EFFECT OF WATER STRESS IMPOSED AT TILLERING, FLOWERING AND GRAIN FILLING IN IRRIGATED WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES

By

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DECLARATION

I, Unathi Liwani, declare that

1. The research reported in this dissertation, except where otherwise indicated, is my original research.
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As the candidate's supervisor(s), I/We have approved this dissertation for submission.

Signed…………………………………Date……………….

Dr. LS Magwaza (Supervisor)

Signed…………………………………Date……………….

Dr. AO Odindo (Co-supervisor)

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GENERAL ABSTRACT

Wheat is one of the most important crops grown in South Africa. However, its production is threatened by the current drought periods the country has been experiencing. This includes a decline in the production of irrigated wheat which boosted the country's wheat production. In South Africa information which could guide irrigated wheat farmers in using less water at water stress tolerant growth stages is still in its infancy. In order to assist these farmers, the International Maize and Wheat Improvement Centre (CIMMYT) bred genotypes reported to withstand very hot and dry conditions. In contribution, this study was undertaken with the following objectives: 1) to select water stress tolerant irrigated genotypes through evaluating the response of their physiological traits after water stress at tillering, flowering and grain filling 2) to determine the growth stage at which limited water supply would have minimal effect on the growth, development and yield of eight newly developed wheat genotypes. An 8 (genotypes) × 2 (water treatments (stress and no stress (control)) × 3 (growth stages (tillering, flowering and grain filling)) factorial experiment was conducted in a randomized complete block design and replicated three times. Results for the first objective indicated that the rate of photosynthesis was only affected ($p < 0.05$) for genotype LM98 after water stress at tillering and LM43 after water stress at flowering. The rest of the genotypes showed tolerance ($p > 0.05$) in these growth stages and at grain filling. Water stress in the three growth stages did not affect ($p > 0.05$) the transpiration rate and stomatal conductance. Only the instantaneous water use efficiency of genotype LM43 and LM35 was not affected ($p > 0.05$) by water stress at tillering but affected ($p < 0.05$) for the same genotypes at flowering. Whereas, water stress at grain filling affected ($p < 0.05$) the instantaneous water use efficiency of genotype LM35, LM79, LM57 and LM98. Water stress imposed at grain filling had no effect ($p > 0.05$) on the relative water content. It, however, had an impact ($p < 0.05$) on the relative water content of LM43 and LM35 when it was imposed at tillering. The genotypes also showed susceptibility ($p < 0.05$) to water stress at the flowering stage with genotype LM98, LM79, LM83 and LM57 affected. It was then recommended that genotype LM35, LM79, LM57 and LM98 maintained a higher water use efficiency after water stress at grain filling. While the instantaneous water use efficiency of LM43 and LM35 was higher after water stress at tillering but reduced by water stress at flowering stage. From the results of the second objective it was discovered that the plant height of the studied genotypes was not affected ($p > 0.05$) by water stress at tillering and grain filling. The number of fertile tillers was reduced ($p < 0.05$) by water stress at tillering in susceptible genotypes while at flowering and grain filling the number of fertile tillers for all the genotypes was not affected ($p > 0.05$). The spike length was affected ($p < 0.05$) by water stress at all growth stages while the harvest index was not affected ($p > 0.05$). Aboveground biomass was only affected ($p < 0.05$) at tillering stage. Grain yield production which was the primary concern declined only after water stress at tillering. Grain yield production was more linked to the number of fertile tillers after water stress at each growth stage. Based on overall findings this study was able to recommend that the studied genotypes can be subjected to water stress at grain filling and flowering. At tillering, genotype LM83 is better at tolerating water stress while genotype LM47, LM79 and LM66 are susceptible.

TABLE OF CONTENT

LIST OF FIGURES

Chapter 3

Chapter 4

LIST OF TABLES

Chapter 4

CHAPTER 1

General Introduction

1.1 Background

South Africa currently faces a wheat production crisis, as the country is experiencing one of its worst droughts since 1982 and water is a very limited resource (Essa and Jazeera, 2015). Water scarcity has resulted in the need to optimize water use in all human activities, especially irrigation agriculture that utilizes more than 60% of the total water consumed in South Africa (Basson, 2011). However, there is still a potential to maximize wheat production under the water-limited conditions through the identification of water stress tolerant wheat growth stages that enable the crop to survive with limited water.

Wheat is one of an essential crops produced in South Africa (DAFF, 2012). Though, in the past five years' wheat farmers have been failing to produce enough wheat to meet the demands of the country's increasing population. For instance, wheat production has declined from 2.5 million tons planted on 974,000 hectares of land in 2001/2002 to 1.78 million tons planted on 551,000 hectares in 2014/2015, drought being the major contributor to this decline (Agricultural Statistics, 2012; Mokhema, 2015; USAD, 2017). Irrigated wheat has however been able to boost the total wheat production compared to dryland wheat in South Africa (Fourie and Botha, 2011). However, during the severe drought period in the year, 2014/2015 many irrigated wheat farmers had insufficient water to fully irrigate their crops which led to the declining of irrigated wheat yields across farmers' fields (Dube et al., 2016).

In many countries, selection of irrigated wheat genotypes with water stress tolerant growth stages through evaluating the response of physiological and morphological traits under water limited conditions have been implemented (Veesar et al., 2006; Akram, 2011; Mirzae et al., 2011; Liu et al., 2015). Such research enabled recommendations to be made to irrigated wheat farmers on irrigation schedules of different genotypes under limited water conditions. In South Africa, such research studies are still in its infancy (ARC-SGI 2014, 2015). This makes it difficult for the country's irrigated wheat farmers to withhold irrigation at any growth stage as some growth stages are more susceptible than others (Boutraa et al., 2010; Akram, 2011). Water is fast becoming a scarce resource and wheat yields are declining in South Africa. This suggests that there is a need to identify water stress tolerant growth stages of irrigated wheat genotypes. This will enable the crop to resume normal plant growth under limited water conditions. Hence, the objective of this study was to determine wheat growth stages that are tolerant to water stress at either flowering, tillering or grain filling.

1.2 Rationale of the study

Maintaining high yields and conserving water in irrigated wheat farming during drought periods may not be conceivable in South Africa if the farmers are not addressed about genotypes that will tolerate water stress at certain growth stages. Based on the work that has been done in South Africa regarding this issue, it is clear that considerable uncertainty exists regarding the tolerance of wheat growth stages to water stress and more research still needs to be done. Knowledge of such growth stages among irrigated wheat genotypes will allow effective prioritization of research development and interventions which are aimed at maintaining high yields under limited water conditions for both commercial and emerging farmers. The information generated from this research will be important for sustainable and profitable wheat farming during the periods of water shortage. As it will contribute to the compilation of production guidelines for South African irrigated wheat farmers by recommending tolerant growth stages along with the genotypes that will assist them to maximize yields under water limited conditions.

1.3 Research aim and objectives

The overall aim of this study was to identify water stress tolerant growth stages of irrigated wheat genotypes that enable the crop to survive under water limited conditions.

The specific objectives of the study were:

a) To select water stress tolerant irrigated wheat genotypes through evaluating the response of their physiological traits after water stress at tillering, flowering and grain filling.
b) To determine the growth stage at which limited water supply would have minimal effect on the growth, development and yield of eight newly developed wheat genotypes.

1.4 Thesis outline

This thesis includes two chapters written in the form of discrete research papers, each following the format of a stand-alone research paper. This is a dominant format adopted by the University of KwaZulu-Natal. As such there is some unavoidable repetition of references and introductory information between chapters. The structure of the thesis is outlined in the table below:

Chapter	Title
1.	General introduction.
2.	Literature review.
3.	Physiological responses of irrigated wheat (*Triticum aestivum* L.) genotypes subjected to water stress at different growth stages.
4.	Growth, morphological and yield responses of irrigated wheat genotypes to imposed water stress at different growth stages.
5.	Overview of research findings and recommendations.

References

Agricultural Statistics (2012) *Abstract of agricultural statistics*. Department of Agriculture, Forestry and Fisheries. Pretoria. South Africa.

Akram M (2011) Growth and yield components of wheat under water stress of different growth stages. *Bangladesh Journal of Agricultural Research* 36: 455-468.

ARC-SGI (Agricultural Research Council-Small Grain Institute) (2015) *Wheat production guidelines for small-scale farmers: Production of small grains in the winter rainfall area*. Bethlehem. Free State. South Africa.

ARC-SGI (Agricultural Research Council-Small Grain Institute) (2014) *Wheat production guidelines for small-scale farmers:Production of small grains in the summer rainfall areas.* Bethlehem. Free State. South Africa.

Basson MS (2011) Water development in South Africa. *Principal 7 of the water use*. http://www.un.org/waterforlifedecade/green_economy_2011/pdf/session_1_economic_instruments_south_africa.pdf. (Accessed August 2015).

Boutraa T, Akhkha A, Al-Shoaibi AA, Alhejeli AM (2010) Effect of water stress on growth and water use efficiency (WUE) of some wheat cultivars (*Triticum durum*) grown in Saudi Arabi. *Journal of Taibah University for Science* 3: 39-48. https://doi.org/10.1016/S1658-3655(12)60019-3.

DAFF (Department of Agriculture Forestry and Fisheries) (2012) Wheat: Production guidelines. Pretoria. South Africa.

Dube E, Willem K, Van Rensburg B, Van Der Merwe M, Van Der Merwe E, Hatting H (2016) *National cultivar evaluation results: Did irrigation wheat beat the heat in 2015*. Agricultural Research Council- Small Grain Institute. Bethlehem. Free State. South Africa.

Essa A, Jazeera AL (2015) South Africa in midst of “epic drought”. http://www.climatesignals.org/headlines/south-africa-midst-epic-drought. (Accessed December 2015).

Fourie P, Botha P (2011) The role of irrigation in grain production. Grain SA. Pretoria. South Africa.

Liu H, Searle RL, Mather ED, Able AJ, Able JA (2015) Morphological, physiological and yield responses of durum wheat to pre-anthesis water deficit stress are genotype dependent. *Crop and Pasture Science* 66: 1024-1038.

Mirzaei A, Naseri R, Soleimani R (2011) Response of different growth stages of wheat to moisture tension in a semi-arid land. *World Applied Science Journal* 12: 83-89.

Mokhema T (2015) South Africa Cuts Output Forecast as drought hurts yields. https://www.bloomberg.com/news/articles/2015-12-15/south-africa-cuts-wheat-output-forecast-as-drought-hurts-yields. (Accessed January 2016).

USAD (United States Department of Agriculture - Foreign agricultural service) (2017) *Commodity intelligence report*- South Africa: Near records yields expected for the 2016/17 wheat crop. https://pecad.fas.usda.gov/highlights/2017/01/SouthAfrica/index. (Accessed February 2017).

Veesar NF, Channa AN, Rind MJ, Larik AS (2006) Influence of water stress imposed at different stages on growth and yield attributes in bread wheat genotypes (*Triticum aestivum* L.). Department of Plant Breeding and Genetics. Sindh Agriculture University. Tandojam, Sindh. Pakistan.

CHAPTER 2

Selection indices to identify water stress tolerant growth stages in irrigated wheat (*Triticum aestivum* L.) genotypes – a review

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2.1 Abstract

Wheat is among the most important crops grown in South Africa. Its production is threatened by many biotic and abiotic factors, but among this, drought is the major devastating stress which results in water stress in crops. This review describes some aspects of water stress induced changes in wheat's physiological and morphological traits which in turn affects yield production. Water stress triggers many response mechanisms in crops such as drought escape, dehydration avoidance and drought tolerance. Understanding the response of wheat genotypes to water stress at each growth stage can assist determine growth stages that can enable the crop to survive under water-limited conditions. The sections of this review focus on the effect of water stress on wheat, the response of wheat genotypes to water stress at different growth stages, the importance of genotype choice under water limited environments and suitable techniques to select water stress tolerant growth stages in irrigated wheat genotypes.

Keywords: water stress, response mechanisms, wheat genotypes.

2.2 Introduction

Wheat is one of the most important grain crops produced in South Africa and is mostly produced for human consumption (Gbetibouo and Hassan, 2004; Shewry, 2009). It is a good source of protein, minerals, B-group vitamins and dietary fiber which is excellent for human health (FAO, 2004; Sarwar et al., 2013). Because of such importance, the crop is utilized as a primary cereal globally (Sarwar et al., 2013).

Despite its importance and high demand, wheat production in South Africa has declined over the past thirty-four years (Agricultural Statistics, 2014; USAD, 2017). South Africa requires about 3.4 million tons of wheat annually but only 1.87 million tons are currently being produced locally (Newsome, 2012; Fourie and Sihlobo, 2016; USAD, 2017). This is a strong contrast compared to 2.4 million tons produced in 1982 resulting in 60% of wheat imported (Newsome, 2012; Fourie and Sihlobo, 2016; USAD, 2017). Production land has also declined across the nation (Mokhema, 2015; USDA, 2017). For instance, about 974,000 hectares was used to grow wheat in the year 2001/2002. The production area declined to 551,000 hectares in the 2014/2015 season (Mokhema, 2015; USDA, 2017).

