importantly grain yield were considered. There were generally no correlations between agro-physiological traits and grain yield. Due to significant differences across two water regimes across sites, AMMI findings revealed that genotypes G21 and G33 were stable across sites and across water regimes.

Determination of the effects of drought stress on wheat grain quality compositions was done in Chapter 4, through the assessment of grain quality traits such as thousand kernel weight; hectolitre mass; fixed protein; and wet gluten. All these quality parameters were taken from the grain yield of the same set of genotypes evaluated under two water regimes at the field over two sites as indicated in Chapter 3. There were generally weak positive and significant correlations among grain quality traits. Therefore, G38 was stable for wet gluten; G31 and G26 were stable for fixed protein; G36 was stable for hectolitre mass; and G11, G15, and G29 were stable for thousand kernel weight at the UFH site. For the ZAN site, G6 was stable for both wet gluten and fixed protein; G13, and G15 were stable for hectolitre mass; and G35, G21 and G40 were stable for thousand kernel weight at the ZAN site.

Drought tolerance indices (Chapter 5) were computed only from the average grain yield data of two seasons under both optimum and drought stress conditions. This was made possible through the use of mathematical equations as indicated in the materials and method section of the Chapter. Appropriate drought tolerance indices that can be used as selection tools under field conditions were the following: mean productivity (MP); geometric mean productivity (GMP); and harmonic mean (HM). These drought tolerance indices were identified based on their correlations with grain yield at both under drought and optimum (stress "Ys" and non-stress "Yp") conditions. However, the principal component analysis also indicated that MP, GMP, and HM are the most effective selection indices, based on the higher positive loadings for these three selection indices under the first principal component (PC-1) and the fact that they had many genotypes around them as illustrated by the principal component biplot. This indicated that they have much influence, and therefore, are recommended as selection criteria under optimum and drought conditions. Therefore, the findings revealed that genotypes G5, G22, G8, and G21 were drought tolerant, while genotypes G19, G16, G2, G20, and G24 were drought sensitive.

6.2 Conclusion

As revealed by a number of different statistical outputs, results showed that genotypes that were more tolerant to drought, and stable across sites were G5; G22; and G21. These genotypes appeared not to change their performance under optimum and drought-stressed conditions, as well as across sites. Therefore, these genotypes can be recommended as potentially suitable for production under drought and/or optimum environmental conditions of the Eastern Cape.

6.3 Recommendations

High-yielding and stable genotypes (G5; G22; G21) across sites and water regimes should be evaluated or tested for drought tolerance in more sites within the Province of the Eastern Cape, and other Provinces of the country. Furthermore, these genotypes should be screened for other prevailing abiotic yield constraints such as soil acidity, soil salinity, and disease resistance in the province. A combination of all these abiotic stress tolerances and/or resistance would ultimately double the yield achievement of such genotypes, and therefore boost the whole wheat value chain income, and overcome the food security crisis of the country, and the world at large. With regard to quality traits, high-yielding genotypes commonly possessed poor quality and therefore, there is a need to find a balance through breeding techniques to incorporate good-quality traits in high-yielding genotypes. One of the ideas to mitigate the gap between yield quantity and quality is to blend high-yielding genotypes with good-quality genotypes.

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Appendix

The ranking of grain yield means from highest to lowest per environment per site.