Dryland and irrigated wheat which are categorized based on the conditions they are grown under are both planted in South Africa (ARC-SGI, 2015). The water intake of dryland wheat depends on the unpredictable seasonal rainfall, while that of irrigated wheat is applied through irrigation. However, the availability of sufficient irrigation water also depends on rainfall availability which has been a major problem in the country (Hedden and Cilliers, 2014). As South Africa has been experiencing a major drought period that has resulted in the country's water supplying rivers and dams being in deficit (Hedden and Cilliers, 2014). The drought crisis has been caused by current low and changing annual rainfall along with high natural evaporation levels resulting in South Africa to be rated as the 30th driest country in the world (Hedden and Ciliers, 2014). To alleviate crop losses and conserve water, many research studies conducted in other parts of the world could determine growth stages within irrigated wheat genotypes that are not responsive to water stress and enable the crop to maximize yield under water limited conditions (Veesar et al., 2007; Jatoi et al., 2011; Mirzae et al., 2011). This allowed recommendations to be made in assisting irrigated wheat farmers to irrigate wisely during this water shortage period.

In South Africa, during the severe drought period, many dryland wheat farmers faced severe crop losses while irrigated wheat farmers switched to full irrigation to prevent crop failure (Mokhema, 2015). Hence, irrigated wheat has contributed more to the total production than dryland wheat (Fourie and Botha, 2011). But the use of full irrigation is more becoming a challenge as the water research commission (2015) has reported water restrictions of up to 60% in most provinces. Consequently, leading to irrigated wheat farmers having insufficient irrigation water hence experiencing a yield decline (Dube et al., 2016). Despite these challenges, availability of sufficient wheat remains important (Le Roux, 1995; Sihlobo and Kapuya, 2016). Hence, research studies in identifying water stress tolerant growth stages in irrigated wheat genotypes can also be useful in South Africa as such studies are still limited in the country (ARC-SGI, 2013, 2014, 2015).

With this view, it was necessary to review the response of wheat genotypes to water stress imposed at different growth stages, to evaluate the importance of genotype choice and to appraisal suitable techniques that can aid in the selection of best performing genotypes. The outcome of this review will generate knowledge that can contribute to further understanding the different mechanisms that enable irrigated wheat to survive under limited water environments.

Most importantly it will guide farmers and researchers on appropriate techniques they can use to evaluate water stress tolerant growth stages on wheat genotypes. This knowledge can be used to produce production guidelines for South African irrigated wheat farmers.

2.3 Effect of water stress at different growth stages of wheat genotypes

Although researchers and breeders have been successful in maintaining wheat production in drought occurring regions, it is still difficult to increase the yield as the drought crisis worsens (Boutraa et al., 2010; Ali et al., 2013; Dube et al., 2016). Drought is a period of dry weather that causes plant water stress, while plant water stress is a term used to describe the strain experienced by plants when they do not receive enough water (Bray, 2001). Due to plant water stress caused by drought many studies have been conducted globally to determine how water stress imposed at different growth stages affects the wheat’s physiological and morphological traits as well as the yield production (Boutraa et al., 2010; Jatoi et al., 2011; Ali et al., 2013). This aimed at examining the reaction of the crop’s physiological and morphological traits that help explain the genotype’s performance and the severity of water stress (Boutraa et al., 2010; Jatoi et al., 2011; Ali et al., 2013). For instance, Ali et al. (2013) investigate the effect of water stress on the physiology and yield of twelve wheat genotypes grown in Pakistan. The genotypes were exposed to four water treatments (T1 - 380 mL, T2 - 190 mL, T3 - 126 mL and T4 - 95 mL), each given at 15 days’ intervals. Morphological (yield per plant and spikelets per spike) and physiological (electrolyte leakage, turgidity and relative water content) traits of each genotype were measured after subjecting the genotypes to these conditions. Findings indicated that one of the consequences of water stress in the physiological traits is an increase in the electrolyte leakage while the relative water content and turgidity are reduced. Yield per plant and spikelets per spike were also reduced by water stress which subsequently reduced the yield.

The ability of a genotype to tolerate water stress differed based on the level of stress and the sensitivity of the genotype's growth stage. Hence, some genotypes (Tarata, ZAS-08 (08), ZAS-42(21), and Ghazanavi-98) were more tolerant than others resulting in high yield. These results imply that water stress affects wheat genotypes, however, the level of tolerance differs among genotypes and depends on the duration of the stressing period and the sensitivity of the genotype's growth stage. These outcomes also give an indication that there is a possibility of planting irrigated wheat genotypes with growth stages that can cope with limited water.

Water stress deteriorates wheat traits but some genotypes can recover and resume normal plant functioning after being subjected to water stress, hence it is important to identify such genotypes. This was also proven by Boutraa et al. (2010), who examined the effect of water stress on the growth and water use efficiency (WUE) on four wheat genotypes (Al-gaimi, Sindy-1, Sindy-2, and Hab-Ahmar) grown in Saudi Arabia. The genotypes were exposed to three water regimes: 80% field capacity (FC) which served as a control, 50% FC as mild stress and 30% FC as severe stress. Significant correlations were evaluated between the type of genotype and the level of water stress. Among the four wheat genotypes, mild water stress affected Sindy-2 by reducing the water use efficiency compared to Sindy-1, while Al-gaimi maintained a higher water use efficiency. The relative water content was however reduced for Al-gaimi and Hab-Ahmar under severe stress whereas Sindy-1 and Sindy- 2 showed tolerance with a high relative water content. It was further discovered that Al-gaimi was the most water stress tolerant genotype among the four. This decision was influenced by the ability of this genotype to maintain a higher water use efficiency compared to other genotypes which is an important trait when evaluating water stress tolerance of a genotype under water limited conditions. The different responses of these traits to water stress can be due to the genetic build up of each genotype, which in most cases determines the sensitivity of the genotypes' growth stage to water stress.

A similar study was conducted in Pakistan under rain shelter, to identify the effect of water stress on physiological and yield parameters at flowering stage in twelve elite spring wheat genotypes (Anmol, Inqilab, Moomal, TJ-83, Sarsabz, Khirman, SKD-1, TD-1, Kiran-95, Abadgar, Marvi and Imdad-05) (Jatoi et al., 2011). The study demonstrated that three of the wheat genotypes (TD-1, SKD-1, and Sarsabz) were more resistant to water stress at flowering. This was indicated by their ability to maintain a higher relative water content and obtain higher yields after being subjected to water stress at this growth stage. Genotype Anmol, Imdad-05 and Inqilab retained the lowest relative water content proving to be susceptible to water stress at flowering. These results highlight that apart from the yield, the relative water content and water use efficiency can be used as genotype selection traits when trying to identify water stress tolerant growth stages in wheat genotypes.

Furthermore, these studies collectively suggest that water stress threatens the growth and development of wheat. However, there is still a possibility of maximizing yields with limited water through evaluating growth stages within irrigated wheat genotypes that are not responsive to water stress and enable the crop to resume normal plant growth under stressful environments. The measuring of biochemical, physiological and morphological traits can be able to give researchers an accurate indication of the level of water stress tolerance at different growth stages of irrigated wheat.

2.4 Sensitivity of wheat growth stages to water stress

Water stress imposed at any developmental stage of wheat genotypes negatively affects the crop's physiological, morphological and leaf gas exchange traits which then lowers the yield and yield components of the crop (Hochman, 1982; Mirzae et al., 2011; Akram, 2011). Flowering, tillering and grain filling stages are some of the most sensitive growth stages to water stress (Mirzae et al., 2011).

2.4.1 Tillering

Tillering has great agronomic importance in wheat since it can partially or totally compensate the difference in plant number after crop establishment or may allow crop recovery after being subjected to water stress (Acevedo et al., 2002). The stage is considered as one of the most water stress sensitive growth stages in wheat (Acevedo et al., 2002). The sensitivity of tillering stage to water stress has been reported by Qadir et al. (1999) who conducted an experiment on how water stress at tillering stage affects growth and yield performance of four wheat genotypes (Pasban-90, Barani-83, Punjab-85, and Rohtas-90). The results revealed that water stress at tillering resulted in less number of fertile tillers for Pasban-90, and Punjan-85 genotypes. Water stress at tillering was also found to be detrimental to the leaf area, plant height, spikelets per spike and grain weight. However, the degree of sensitivity of tillering stage to water stress varied among genotypes and the intensity of the stress. This suggests that the tillering stage cannot survive without water, nevertheless, wheat genotypes that have been bred for high tolerance can recover after water stress and resume normal plant growth.

Veesar et al. (2007), also investigated the sensitivity of the tillering stage to water stress on Sindh-81, Mutant of Sindh-81, Indus-66 and Mutant of Indus-66 genotypes. Findings justified the sensitivity of the tillering stage to water stress as a significant reduction in plant height, the number of fertile tillers and spikelets per spike were evident. In terms of genotype performance, Sindh-81 was found to be the most tolerant genotype as its plant height, the number of fertile tillers and spikelets per spike were not reduced after water stress at tillering and similar to when this genotype is grown under full irrigation. This justifies that the tillering stage is one of most susceptible growth stages in wheat genotypes. Nevertheless, tolerant wheat genotypes have a tillering stage that does not disrupt the crop’s development and yield production when subjected to water stress. Hence, identifying such growth stages can contribute in increasing yields while saving water.

2.4.2 Flowering

The flowering stage is also considered as one of the most sensitive growth stages to water stress in wheat genotypes (Farooq et al., 2014). Water stress at this growth stage has been reported to result in a massive yield loss (Farooq et al., 2014). In a research study conducted in Turkey by Kilic and Yagbasanlar (2010), on the effect of water stress imposed at flowering stage of 14 durum wheat genotypes (Altintoprak-98, Aydin-93, Ceylan-95, Dicle-74, Diyarbakir-81, D.5456, Ege-88, Firat-93, Gidara-II, Ozberk, Harran-95, Saricanak-98, Sorgul, and Balcali-2000). Water stress resulted in reduced grain yield in sensitive genotypes. However, some genotypes were able to recover and produce high yield after being subjected to water stress.

A similar study conducted by Khakwani et al. (2012) on growth and yield response of wheat genotypes to water stress at flowering stage indicated that Zam-04 and Hashim-8’s flowering stage was highly resistant to water stress. These genotypes produced the highest yield compared to other genotypes stressed at flowering. This is an indication that flowering is the most critical growth stage to water stress in wheat but genotypes that are bred to withstand water stress may not be affected by water stress at flowering. Therefore, monitoring the response of wheat genotypes to water stress imposed at flowering can aid maximize wheat yields and conserve water without the crop suffering when irrigation is withheld at this growth stage.

2.4.3 Grain filling

Water stress at the grain filling results in reduced grain yield and yield components such as plants per unit area, tillers per plant, spikelets per head, and kernels per spikelet (Veesar et al., 2007; Mirzaei et al., 2011). Despite these challenges, there are genotypes that can endure water stress at grain filling and this response is associated with the genetic features of each genotype (Veesar et al., 2007; Mirzaei et al., 2011). This was proven by Ahmadi and Baker (2001), who conducted a glasshouse experiment to determine the sensitivity of the grain filling stage to water stress. The grain filling stage was found to be very sensitive to water stress as the relative water content, leaf water potential, and grain yield were significantly lower under water stressed conditions than in well-watered conditions.

The sensitivity of grain filling stage to water stress was also tested by Saeidi and Abdoli (2015), who evaluated the response of yield, gas exchange variables and some physiological traits of wheat genotypes to water stress imposed at grain filling. The genotypes evaluated were Bahar, Parsi, Pishtaz, Pishgam, Chamara, Zarin, Sivand, Marvdasht and DN-11. Results revealed that genotypes differed in their response to water stress at grain filling, for example, some genotypes indicated to be vulnerable to water stress at this growth stage. Thus, sensitive genotypes exposed to water stress at grain filling had a lower stomatal conductance, the rate of photosynthesis and transpiration rate while these were higher for water stress tolerant genotypes. Likewise, Veesar et al. (2007) observed that a significant reduction in yield was evident when some wheat genotypes (Sindh-81, a mutant of Sindh-81, Indus-66 and mutant of Indus-66) were stressed at grain filling.

Mushtaq et al. (2011) evaluated the performance of two wheat genotypes (Farooq-2006 and Mairaj-2008) when water stress was imposed at four growth stages; tillering, jointing, spike emergence and grain filling. Yield reduction was most evident after water stress at grain filling stage. These studies collectively suggest that the grain filling stage is the most sensitive growth stage but there is still a possibility of identifying wheat genotypes that have a water conserving grain filling stage while maximizing production.

2.5 Mechanism of water stress tolerance

The sensitivity of the crop’s growth stages to water stress negatively affects its growth and development (Fang and Xiong, 2015). This allows them to develop several response mechanisms which help tackle water stress and survive under water limited environments (Blum, 2010). These include drought tolerance, dehydration avoidance and drought escape (Tekle and Alemu, 2016).

2.5.1 Drought tolerance

Control of drought stress in crops is not only very complex but it is also influenced by other environmental factors and by the development stage of the crop (Waseem et al., 2011). Plants can tolerate drought stress by modifying their morphological and physiological characteristics. These include a cuticle that decreases transpiration, closing of the stomata, reduction in leaf surface area, and the ability to accelerate senescence (Ribichich and Chan, 2015).

To determine the ability of the plant to tolerate drought, several indices are used, these include stress tolerance index, susceptibility index, and yield stability index (Khan and Naqvi, 2011). These indices provide meaningful measures in drought stress conditions, in terms of comparing yield loss under water stress with that at suitable conditions (Golabadi et al., 2006). For instance, a higher value of tolerance index is an indication that the plant is more sensitive to water stress, whereas a lower value indicates the ability of the plant to tolerate water stress (Bogale and Tesfaye, 2011).

The indicators associated with drought tolerance in the crop mainly cover the physiological parameters which are related to osmotic adjustment (OA), which is a major component of drought resistance (Fang and Xiong, 2015). Osmotic adjustment is the lowering of osmotic potential that arises from the net accumulation of solutes in response to water stress and in turn maintains turgor in plants (Guei and Wassom, 1993). For example, the accumulation of proline content in wheat after water stress has also been associated with drought tolerance (Mwadzingeni et al., 2016). Proline has been reported to accumulate in wheat genotypes after prolonged water stress which is an important component of drought resistance (Farooq et al., 2014; Mwadzingeni et al., 2016). Hence, many research studies determining the effect of water stress on wheat have used the proline content as an indication of the level of water stress the crop has suffered (Johari-Pireivatlou, 2009; Maralian et al., 2010; Bilal et al., 2015).

2.5.2 Dehydration avoidance

Dehydration avoidance can be defined as the ability of the plant to sustain high plant water status or cellular hydration under drought stress conditions (Blum, 2005). The dehydration avoidance strategies are diverse and mostly depend on the severity of stress. These strategies can take place in the whole plant, the organs or cellular level (Toriyama et al., 2004). Generally, plants avoid dehydration during water stress by closing the stomata that regulate water loss from the leaves, thereby restricting the transpiring area and maintaining the root water uptake as the soil becomes dry (Dodd and Ryan, 2016). Comas et al. (2013) reported that plants with higher root density and deep rooting system are associated with higher water absorption. Therefore, crops with a deep rooting system are favored where deep soil water is available in the profile. Ehlers and Goss (2016) stated that during dehydration avoidance plants avoid desiccation of their tissues by increasing water uptake, reducing water loss, or enhancing the internal storage of water. While, Levitt (1985) stated that dehydration avoidance due to cuticle control increases with leaf number to a maximum in the intermediate leaf, decreasing to a minimum in the upper leaves. The indicators associated with dehydration avoidance are usually related to moisture maintenance, water uptake and water use efficiency. Suggesting that plants can tolerate dehydration and resume normal plant growth (Fang and Xiong, 2015).

2.5.3 Drought escape

Drought escape is defined as the ability of the plant to complete its life cycle before major water stress occurs (Douglas and Asay, 1993). Plants with drought escape traits will germinate from dormant seed only when there is enough water (Ehlers and Goss, 2016). Afterward, they will survive with limited water supply because they can terminate vegetative growth and become reproductive after a very short life cycle or after few weeks. Early maturing is an important attribute of drought escape (Blum, 2005).

2.6 Parameters that assist detect the level of water tolerance in wheat genotypes

Morphological (plant height, the number of fertile tillers, spike length and spikelets per spike) and physiological (rate of photosynthesis, stomatal conductance, intercellular CO_2 concentration and transpiration rate) traits can be good indicators when evaluating the level of water stress tolerance of wheat genotypes (Allahverdiyed, 2015). This was validated by an experiment conducted by Allahverdiyed (2015) who tested these parameters on six durum wheat genotypes (Garagylchyd 2, Shiraslan 23, Barakatli-95, Alinja-84, Vugar and Tartar) and seven bread wheat genotypes (Gobustan, Giymatli-2/17, Grmyxygul 1, Azamatli-95, Tale-38, 12nd FAWWONN^{0}97 and 4th FEFWSNN^{0}50) that are usually grown in Portugal. Water stress affected both the durum and bread wheat genotypes. For instance, leaf area was reduced in all the water stressed genotypes, however, the effect of water stress differed amongst genotypes as some genotypes were highly tolerant. Genotypes that are better adapted to water stress had a higher relative water content, leaf area index, rate of photosynthesis and transpiration rate, while these traits were low in water stress vulnerable genotypes.

Allahverdiyed (2015) was supported by Anjum et al. (2011), who based on their findings concluded that water stress progressively reduced CO_2 assimilation rates due to the reduction of stomatal conductance. Plant height, spike lengths, spike weight, spikelets per spike, grain yield, and chlorophyll content, are also components that can be used to determine the effect of water stress on wheat (Kilic and Yagbasanlar, 2010). A reduction in these parameters indicates the poor genotype performance (Kilic and Yagbasanlar, 2010). Boutraa et al. (2010) tested the effect of water stress on growth and water use efficiency of wheat genotypes grown in Saudi Arabia. Plant height, water use efficiency and leaf area showed to be good water stress tolerance indicators in this experiment. They were found to be reduced after the water stress period in susceptible genotypes. Whereas, they were higher in better adapted genotypes and performed the same as genotypes subjected to full irrigation. The proline content is also reported to be an important solute that can determine the level of water stress tolerance in a genotype when it is experiencing water stress (Farooq et al., 2014; Mwadzingeni et al., 2016). It is important to measure as many physiological and morphological parameters as possible to be able to validate if a genotype is tolerant or susceptible to water stress.

2.7 The importance of identifying water conserving irrigated wheat genotypes in South Africa

Genotype choice is an important production decision and could aid in reducing the risk of crop failure (Otto, 2016). The decision is compounded by several factors which include yield potential, agronomic characteristics, the region considered and adaptability of the genotype (DAFF, 2010). The ability to survive under water stressed environments is among the many characteristics that should be considered when selecting a suitable genotype as wrong genotype choice is a yield-limiting factor (DAFF, 2010; Barnard, 2012; Liu et al., 2013). Better adapted genotypes will yield higher even under water limited conditions (DAFF, 2010; Barnard, 2012; Liu et al., 2013). To accurately determine water stress tolerant genotypes, a criterion for selection is used which involves rating the response of wheat genotypes to water stress based on three characteristics: (1) good tolerance, (2) reasonable tolerance and (3) poor tolerance (ARC-SGI, 2009).

In South Africa, research studies focusing on identifying water stress tolerant growth stages in irrigated wheat genotypes is still in its infancy. The national wheat cultivar evaluation program on irrigated wheat conducted by the Agricultural Research Council-Small Grain Institute every year characterizes commercially released genotypes to recommend genotypes which will perform best in different production regions (Kilian et al., 2014, 2015). Trials are planted in all the major wheat production regions of South Africa, namely; Cooler Central irrigation areas, the Warmer Northern irrigation areas, KwaZulu-Natal and Highveld irrigation areas (Kilian et al., 2014, 2015; ARC-SGI, 2014, 2015). At each production region, there are more than 16 localities (test sites) where the trials are planted (Kilian, 2014, 2015). The number of genotypes tested differs yearly and it depends on the number of new entries received in a certain year. For instance, in 2015, 24 genotypes were planted in each locality under each production region, while in 2014, 19 genotypes were planted (Kilian, 2014, 2015). The genotypes are all getting sufficient water based on the farmers' irrigation scheduling, irrigation is not restricted. This is to recommend genotypes to farmers that will perform well in their environments under their management schedule. But this still does not provide a solution for irrigated wheat farmers should they be faced with a crisis of water shortage. Moreover, this limited information restricts farmers from withholding irrigation at any growth stage as some growth stages are more sensitive than others.

2.8 Techniques to select water stress tolerant genotypes

Several methods have been applied in different studies globally for identifying water stress tolerant genotypes (Mirbahar et al., 2009; Boutraa et al., 2010; Hammad and Ali, 2014). Hammad and Ali (2014) carried out an experiment on both the field and glasshouse in an attempt to detect the best performing wheat genotypes exposed to three water regimes: irrigation depletion of 50%, 65%, and 80% of available soil water. Their findings showed that crops under water stress experienced a reduction in grain yield, number of spikes, grain weight, and the number of grains per spike but concluded that the were genotypes that performed better than others. Furthermore, the severity of the effect on these traits dependent on the period of water stress. The method used in this study signified the effects of the different water stress levels and how each wheat genotype reacts to water stress.

Boutraa et al. (2010) studied the effect of water stress on growth and water use efficiency using four wheat genotypes (Sindy-1, Sindy-2, Al-gaimi and Hab-Ahmar) and concluded that wheat genotypes differed in the response to water stress hence some were water stress tolerant. Among the four genotypes used, Al-gaimi was more tolerant. This experiment was successfully conducted in the glasshouse. Mibahar et al. (2009) demonstrated that even though water stress influences wheat traits such as plant height and number of tillers, the wheat genotypes are still able to adjust and adapt to these conditions.

Lastly, Saeidi et al. (2015) who also planted greenhouse trials of different bread wheat genotypes indicated that water stress decreased grain yield by reducing the number of grains per spike. Furthermore, water stress significantly reduced the chlorophyll content and the relative water content in wheat genotypes. However, Pishtaz and Marvdasht among the tested genotypes showed tolerance to water stress as they recovered very well after water stress was eliminated. Findings in these studies suggest that there are many different techniques to evaluate the performance of wheat genotypes under water stressed conditions. The technique to use can depend on the objective of the study or the availability of the equipment but whichever technique used; useful information can be acquired.

2.9 Research gaps and future prospects for South Africa's wheat industry

In this review, the focus was primarily on the effect of water stress imposed at different growth stages of wheat genotypes, the strategies of adaptation of these genotypes to water stress at different growth stages and the various methods of selecting tolerant wheat genotypes with water stress adaptable growth stages. It is evident that water stress directly affects wheat genotypes at different growth stages and identifies yield as a predominant consequence. In fact, the higher wheat yields are obtained in wheat genotypes that could tolerate water stress compared to the sensitive ones.

The review further showed that more in-depth research still needs to be done in South Africa to better understand the response of irrigated wheat genotypes to water stress induced at certain growth stages. This was also proven by the fact that most research studies on wheat conducted in the country focus on breeding and evaluating tolerant genotypes under dryland conditions. While, research on irrigated wheat genotypes evaluates their performance at various geographical regions and management strategies. This review was also able to justify that whichever technique used whether it's a field experiment or at the glasshouse, identification of irrigated wheat genotypes with water stress tolerant growth stages is attainable.

References

Acevedo E, Silva P, Silva H (2002) *Wheat growth and physiology*. Food and Agriculture Organization of the United Nations. Rome: FAO. http://www.fao.org/docrep/006/Y4011e.htm. (Accessed August 2016).

Agricultural Statistics (2014) Abstract of Agricultural Statistics. Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa.

Ahmadi A, Baker DA (2001) The effect of water stress on grain filling processed in wheat. *Journal of Agricultural Science* 136: 257-269.

Akram M (2011) Growth and yield components of wheat under water stress of different growth stages. *Bangladesh Journal of Agriculture Resources* 36: 455-468.

Ali A, Ali N, Ullah N, Ullah F, Adnan M, Swati ZA (2013) Effect of drought stress on the physiology and yield of the Pakistani wheat germplasms. *International Journal of Advancements in Research and Technology* 2: 419-430.

Allahverdiyed T (2015) Effect of drought stress on some physiological traits of durum (*Triticum durum* Desf.) and bread (*Triticum aestivum* L.) wheat genotypes. *Journal of Stress Physiology and Biochemistry* 11: 29-38.

Anjum SA, Xie XY, Wang LC, Seleem MF, Man C, Lei W (2011) Morphological, physiological and biochemical responses of plants to drought stress. *African Journal of Agricultural Research* 6: 2026-2032. https://doi.org/10.5897/AJAR10.027.

ARC-SGI (Agricultural Research Council – Small Grain Institute) (2013) *Guidelines of small grain in the winter rainfall areas*. Bethlehem, Free State, South Africa.

ARC-SGI (Agricultural Research Council- Small Grain Institute) (2015) *Wheat production guidelines for small-scale farmers: Production of small grains in the winter rainfall area*. Bethlehem, Free State, South Africa.

ARC-SGI (Agricultural Research Council-Small Grain Institute) (2009) *Wheat production guidelines for small-scale farmers:Production of small grains in the summer rainfall areas*. Bethlehem, Free State, South Africa.

ARC-SGI (Agricultural Research Council-Small Grain Institute) (2014) *Wheat production guidelines for small-scale farmers:Production of small grains in the summer rainfall areas*. Bethlehem, Free State, South Africa.

Barnard A (2012) *Physiological changes in the wheat crop*. Agricultural Research Council – Small Grain Institute, Bethlehem, Free State, South Africa.