ufh20				**ufh21**				**zan20**				**zan21**			
G_C	**drt**	**G_C**	**opt**	**G_C**	**drt**	**G_C**	**opt**	**G_C**	**drt**	**G_C**	**opt**	**G_C**	**drt**	**G_C**	**opt**
G5	2356	**G5**	3270	**G9**	4385	**G5**	6914	**G35**	2548	**G23**	2819	**G33**	6025	**G28**	4844
G35	2260	**G29**	3226	**G22**	4304	**G22**	6278	**G34**	2260	**G11**	2197	**G12**	4829	**G21**	4505
G40	2193	**G22**	2973	**G15**	4016	**G8**	5793	**G33**	2120	**G13**	1925	**G38**	4564	**G33**	3947
G36	2161	**G36**	2772	**G21**	3596	**G15**	5515	**G37**	2062	**G32**	1809	**G21**	4346	**G35**	3859
G21	2044	**G33**	2716	**G39**	3519	**G40**	5441	**G16**	1943	**G36**	1748	**G7**	4165	**G5**	3428
G27	2010	**G30**	2685	**G5**	3488	**G30**	5313	**G22**	1889	**G28**	1746	**G14**	4145	**G13**	3386
G29	1988	**G14**	2554	**G37**	3402	**G33**	5195	**G39**	1838	**G21**	1725	**G18**	4002	**G32**	3310
G37	1941	**G37**	2553	**G31**	3401	**G21**	5135	**G5**	1802	**G16**	1707	**G28**	3927	**G11**	2927
G12	1867	**G38**	2534	**G30**	3168	**G9**	5116	**G8**	1758	**G9**	1689	**G5**	3924	**G14**	2680
G8	1855	**G40**	2484	**G18**	3157	**G29**	5068	**G29**	1703	**G39**	1502	**G31**	3803	**G36**	2631
G28	1843	**G8**	2462	**G8**	3053	**G13**	4940	**G7**	1680	**G35**	1495	**G13**	3707	**G15**	2570
G34	1783	**G7**	2432	**G36**	2940	**G32**	4483	**G31**	1575	**G14**	1493	**G15**	3683	**G38**	2541
G4	1723	**G39**	2412	**G7**	2903	**G1**	4425	**G11**	1545	**G7**	1474	**G3**	3678	**G9**	2513
G7	1722	**G32**	2340	**G29**	2845	**G39**	4183	**G1**	1508	**G1**	1468	**G32**	3536	**G34**	2424
G11	1712	**G21**	2271	**G12**	2808	**G28**	4138	**G36**	1495	**G40**	1419	**G35**	3521	**G30**	2406
G10	1680	**G35**	2212	**G40**	2795	**G18**	3983	**G38**	1429	**G31**	1415	**G11**	3497	**G8**	2404
G38	1670	**G31**	2204	**G1**	2743	**G7**	3979	**G30**	1379	**G5**	1414	**G34**	3323	**G22**	2362
G22	1635	**G11**	2195	**G28**	2684	**G31**	3784	**G13**	1354	**G29**	1364	**G36**	3254	**G1**	2216
G31	1564	**G15**	1776	**G11**	2645	**G14**	3731	**G17**	1305	**G38**	1345	**G23**	3117	**G31**	1933
G13	1536	**G1**	1740	**G13**	2598	**G36**	3569	**G10**	1298	**G30**	1343	**G22**	3061	**G29**	1901
G1	1488	**G28**	1693	**G10**	2469	**G35**	3465	**G14**	1239	**G37**	1338	**G9**	3039	**G12**	1897
G9	1471	**G34**	1658	**G32**	2416	**G6**	3448	**G9**	1229	**G17**	1337	**G8**	3001	**G25**	1671
G32	1463	**G4**	1611	**G35**	2386	**G12**	3435	**G21**	1175	**G33**	1281	**G40**	2988	**G7**	1597

G33	1459	**G12**	1482	**G38**	2352	**G38**	3411	**G12**	1168	**G6**	1253	**G26**	2788	**G26**	1506
G3	1458	**G9**	1480	**G33**	2286	**G26**	3227	**G28**	1055	**G12**	1224	**G27**	2723	**G23**	1448
G17	1397	**G26**	1250	**G34**	1855	**G11**	3171	**G32**	1047	**G26**	1207	**G6**	2712	**G6**	1315
G14	1392	**G17**	1205	**G14**	1566	**G10**	2703	**G6**	993	**G2**	1183	**G37**	2698	**G10**	1196
G30	1371	**G10**	1188	**G27**	1474	**G27**	2572	**G40**	831	**G25**	1110	**G39**	2345	**G3**	1140
G39	1353	**G25**	1148	**G19**	1359	**G3**	2547	**G23**	777	**G8**	1092	**G10**	2191	**G18**	1056
G6	1289	**G6**	948	**G26**	1310	**G25**	2496	**G27**	763	**G34**	1011	**G29**	2175	**G19**	979
G26	1094	**G16**	917	**G23**	1208	**G23**	2433	**G18**	713	**G22**	901	**G19**	2078	**G27**	951
G25	1022	**G3**	855	**G25**	1144	**G37**	2221	**G25**	464	**G19**	894	**G1**	1933	**G17**	880
G2	978	**G27**	791	**G6**	1134	**G19**	2179	**G3**	456	**G3**	869	**G17**	1835	**G16**	839
G16	967	**G20**	686	**G24**	945	**G2**	1976	**G2**	407	**G27**	721	**G30**	1827	**G39**	793
G20	933	**G19**	649	**G16**	911	**G34**	1876	**G20**	386	**G20**	667	**G16**	1734	**G37**	753
G18	829	**G24**	644	**G3**	895	**G4**	1860	**G24**	203	**G10**	595	**G25**	1729	**G4**	695
G23	655	**G2**	615	**G4**	753	**G17**	1857	**G19**	149	**G18**	458	**G2**	1686	**G20**	619
G15	622	**G23**	553	**G2**	746	**G16**	1641	**G26**	89	**G15**	433	**G4**	1620	**G24**	608
G19	528	**G18**	167	**G17**	717	**G24**	1415	**G4**	27	**G24**	224	**G24**	1456	**G40**	458
G24	197	**G13**	111	**G20**	594	**G20**	1040	**G15**	17	**G4**	65	**G20**	1429	**G2**	455
G-mean	**1488**	**G-mean**	**1737**	**G-mean**	**2324**	**G-mean**	**3649**	**G-mean**	**1192**	**G-mean**	**1274**	**G-mean**	**3052**	**G-mean**	**1991**
G-mean^1	**1612**			**G-mean^1**	**2987**			**G-mean^1**	**1233**			**G-mean^1**	**2522**		
G-mean^2	**2299**							**G-mean^2**	**1877**						
G-mean^3	**2088**														