Bilal M, Iqbal I, Rana RM, Rehman SU, Haidery Q, Ahmad F, Ijaz A, Umar HMI (2015) A comprehensive review of effects of water stress and tolerance in wheat (*Triticum aestivum* L.). *Tropical Plant Research* 2: 271-275.

Blum A (2005) Drought resistance, water-use efficiency, and yield potential- are they compatible, dissonant, or mutually exclusive? *Australian Journal of Agricultural Research* 56: 1159-1168. https://doi.org/10.1071/AR05069.

Blum A (2010) *Plant breeding for water limited environments*. Plant water relations, plant stress and plant production. In: Plant breeding for water-limited environments. Springer Dordrecht Heidelberg London New York. https://doi.10.1007/978-1-4419-7491-4.

Bogale A, Tesfaye K, Geleto T (2011) Morphological and physiological attributes associated to drought tolerance of Ethipian durum wheat genotypes under water deficit conditions. *Journal of Biodiversity and Environmental Sciences (JBES)* 1: 22-36.

Boutraa T, Akhkha A, Al-Shoaibi AA, Alhejeli AM (2010) Effect of water stress on growth and water use efficiency (WUE) of some wheat cultivars (*Triticum durum*) grown in Saudi Arabi. *Journal of Taibah University for Science* 3: 39-48. https://doi.org/10.1016/S1658-3655(12)60019-3.

Bray EA (2001) Plant response to water-deficit stress. *Encyclopedia of Life Sciences*: 1-5. https://doi.org/10.1002/9780470015902.a0001298.pu.

Comas LH, Becker SR, Cruz VMV, Byme PF, Dieng DA (2013) Root traits contributing to plantproductivity under drought. *Frontiers in Plant Science* 4: 1-16. https://doi.org/10.3389/fpls.2013.00442.

DAFF (Department of Agriculture, Forestry and Fisheries) (2010) Wheat: *Production guideline*. DAFF, South Africa.

Dodd IC, Ryan AC (2016) Whole-plant physiological responses to water deficit stress. *eLS:* 1 – 9. https://doi.org/10.1002/9780470015902.a0001298.pub3.

Douglas AJ, Asay KH (1993) Viewpoint: Selection for improved drought response in cool-season grasses. *Journal of Range Management* 46: 194-204.

Dube E, Willem K, van Rensburg B, van der Merwe M, van der Merwe E, Hatting H (2016) *National cultivar evaluation results: Did irrigation wheat beat the heat in 2015?* Agricultural Research Council- Small Grain Institute, Bethlehem, Free State, South Africa.

Ehlers W, Goss M (2016) Water stress in plant. In: Water dynamics in plant production. Gottingen, Germany. https://doi.org/10.1079/9780851996943.0000.

Fang Y, Xiong L (2015) General mechanisms of drought response and their application in drought resistance improvements in plants. *Cellular and Molecular Life Sciences* 72: 673 - 689. https://doi.org/10.1007/s00018-014-1767-0.

FAO (Food and Agriculture Organization of the United Nations) (2004) *Rice and human nutrition. International year of rice: Rice is life*. http://www.fao.org/ Newsroom/en/ focus/ 2004/ 36887/ index.html. (Accessed October 2016).

Farooq M, Hussain M, Kadambot HM (2014) Drought stress in wheat during flowering and grain filling periods. *Critical Reviews in Plant Science* 33: 331-349. https://dx.doi.org/10.1080/07352689.2014.875291.

Fourie P, Botha P (2011) The role of irrigation in grain production. Grain SA. Pretoria. South Africa.

Fourie P, Sihlobo W (2016) *Wheat import tariff does not lead to a significant increase in the price of white bread*. Grain SA. Pretoria. South Africa.

Gbetibouo GA, Hassan RM (2004) Measuring the economic impact of climate change on major South African field crops: A Ricardian approach. *Global and planetary change* 47: 143-152. https://doi.org/10.1016/J.gloplacha.2004.10.009.

Golabadi M, Arzani A, Mirmohammadi Maibody SAM (2006) Assessment of drought tolerance in segregating populations in durum wheat. *African Journal of Agricultural Research* 1: 162-171.

Guei RG, Wassom CE (1993) Genetics of osmotic adjustment in breeding maize for drought tolerance. *Heredity* 71: 436-441. https://doi.org/10.1038/hdy.1993.158.

Hammad SAR, Ali OAM (2014) Physiological and biochemical studies on drought tolerance of wheat plants by application of amino acids and yeast extract. *Annals of Agricultural Sciences* 59: 133-145. https://doi.org/10.1016/J.aosas.2014.06.018.

Hedden S, Cilliers J (2014) Parched Prospects: The emerging water crisis in South Africa. Institute for security studies: African future paper 11. https://issafrica.s3.amazonaws.com/site/uploads/AF11.15Sept2014.pdf. (Accessed September 2016).

Hochman Z (1982) Effect of water stress with phasic development on yield of wheat grown in a semi-arid environment *Field Crops Research* 5: 55-67. https://doi.org/10.1016/0378-4290(82)90006-5.

Jatoi WA, Kumbhar BMB, Khan NU, Kerio MI (2011) Effect of water stress on physiological and yield parameters at anthesis stages in elite spring wheat cultivars. *Sarhad Journal of Agriculture* 27: 59-65.

Johari- Pireivatlou M (2009) Effect of soil water stress on yield and proline content of four wheat line. *African Journal of Biotechnology* 9: 036–040. https://doi.org/10.5897/ajb09.521.

Khakwani AA, Dennett MD, Munir M, Abid M (2012) Growth and yield response of wheat varieties to water stress at booting and anthesis stages of development. *Pakistan Journal of Botany* 44: 879-886.

Khan N, Naqvi FN (2011) Heritability of morphological traits in bread wheat advanced lines under irrigated and non-irrigated conditions. *Asian Journal of Agricultural Sciences* 3: 215-222.

Kilian WH, Dube E, Du Plessis D, van der Merwe HSCA, van der Merwe ME, van Rensburg B, Hatting MHA, Moapeli MH, Miles MCE (2014) *National wheat cultivar evaluation programme under irrigation report*. Agricultural Research Council-Small Grain Institute. Bethlehem, Free State, South Africa.

Kilian WH, Dube E, Du Plessis D, van der Merwe HSCA, van der Merwe ME, van Rensburg B, Hatting MHA, Moapeli MH, Miles MCE (2015) *National wheat cultivar evaluation programme under irrigation report*. Agricultural Research Council-Small Grain Institute. Bethlehem, Free State, South Africa.

Kilic H, Yagbasanlar T (2010) The effect of drought stress on grain yield, yield components, and some quality traits of durum wheat (*Triticum turgidum ssp.durum)* cultivars. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 38: 164-170. http://dx.doi.org/10.15835/nbha3814274.

Le Roux J (1995) Current trends in wheat research in South Africa. In: proceedings of 9th regional wheat workshop: for Eastern, Southern and Central. Paper presented at the ninth regional wheat workshop: for Eastern, Central and Southern Africa, Addis Ababa, Ethiopia, 2-6 October 1995. http://libcatalog.cimmyt.org/download/cim/65763.pdf. (Accessed January 2017).

Levitt J (1985) Relationship of dehydration rate to drought avoidancve, dehydration avoidance of cabbage leaves, and to their acclimation during drought-induced water stress. *Plant, Cell and Environment* 8: 287-296.

Liu H, Sultan MARF, Zhao HX (2013) The Screening of water stress tolerant wheat cultivars with physiological indices. *Global Journal of Biodiversity Science and Management* 3: 211-218.

Maralian H, Ebadi A, Didar TR, Eghrari BH (2010) Influence of water deficit stress on wheat grain yield and proline accumulation rate. *African Journal of Agricultural Research* 5: 286-289.

Mirbahar AA, Markhand GS, Mahar AR, Abro AA, Kanhar NA (2009) Effects of water stress on yield and yield components of wheat (*Triticum aestivum* L.) varieties. *Pakistan Journal of Botany* 41: 1303-1310.

Mirzaei A, Naseri R, Soleimani R (2011) Response of different growth stages of wheat to moisture tension in a semiarid land. *World Applied Sciences journal* 12: 83-89.

Mokhema T (2015) South Africa Cuts Output Forecast as drought hurts yields. Bloomberg. https://www.bloomberg.com/news/articles/2015-12-15/south-africa-cuts-wheat-output-forecast-as-drought-hurts-yields (Accessed June 2016).

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

Mwadzingeni L, Shimelis H, Tesfay S, Tsilo TJ 2016 Screening of bread wheat genotypes for drought tolerance using phenotypic and proline analyses. *Frontiers in Plant Science* 7: 1 -12. https://doi.org/10.3389/fpls.2016.01276.

Newsome M (2012) Report claims Africa could meet its own wheat needs. http://mg.co.za/article/2012-10-10-report-claims-Africa-could-meet-its-own-wheat-needs. (Accessed July 2016.).

Otto W (2016) How to choose the "Mr right" of wheat cultivars. Pula/imvula. Grain SA, South Africa.

Qadir G, Saeed M, Cheema MA (1999) Effect of water stress on growth and yield performance of four wheat cultivars. *Pakistan Journal of Biological Sciences* 2: 239-239.

Ribichich KF, Chan RL (2015) How do plants deal with flood and drought? Biotechnological strategies to enhance the natural response and improve yield. ISB news report. http://www.isb.vt.edu/news/2015/AugSep/RibichichChan.pdf. (Accessed November 2016).

Saedi M, Abdoli M (2015) Effect of drought stress during grain filling on yield and its components, gas exchange variables, and some physiological traits of wheat cultivars. *Journal of Agricultural Science and Technology* 17: 885-895.

Saeidi M, Ardalani S, Honarmand SJ, Ghobadi ME, Abdoli M (2015) Evaluation of drought stress at vegetative growth stage on the grain yield formation and some physiological traits as well as fluorescence parameters of different bread wheat cultivars. *Acta Biologica Szegediensis* 59: 35-44.

Sarwar MH, Sarwar MF, Sarwar M, Qadri NA, Moghal S (2013) The importance of cereals (*Poaceae: Gramineae*) nutrition in human health: A review. *Journal of Cereals and Oilseeds* 4: 32-35. https://doi.org/10.5897/JCO.12.023.

Shewry PR (2009) Wheat. *Journal of Experimental Botany* 60: 1537-1553. https://doi.org/10.1093/jxb/erp058.

Sihlobo W, Kapuya T (2016) *How far off is South Africa from the current season's targeted grain imports?* Grain SA, Pretoria, South Africa.

Tekle AT, Alemu MA (2016) Drought tolerance mechanisms in field crops. *World Journal of Biology and Medical Sciences* 23: 15-39.

Toriyama K, Heong KL, Hardy B (2004) Rice is life: scientific perspectives for the 21st century. In: Proceedings of World Rice Research Conference, 4-7 November 2004, Tokyo and Tsukuba, Japan.

USAD (United States Department of Agriculture - Foreign agricultural service) (2017) *Commodity intelligence report*- South Africa: Near records yields expected for the 2016/17 wheat crop. https://pecad.fas.usda.gov/highlights/2017/01/SouthAfrica/index. (Accessed February 2017).

Veesar NF, Channa AN, Rind MJ, Larik AS (2007) Infuence of water stress imposed at different stages on growth and yield attributes in bread wheat genotypes (*Tritcum aestivum* L.). Department of Plant Breeding and Genetics, Sindh Agriculture University, Tandojam, Sindh, Pakistan.

Waseem M, Ali A, Tahir M, Nadeem MA, Ayub M, Tanveer A, Ahmad R, Hussain M (2011) Mechanism of drought tolerance in plant and its management through different methods. *Continental Journal of Agricultural Science* 5: 10-25.

WATER RESEARCH COMMISSION (2015) Water restrictions. *Drought: your gateway to managing water scarcity in times of drought*. Water Research Commission. Pretoria. South Africa.

CHAPTER 3

Physiological responses of irrigated wheat (T*riticum aestivum* L.) genotypes to water stress at different growth stages

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3.1 Abstract

The recent drought in South Africa, over the past decade, has not only reduced the production of dryland wheat but has also decreased yields of irrigated wheat. This study evaluated physiological traits of irrigated wheat genotypes in response to water stress imposed at different growth stages. A factorial experiment [2 water treatment (stressed vs non-stresses) x 3 growth stages (tillering, flowering & grain filling) x 8 (genotypes)] based on a randomized complete block design with three replicates was conducted. The results indicated that the rate of photosynthesis was affected by water stress for genotype LM98 at tillering and LM43 at flowering while the other 6 genotypes tested were not affected. Transpiration rate and stomatal conductance were not affected across growth stages. Genotype LM43 and LM35 instantaneous water use efficiency was not reduced by water stress at tillering but lower after water stress at flowering. In genotype LM35, LM79, LM57 and LM98 it was affected at grain filling. The relative water content was not affected at grain filling but reduced for LM43 and LM35 at tillering. At flowering, it was affected in LM98, LM79, LM83 and LM57. In conclusion, LM35, LM79, LM57 and LM98 was not affected by the imposed water stress at grain filling. LM43 and LM35 were tolerant to water stress at tillering but sensitive at flowering.

Keywords: genotypes, growth stages, physiological traits, water stress, drought tolerant wheat genotypes.

3.2 Introduction

Drought is the major factor limiting wheat (*Triticum aestivum* L.) production worldwide and has been the main reason for reduced yield in recent years (Mancosu et al. 2015; Daryanto et al. 2016). The production of irrigated wheat under water limiting conditions has become a major contributor to the total wheat production in South Africa compared with dryland wheat production (Fourie and Botha 2011). However, increasing drought occurrences in recent years has caused water shortages which pose a challenge to the frequent use of irrigation water (WRC 2015, 2016). Drought incidences have resulted in the lowering of the country's major water supplying dams and some parts of the country being listed as disaster areas (WRC 2015, 2016). Therefore, leading to the restriction of water use across the country (Turton et al. 2016).

Alternative strategies such as withholding water at certain irrigation intervals can assist in maximizing wheat production under limited water conditions. On the other hand, this could result in crop failure as insufficient water at water stress sensitive growth stages reduces productivity (Tabassam et al. 2014). Tillering, flowering and grain filling have been reported to be sensitive growth stages in wheat production (Sokoto and Sigh 2013; Nayyar and Walia 2003). However, due to the physiological mechanisms which are governed by the genetic makeup of each genotype some of these growth stages can cope with water stress in tolerant wheat genotypes (Blum 2005; Farooq et al. 2009; Hanin et al. 2011). These are referred to as response mechanisms and include; drought escape, dehydration avoidance, and drought tolerance (Blum 2005; Farooq et al. 2009; Tekle and Alemu 2016). Accumulation of proline and oxygen free radicals are other indicators of responses of plants to water stress.

Proline content is the amino acid that accumulates in plants when they are subjected to water stressed conditions together with the relative water content, rate of photosynthesis, stomatal conductance, transpiration rate and water use efficiency have been used as water stress physiological indices in many studies (van Heerden and de Villiers 1996; Hafid et al. 1998; Hayat et al. 2012). The different reactions of these traits to water stress at different growth stages of wheat genotypes have been able to assist researchers in identifying water stress tolerant growth stages in irrigated wheat genotypes (Ashraf and Khan 1993; Akram 2011; Saeidi et al. 2015). However, such research is still lacking in South Africa. Therefore, it is important to study the physiological responses of the newly developed wheat genotypes to further improve their performance under water stress conditions. Hence, the present study was conducted to select water stress tolerant irrigated wheat genotypes through evaluating the response of their physiological traits after water stress at tillering, flowering and grain filling.

3.3 Materials and Methods

3.3.1. Experimental description, design, plant material and agronomic practices

A greenhouse experiment was conducted during the 2015/16 winter season in a tunnel at the University of KwaZulu-Natal, Pietermaritzburg, South Africa (29˚ 37' S, 30˚ 24' E). The average day and night temperatures in the tunnel were 30 °C and 18 °C, respectively, while the relative humidity ranged between 45 - 55%. Temperature and relative humidity were monitored electronically using a data logger (HOBO 2K logger, Onset Computer Corporation, Bourne, USA). Based on soil analysis conducted prior to planting, the soil texture was clay loam with P (18 mg/L), K (188 mg/L), Ca (1300 mg/L), Zn (5 mg/L), Mn (73 mg/L), Cu (15.1 mg/L), pH (4.64), organic carbon (2.1%) and total N (0.19%). Nitrogen, phosphorus and potassium were applied in 160 kg ha^{-1}; 20 kg ha^{-1} and 0 kg ha $^{-1}$, respectively using urea (46%) and single superphosphate (10.2%) to meet crop nutritional requirement (Brady and Weil, 2008). The weeds were removed by hand, weekly.

Eight wheat genotypes (LM35, LM66, LM47, LM83, LM79, LM57, LM43 and LM98) were evaluated under two water regimes (well irrigated and water-stressed). An 8 × 2 × 3 factorial arrangement in a randomized complete block design (RCBD) was laid out with three replicates. Crops were subjected to water stress at the beginning of each growth stage (tillering, flowering and grain filling) by removing the drippers and relieved of water stress at the end of these growth stages by placing back the drippers.The beginning and final phase of each growth stage were determined using a high descriptive growth scale referred to as the Zadoks scale (Figure 1) (Zadoks et al. 1974; ARC-SGI 2014). The control was irrigated to field capacity. Soil moisture was monitored with a Time Domain Reflectometer probe (Campbell Scientific Inc. USA) in both water regimes.

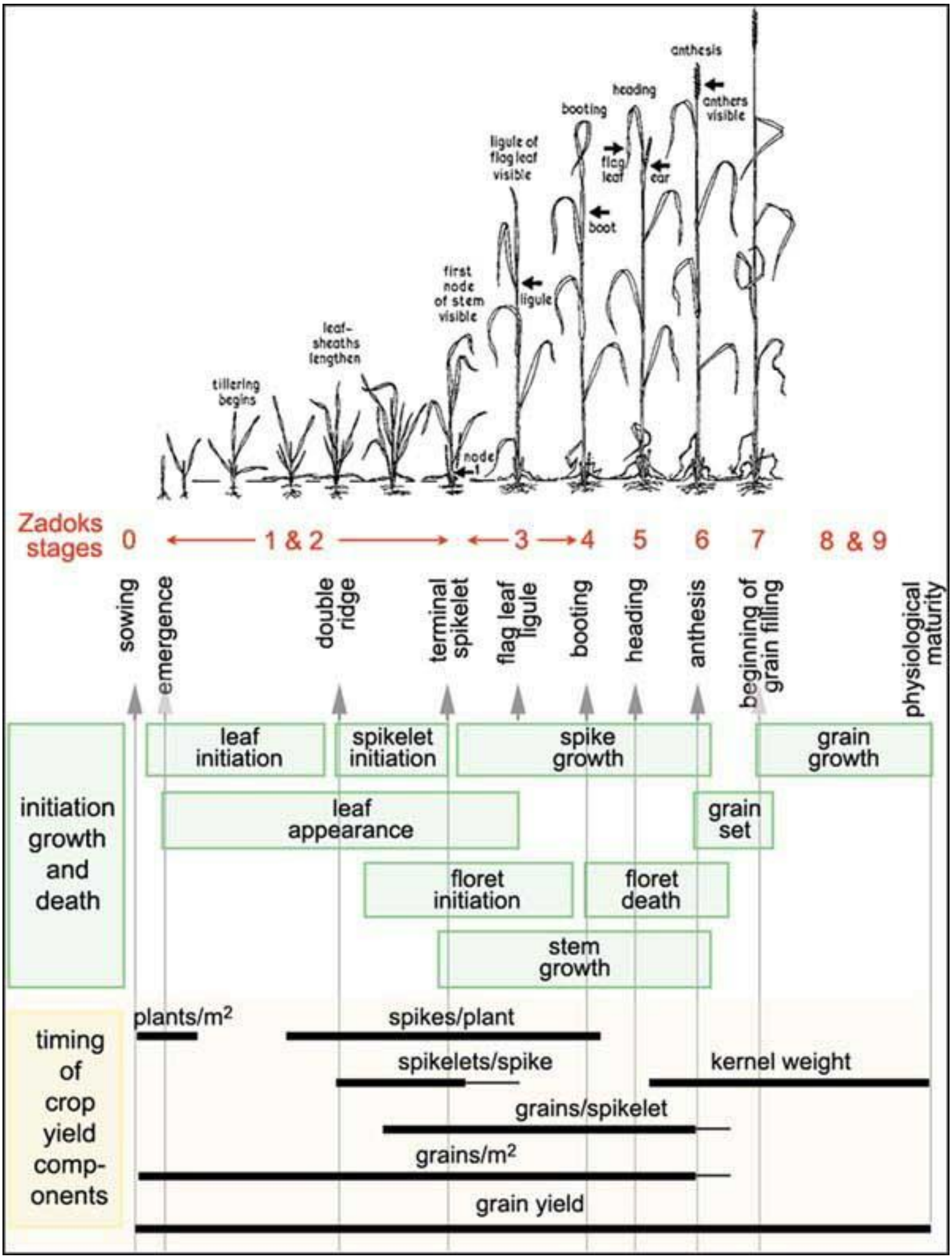

Figure 1: A guide to the identification of growth stages in grain crops (Zadoks et al. 1974; Rawson and Macpherson 2000).

3.3.2 Data collection

3.3.2.1 Leaf gas exchange traits

The stomatal conductance (g_s), the rate of photosynthesis (P_n), transpiration rate (T_r) and instantaneous water use efficiency (IWUE) were measured under full sunlight using the LI-6400 XT Portable Photosynthesis System (Licor Bioscience, Inc. Lincoln, Nebraska, USA) (Allahverdiyev et al. 2015). The flag leaf was placed on the sensor of the machine, this was done at the end of the stressing period in each growth stage for both stressed and well-watered genotypes.

3.3.2.2 Relative water content

The relative water content was measured in daytime using the flag leaf of each genotype in all growth stages (tillering, flowering, and grain filling) after water stress for the stressed and non-stressed genotypes. Three plants per genotype were selected randomly. The flag leaf was removed from each plant using secateurs. The leaves were placed in sealed plastic bags and transferred to the laboratory immediately to minimize the loss of leaf moisture. Fresh weights were measured within two hours after removal. The turgid weight was weighed after soaking the leaves in distilled water for 18 hours at room temperature below 20 °C and under low light conditions. The soaked leaves were then quickly and carefully blotted with tissue paper and the turgid weight was determined. Dry weight was measured after oven drying the leaf samples for 48 hours at 85 °C (Boutraa et al. 2010). The relative water content was calculated using Eq.1 (Boutraa et al. 2010).

$$\text{RWC (\%)} = \frac{fresh\ weight - dry\ weight(g)}{turgid\ weight - dry\ weight(g)} \times 100\% \qquad 1$$

3.3.2.3 Proline analysis

The proline was determined on the flag leaves of each genotype after the water stress period in each growth stage and replicate under both treatments in the three growth stages (Bates et al. 1973). The leaves were collected in daytime, placed in labeled zip lock bags and transferred to the laboratory. The samples were preserved in a freezer at -65 °C. After a week, the leaves were then removed from the freezer and freeze dried (lyophilisation; cryodesiccation) for 48 hours at -56 °C using Virtis Benchtop freeze dryer system (ES Model, SP Industies Inc., Warminster, USA) (Bates et al. 1973). The dried samples were ground to a powder using a mortar and pestle containing liquid nitrogen and 0.5 g of each sample was inserted in labeled test tubes (Bates et al. 1973). A 10 mL of 3% sulfosalicylic acid was prepared by mixing 30 g of sulfosalicylic acid powder with 1 L of water. The mixture was added to the 0.5 g ground leaf samples. The mixture of each sample was then homogenized for one minute using a stirrer (ULTRA-TUR-RAX, IKA® T25 digital, Staufen, Germany) to completely break down the cells in the ground leaf samples (Bates et al. 1973). The homogenized mixture was then filtered using glass wool. A 2 mL of the filtrate was reacted with 2 mL of acid ninhydrin and 2 mL of glacial acetic acid (Bates et al., 1973). The reaction mixture was then placed in an ultrasonic bath (Labotec, Model No. 132, Labotec (PTY) LTD, Johannesburg, South Africa) at 100 °C for one hour thereafter, 4 mL of toluene was added to each sample (Bates et al. 1973). The reaction mixture was then shaken vigorously for 15 - 20 seconds using Vortex mixer (Heidolph, Germany). Using toluene for blank, the absorbance was read at 520 nm in a UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The proline concentration was calculated using Eq. 2.

Proline content (µg per gram of dry leaf tissue)

$$= [(\mu g proline/ml) \times mltoluene)/115.5\mu g/\mu mole]/ [(gsample)/5] \qquad 2$$

3.3.3 Data analysis

The collected data was subjected the analysis of variance (ANOVA) using GenStat® 18th Edition (VSN International, Hemel Hempstead, UK). Mean separation was done using Tukey's test at 5% probability level. Correlation analysis of the measured traits was done with the principal component analysis (PCA) based on correlation matrix and biplots were plotted for both water stress and well-irrigated conditions.

3.4 Results and discussion

3.4.1 Rate of photosynthesis

The genotypes maintained similar ($p > 0.05$) rate of photosynthesis in all growth stages except for LM98 and LM43 stressed at tillering and grain filling stage, respectively (Fig. 2). This may imply that all the studied genotypes were tolerant to water stress in all growth stages but LM98 may be sensitive to water stress at tillering stage while on the other hand, LM43 may be sensitive to water stress at flowering stage. These results contradict those of Wang et al. (2016) who found that there was a significant decrease in the rate of photosynthesis when wheat genotypes (Xinong 979 and larger-spike wheat) are subjected to water stress at tillering, flowering and grain filling. However, they are supported by some of the results and conclusion made by these authors that the rate of photosynthesis is not affected by water stress if the genotypes' growth stage to which water stress is imposed is tolerant to water stress.