Where: ufh20: UFH site 2020 (season 1); ufh21: UFH site 2021 (season 2); zan20: Zanyokwe site 2020 (season 1); zan21: Zanyokwe site 2021 (season 2); G_C: genotype code; G-mean: grand mean for each site and season; G-mean^1: grand mean for each season per site; G-mean^2: grand mean for seasons combined per site; G-mean^3: overall grand mean for sites and seasons combined; drt: drought; opt: optimum.

TABLE OF AGRO-PHYSIOLOGICAL TRAITS MEAN FOR COMBINED SITES

WR	CC	CT	DTH	DTF	DTM	HT	SL	SPS	KPS	GY
OPT	46,09	26,06	98,54	104,6	144,1	101,9	10,2	17,25	40,02	2181
DRT	44,36	28,84	99,21	105,4	135,7	97,19	9,81	17,71	38,05	2019
GM	45,225	27,45	98,875	105	139,9	99,545	10,005	17,48	39,035	2100

TABLE OF MEANS FOR THE UFH SITE

WR	CC	CT	DTH	DTF	DTM	HT	SL	SPS	KPS	GY
OPT	49,4	23,42	99,4	104,8	145,8	101,61	10,15	17,2	39,64	2693
DRT	47,67	28,55	100,06	106	137,3	95,56	9,99	17,19	38,95	1907
GM	48,535	25,985	99,73	105,4	141,55	98,585	10,07	17,195	39,295	2300

TABLE OF MEANS FOR THE ZAN SITE

WR	CC	CT	DTH	DTF	DTM	HT	SL	SPS	KPS	GY
OPT	42,79	28,69	97,67	104,4	142,4	102,2	10,259	17,31	40,39	1643
DRT	41,05	29,14	98,36	104,8	134	98,8	9,634	18,22	37,15	2140
GM	41,92	28,915	98,015	104,6	138,2	100,5	9,9465	17,765	38,77	1891,5

Where WR: water regime; OPT optimum condition; DRT: drought condition; CC: chlorophyll content; CT: canopy temperature; DTH, DTF, DTM: duration to heading, flowering, and maturity respectively; HT: plant height; SL: spike length; SPS: spikelet per spike; KPS: kernel per spike; GY: grain yield; GM: grand mean.

TABLE OF QUALITY TRAITS MEANS FOR COMBINED SITES

WR	HLM	PF	TKW	WG
OPT	76,97	14,75	36,21	27,59
DRT	77,27	14,24	34,91	28,06
GM	77,12	14,5	35,56	27,825

TABLE OF MEANS FOR THE UFH SITE

WR	HLM	PF	TKW	WG
OPT	75,42	16,25	35,46	26,92
DRT	75,72	16,18	31,51	28,46
GM	75,57	16,22	33,49	27,69

TABLE OF MEANS FOR THE ZAN SITE

WR	HLM	PF	TKW	WG
OPT	76,64	13,25	35,48	28,28
DRT	78,46	12,22	36,65	27,70
GM	77,55	12,74	36,07	27,99

Where WR: water regime; OPT: optimum conditions; DRT: drought conditions; HLM: hectolitre mass; PF: protein fixed; TKW: thousand kernel weight; WG: wet gluten; GY: grain yield.