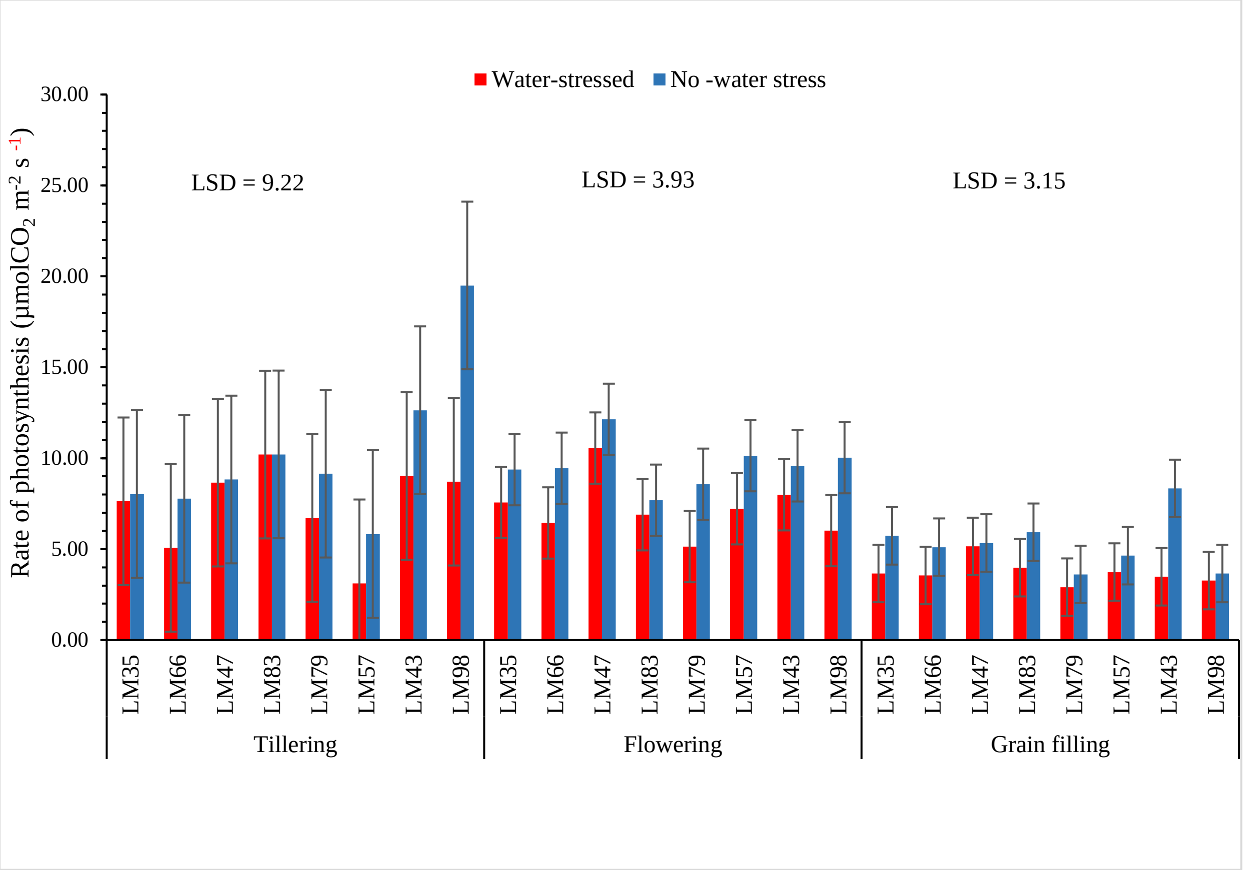

Figure 2: The rate of photosynthesis after water stress at different growth stages of wheat genotypes.

3.4.2 Transpiration rate and stomatal conductance

Water stress at tillering, flowering and grain filling did not have any significant effect *(p* > 0.05) on the stomatal conductance and transpiration rate (Fig. 3). These results suggest that the studied genotypes were tolerant to water stress at the three growth stages. Allehverdiyev et al. (2010) indicated that there is a strong relationship between the transpiration rate and stomatal conductance which explains the trend observed in this study (Fig. 3).

Genotypes that maintain a high stomatal conductance and transpiration rate after water stress at each growth stage are considered tolerant (Gomes et al. 2004; Lisar et al. 2012). Hence, it may be concluded in this study that the studied genotypes may withstand water stress at all growth stages of wheat development. The insignificant effect of water stress on the transpiration rate at all growth stages contradict those of Heinemann et al. (2011), who found that when water stress is imposed at either tillering, flowering or grain filling, the most effective response of the crops is a decline in the transpiration rate and stomatal conductance to reduce the rate of water loss. These results also contradict those of Lisar et al. (2012) and Boutraa et al. (2010) who discovered that the rate of transpiration is reduced when wheat genotypes (Al-gaimi, Sindy-1, Sindy-2, Hab-Ahmar) are exposed to limited water conditions at flowering, tillering or grain filling. However, they are in line with those of Damayanthi et al. (2010), who justified that genotypes (TRI 2025, DN, CY9, DG7, DG39, TRI 2024, TRI 2023 and TRI 2026) that maintain a high rate of transpiration and stomatal conductance after water stress at flowering, tillering and grain filling growth stage are water stress tolerant. The results of this study can be further explained by the fact that the studied genotypes were bred to be highly tolerant to water stress and the duration of the stress imposed in each growth stage may not have been severe enough to result in a significant effect.

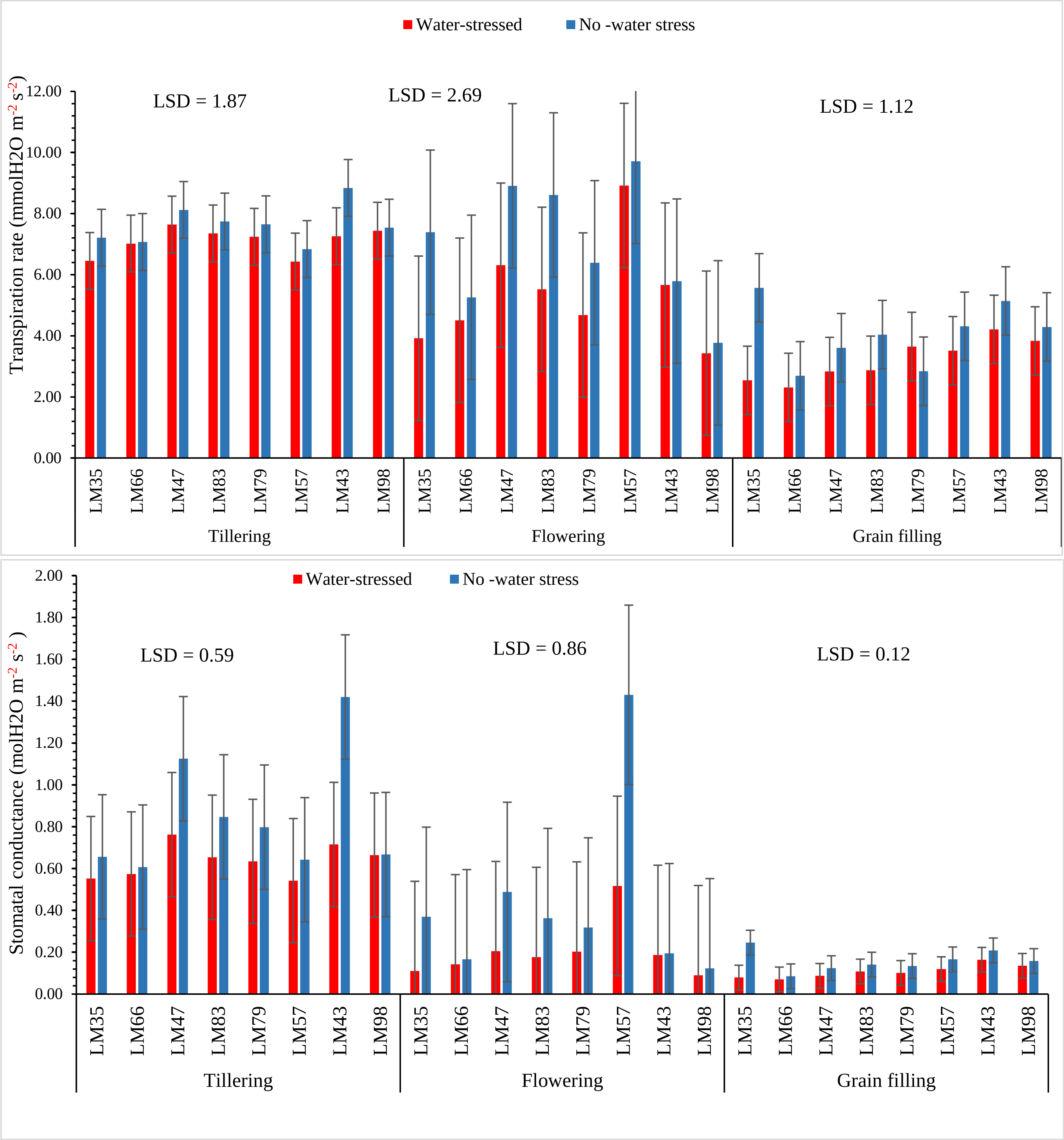

Figure 3: Effect of water stress at different growth stages on the stomatal conductance and transpiration rate of irrigated wheat genotypes.

3.4.3 Instantaneous water use efficiency

Higher instantaneous water use efficiency was recorded in the well-irrigated genotypes at all growth stages (Fig. 4). However, when water stress was imposed at tillering the instantaneous water use efficiency of genotype LM66, LM47, LM83, LM57 and LM98 were reduced ($p < 0.05$) by 71%, 33%, 34%, 46% and 57% (Fig. 4). Water stress at flowering stage only influenced ($p < 0.05$) genotype LM35 and LM43 with 71% and 74% less instantaneous water use efficiency correspondingly (Fig. 4). Whereas, after water stress at grain filling a reduction ($p < 0.05$) in instantaneous water use efficiency was evident in genotype LM35 (58% reduction in instantaneous water use efficiency), LM79 (55% reduction in instantaneous water use efficiency), LM57 (45% reduction in instantaneous water use efficiency) and LM98 (57% reduction in instantaneous water use efficiency) (Fig.4). The rest of the genotypes subjected to water stress at these growth stages showed tolerance to water stress. These results were in line with the findings of Boutraa et al. (2010) and Li et al. (2017) who concluded that the instantaneous water use efficiency was affected by water stress at different growth stages depending on the susceptibility of the genotype and the duration of the stress period. It can, therefore, be concluded that withholding irrigation at any growth stages should only be implemented if the genotypes that showed no effect to water stress in each growth stage are planted.

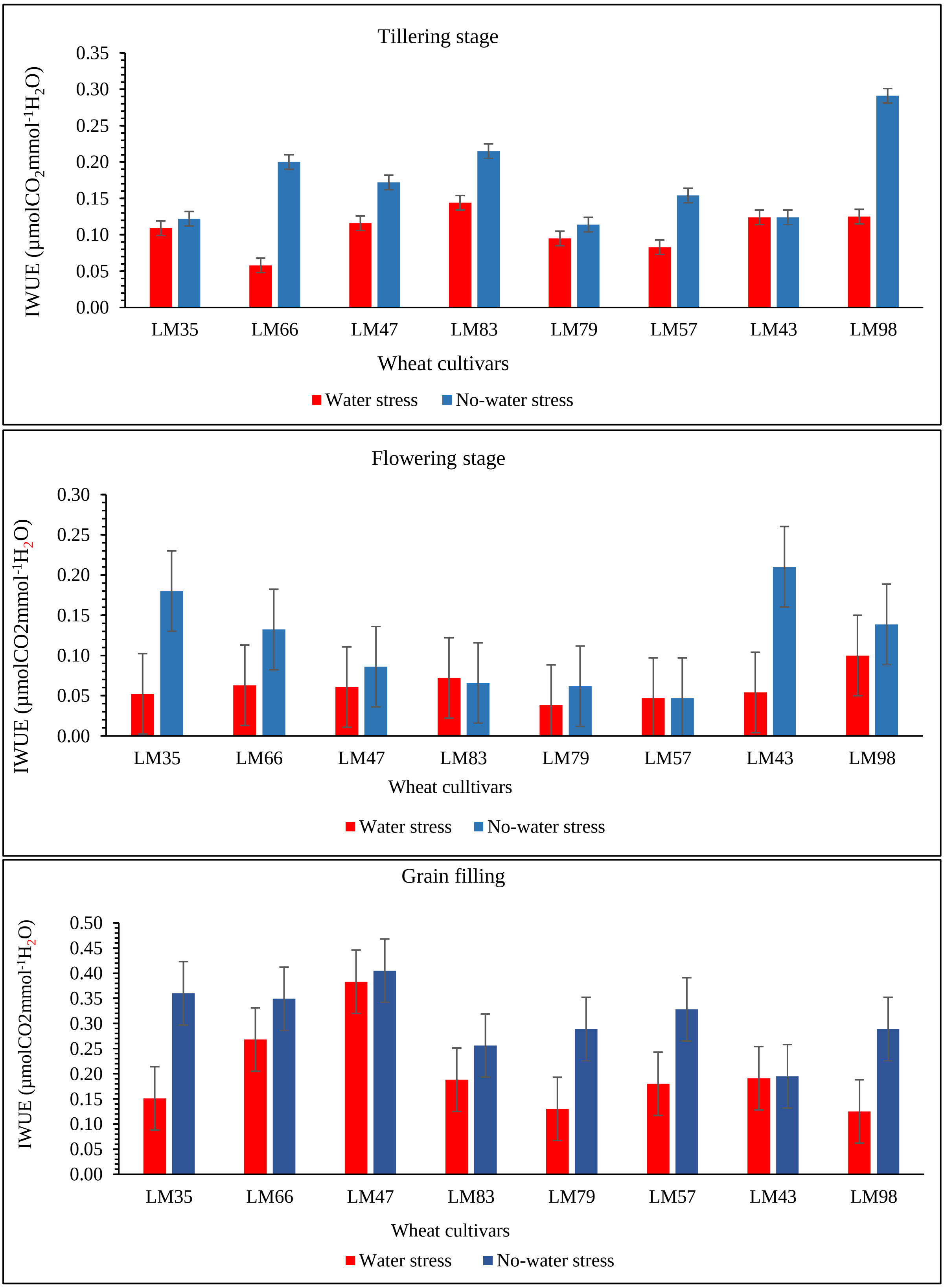

Figure 4: Instantaneous water use efficiency of irrigated wheat genotypes after water stress at different growth stages. [*LSD= 0.11 (tillering), LSD= 0.10 (flowering) and LSD= 0.13 (grain filling)*].

3.4.4 Relative water content

A high relative water content was evident in the genotypes subjected to full irrigation whereas when water stress was imposed the relative water content of the genotypes subjected to water stress at grain filling was not affected ($p > 0.05$) (Fig.5). Genotype LM35 (18% lower relative water content) and LM47 (13% lower relative water content) were the only ones affected by water stress at tillering stage while the rest of the genotypes subjected to water stress at this growth stage were tolerant (Fig. 5). The lowest relative water content obtained after water stress at the flowering stage was from genotype LM98 with a 46% reduction in the relative water content followed by LM79 with 42% lower relative water content, LM83 (35% lower relative water content and LM57 (31% lower relative water content). These findings contradict those of Khakwani et al. (2012) and Keyvan (2010) who discovered that wheat genotypes (Damani, Hashim-8, Gomal-8, DN-73, Zam-04, Dera-98, Chamran, Marvdasht, and Shahriar) were all affected when water stress was experienced at flowering, tillering or grain filling. However, they are supported by the some of the findings and discussion made by these authors that genotypes that retain a high relative water content after water stress at each growth stage are tolerant.

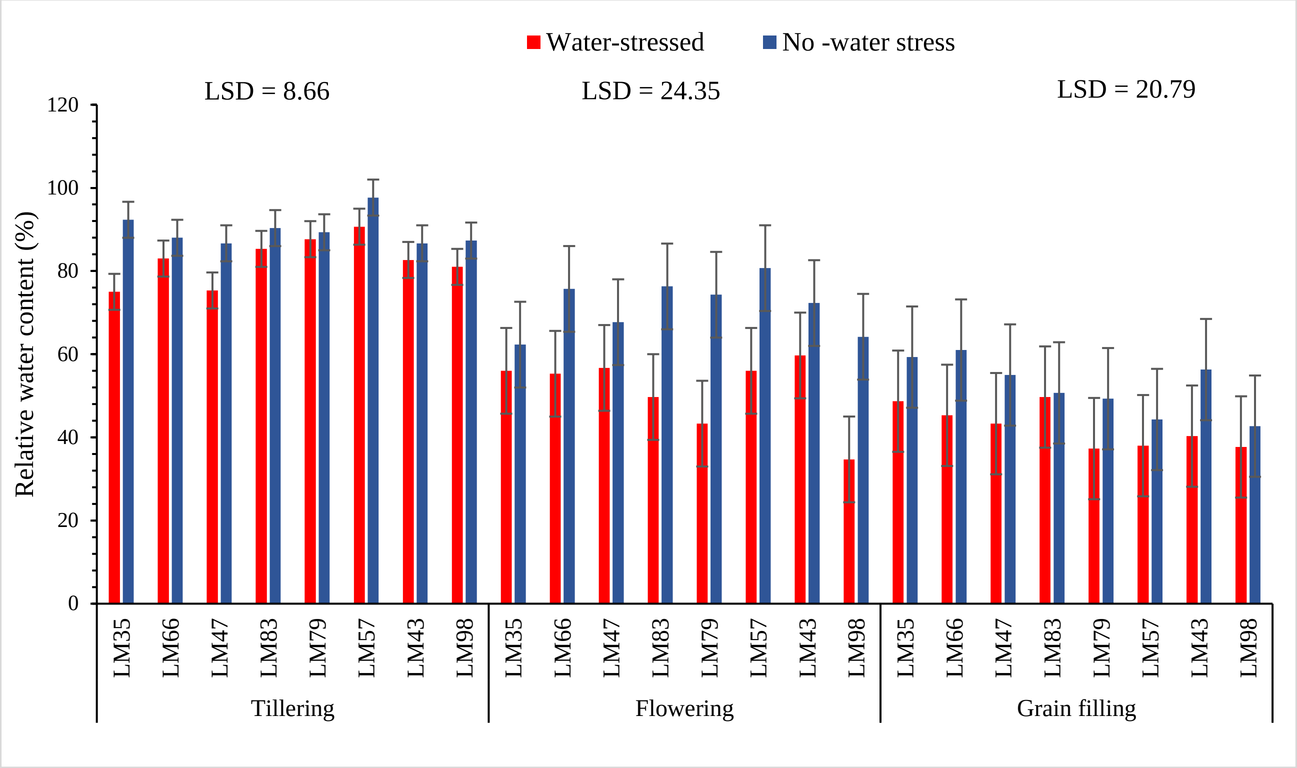

Figure 5: Influence of water stress imposed at three growth stages on the relative water content of wheat genotypes.

3.4.5 Proline content

Water stress imposed at the three growth stages did not cause a significant ($p > 0.05$) accumulation of the proline content (Fig. 6). However, the stressed treatment showed a trend towards higher levels. This may suggest that the studied genotypes were not severely affected by water stress which might have led in a non-significant increase in the proline content. These findings differ from those reported by Man et al. (2011), Maralian et al. (2010), Mwandzingeni et al. 2016) who found that proline content accumulates significantly in crops under water stress. Whereas, they are supported by the fact that proline content only shows a significant accumulation if the crops under water stress are being severely affected (Sultan et al. 2012; Boudjabi et al. 2015; McMichael and Elmore 1997). Hence, the reaction of the proline content could be because the duration of the stress imposed in the studied genotypes at the three growth stages might not have been long enough to cause a significant accumulation in the proline content.

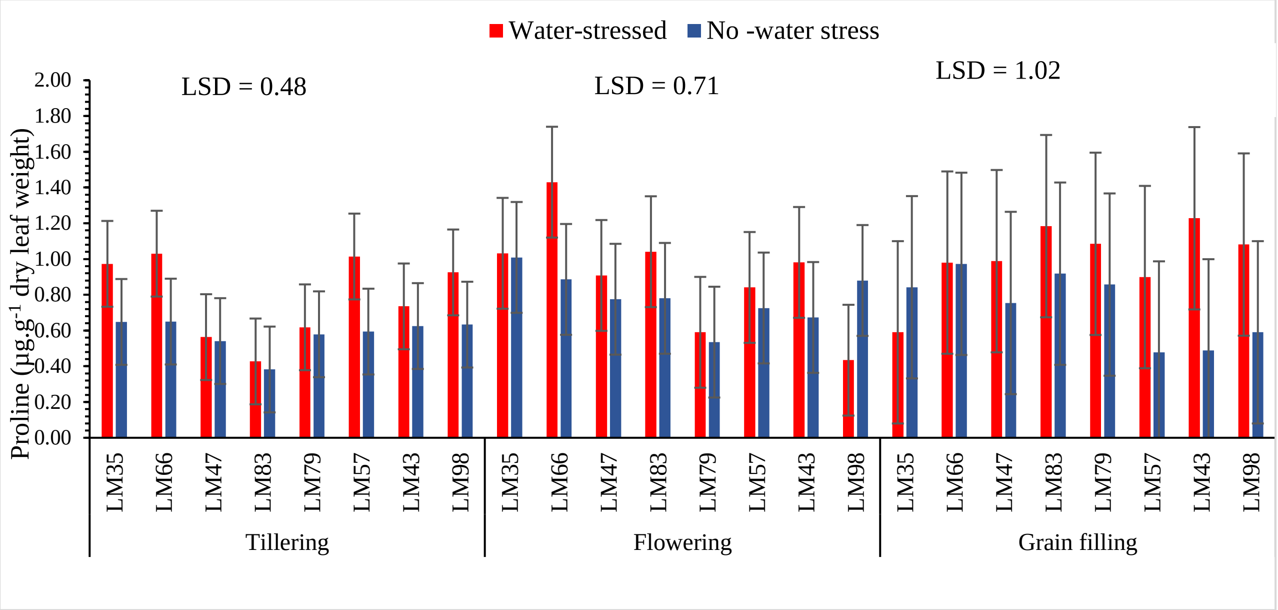

Figure 6: Proline accumulation after water stress at different growth stages of wheat development.

3.4.6 Correlation analysis

Results of the current study show that there were strong positive correlations between proline content, transpiration rate, stomatal conductance and instantaneous water use efficiency (Fig. 7). However, these were negatively correlated with the rate of photosynthesis. The negative correlation in this study between photosynthesis and stomatal conductance is supported by Siddique et al. (1999) who explained that the reduction in the rate of photosynthesis under water stress may not be regulated by the stomatal conductance but rather by non-stomatal factors. However, they contradict those of Wang et al. (2016) who found a correlation between the rate of photosynthesis and stomatal conductance. Hidayati et al. (2016) also discovered that the rate of photosynthesis was not always correlated with transpiration rate and justified his findings with an explanation by Maxwell and Johnson (2000) who indicated that the rate of photosynthesis is mostly influenced by the energy received from light that is absorbed by the chlorophyll and may not be associated with the transpiration rate or stomatal conductance. The positive correlation between the rate of transpiration and stomatal conductance may be due to the reaction of these traits to water stress at tillering, flowering and grain filling which indicated no significant *(p* < 0.05) effect of water stress to these traits. (Fig. 3, 7). This was also the case for the proline content which did not accumulate in any of the growth stages after water, hence signaling the less severe effect water stress had on the measured traits (Fig.7).

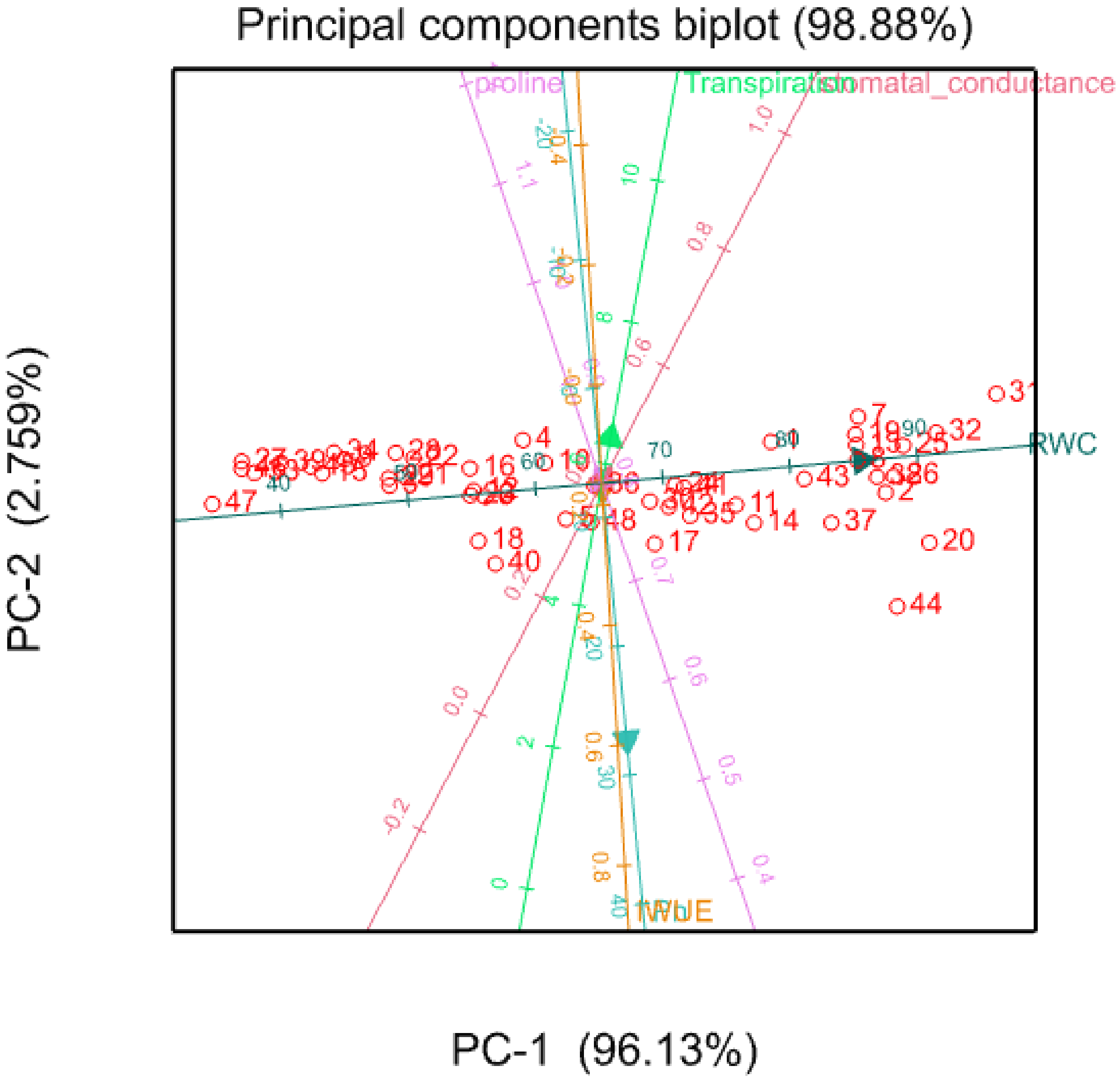

Figure 7: Biplot graphical display of the measured traits in wheat genotypes under both water stress and full irrigation conditions at tillering, flowering and grain filling. *IWUE(▲), Instantaneous Water Use Efficiency; RWC (▲), Relative Water Content; Pn (▲), Rate of photosynthesis*

3.5 Conclusion

Due to studies that demonstrate that despite the reaction of other traits such as the rate of photosynthesis, stomatal conductance, transpiration rate, and relative water content; genotypes with a higher water use efficiency are best suited to be planted in environments with limited water. It can, therefore, be suggested from the studied genotypes that LM35, LM79, LM57 and LM98 are sensitive to water stress at grain filling. But for irrigation that is to be withheld at tillering, LM43 and LM35 are the only ones suitable for planting whereas they are the only ones' sensitive to water stress at flowering stage.

References

Akram M. 2011. Growth and yield components of wheat under water stress of different growth stages. *Bangladesh Journal of Agricultural Research* 36: 455-468.

Allahverdiyev TI, Talai JM, Huseynova IM, Aliyev JA. 2015. Effect of drought stress on some physiological parameters, yield, yield components of durum (*Triticum durum* desf.) and bread (*Triticum aestivum* L.) wheat genotypes. E*kin Journal of Crop Breeding and Genetics* 1: 50-62.

ARC-SGI (Agricultural Research Council - Small Grain Institute). 2014. *Guidelines for production of small grain in the winter rainfall areas*. Bethlehem. Free State. South Africa.

Ashraf MY, Khan AH. 1993. Effect of drought stress on wheat plant in response in early stage. *Pakistan Journal of Agricultural Resource* 36: 455-468.

Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil* 39: 205-207. https://doi.org/10.1007/BF00018060.

Blum A. 2005. Drought resistance, water-use efficiency, and yield potential: are they compatible, dissonant or mutually exclusive? *Australian Journal of Agricultural Research* 56: 1159-1168. https://doi.org/10.1071/AR05069.

Boudjabi S, Kribaa M, Chenchouni H. 2015. Growth, physiology and yield of durum wheat (*Triticum Durum*) treated with sewage sludge under water stress conditions. *EXCLI Journal* 14: 320-334. https://doi.org/10.17179/excli2014-715.

Boutraa T, Akhkha A, Al-shoaibi AA, Alhejeli AM. 2010. Effect of water stress on growth and water use efficiency (WUE) of some wheat cultivars (*Triticum durum*) grown in Saudi Arabi. *Journal of Taibah University for Science* 3: 39-48. https://doi.org/10.1016/S1658-3655(12)60019-3.

Brady NC, Weil RR. 2008. The nature and properties of soils (13th edition).Prentice Hall: New Jeesey.

Damayanthi MMN, Mohotti AJ, Nissanka SP. 2010. Comparison of tolerant ability of mature field grown tea (*Camellia sinesis* L.) cultivars exposed to a drought stress in Passara area. *Tropical Agricultural Research* 1: 66-75. http://doi.org/10.4038/tar.v22i1.2671.

Daryanto S, Wang L, Jacinthe PA. 2016. Global synthesis of drought effects on maize and wheat production. *PLoS ONE* 11: 1-15. https://doi.org/10.1371/journal.pone.0156362.

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA. 2009. Plant drought stress: effects, mechanism and management. *Agronomy for Sustainable Development* 29: 185-212. https://doi.org/10.1051/agro:2008021.

Fourie P, Botha P. 2011. *The role of irrigation in grain production*. Grain SA, Pretoria, South Africa.

Gomes MMA, Lagôa AMMA, Medina CL, Machado EC, Machado MA. 2004. Interactions between leaf water potential, stomatal conductance and absicisic acid contetnt oforange tress submitted to drought stress. *Brazilian Journal of Plant Physiology* 16: 155-16. http://dx.doi.org/10.1590/S1677-04202004000300005.

Hafid RE, Smith DH, Karrou M, Samir K. 1998. Physiological responses of spring durum wheat cultivars to early season drought in a Mediterranean environment. *Annals of Botany* 841: 363-370. https://doi.org/10.1006/anbo.1997.0567.

Hanin M, Brini F, Ebel C, Toda Y, Takeda S, Masmoudi K. 2011. Plant dehydrins and stress tolerance: Versatile proteins for complex mechanisms. *Plant Signaling & Behavior* 6: 1503-1509. https://doi.org/10.4161/psb.6.10.17088.

Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A. 2012. Role of proline under changing environments. *Plant Signaling and Behavior* 7: 1456-1466. http://dx.doi.org/10.4161/psb.21949.

Heinemann AB, Stone LF, Fageria NK. 2011.Transpiration rate response to water deficit during vegetative and reproductive phases of upland rice cultivars. *Scientia Agricola* 68: 24-30. http://dx.doi.org/10.1590/S0103-90162011000100004.

Hidayati N, Triadiati, Anas I. 2016.Photosynthesis and transpiration rates of rice cultivated under the system of rice intensification and the effects on growth and yield. *HAYAT Journal of Biosciences* 23: 67-72.

Keyvan S. 2010. The effects of drought stress on yield, relative water content, proline, soluble carbohydrates and chlorophyll of bread wheat cultivars. *Journal of Animal and Plant Sciences* 8: 1051-1060.

Khakwani AZ, Dennet MD, Munir M, Abid M. 2012. Growth and yield response of wheat varieties to water stress at booting and anthesis stages of development. *Pakistan Journal of Botany* 44: 879-886.

Li Y, Li H, Li YU, Zhang S. 2017. Improving water use efficiency by decreasing stomatal conductance and transpiration rate to maintain a higher ear photosynthetic rate in drought resistance wheat. *The Crop Journal* 5: 231-239. https://doi.org/10.1016/j.cj.2017.01.001.

Lisar SYS, Motafakkerazad R, Hossain MM, Rahman IMM. 2012.Water Stress in Plants: Causes, Effects and Responses, Water Stress, Prof. Ismail Md. Mofizur Rahman (Ed.). ISBN: 978-953-307-963-9. https://doi.org/10.5772/39363.

Man D, Bao YZ, Han LB. 2011. Drought tolerance associated with proline and hormone metabolism in two tall fescue cultivars. *HortScience* 46: 1027-1032.

Mancosu N, Snyder RL, Kyriakakis G, Spano D. 2015. Water scarcity and future challenges for food production. *Water* 7: 975-992. 10.3390/w7030975.

Maralian H, Ebadi A, Didar TR, Haji-Eghrari B. 2010. Influence of water deficit stress on wheat grain yield and proline accumulation rate. African Journal of Agricultural Research 5: 286-289.

Maxwell K, Johnson GN. 2000. Chlorophyll fluorescence-a practical guide. Journal of Experimental Botany 51: 659-668. https://doi.org/10.1093/jexbot/51.345.659.

McMichael BL, Elmore CD. 1977. Proline accumulation in water stressed cotton leaves. *Crop Science* 17:905-908. https://doi.org/10.2135/cropsci1977.0011183X0017000600x.

Mwadzingeni L, Shimelis H, Tesfay S, Tsilo T. 2016. Screening of bread wheat genotypes for drought tolerance using phenotypic and proline analysis. Frontiers in Plant Science 7: 1276. https://doi.org/10.3389/pls.2016.01276.

Nayyar H, Walia D. 2003. Water stress induced proline accumulation in contrasting wheat genotypes as affected calcium and abscisic acid. *Biologia Plantarum* 46: 275-279. https://doi.org/10.1023/A:1022867030790.

Rawson HM, Macpherson HG. 2000. Irrigated wheat: Section 2: Describing the growth of your crop as stages. During which stages is yield determined? Food and Agriculture

Organization of the United Nations. Rome. http://www.fao.org/docrep/006/X8234E/x8234e05.htm. (Accessed April 2017).

Saeidi M, Ardalani S, Honarmand SJ, Ghobadi ME, Abdoli M. 2015. Evaluation of drought stress at vegetative growth stage on the grain yield formation and some physiological traits as well as fluorescence parameters of different bread wheat cultivars. *Acta Biologica Szegediensis* 59: 35-44.

Siddique MRB, Hamid A, Islam MS. 1999. Drought stress effects on photosynthetic rate and leaf gas exchange of wheat. *Botanical Bulletin- Academia Sinica Taipei* 40: 141-145.

Sokoto MB, Singh A. 2013. Yield and yield components of bread wheat as influenced by water stress, sowing date and cultivar in Sokoto, Sudan Savanna, Nigeria. *American Journal of Plant Sciences* 4: 122-130.

Sultan MARF, Hui L, Yang LJ, Xian ZH. 2012. Assessment of drought tolerance of some *Triticum* L. species through physiological indices. *Czech Journal of Genetics and Plant Breeding* 48: 178-184.

Tabassam MAR, Hussain M, Sami A, Shabbir I, Bhutta MAN, Mubusher M, Ahmad S. 2014. Impact of drought on growth and yield of wheat. *Scientia Agriculturae* 7: 11-18. https://doi.org/10.15192/PSCP.SA.2014.3.1.1118.

Tekle AT, Alemu MA. 2016. Drought tolerance mechanisms in field crops. *World Journal of Biology and Medical Sciences* 23: 15-39

Turton A, Du Plessis A, Walker J, Swanepoel S. 2016. Running on empty: what business, government and citizens must do to front South Africa's water crisis. Water interrupted campaign. South Africa. http://actionaid.org/sites/files/actionaid/running_on_empty_-_water_efficiency_report.pdf. (Accessed November 2016).

van Heerden PDR, de Villiers OT. 1996. Evaluation of proline accumulation as an indicator of drought tolerance in spring wheat cultivars. *South African Journal of Plant and Soil* 13: 17-21. http://dx.doi.org/10.1080/02571862.1996.10634368.

Wang X, Wang L, Shangguan Z. 2016. Leaf gas exchange and fluorescence of two winter wheat varieties in response to drought stress and nitrogen supply. *PLOS One* 11: 1-15. https://doi.org/10.1371/journal.pone.0165733.

WRC (Water Research Commission). 2015. Water restrictions. *Drought: your gateway to managing water scarcity in times of drought*. Water Research Commission. Pretoria. South Africa.

WRC (Water Research Commission). 2016. Water restrictions. *Drought: your gateway to managing water scarcity in times of drought*. Water Research Commission. Pretoria. South Africa.

Zadoks JC, Chang TT, Konzak CF. 1974. A decimal code for the growth stages of cereals. *Weed Research* 14: 415-421. https://doi.org/10.1111./j.1365-1380.1974.tb01084.x.

CHAPTER 4

Growth, morphological and yield responses of irrigated wheat (*Triticum aestivum* L.) genotypes to imposed water stress at different growth stages

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4.1 Abstract

Water shortage is one of the major constraints that have resulted in the decline of wheat production in South Africa. Therefore, to save water while improving yield, it is important to assist irrigated wheat farmers to identify water stress tolerant growth stages in irrigated wheat genotypes. This study evaluated newly introduced water stress tolerant wheat genotypes for water stress tolerance at different stages of crop growth and development namely tillering, flowering and grain filling. An 8 (genotypes) × 2 (water treatments) × 3 (growth stages) factorial experiment was laid out in a randomized complete block design with three replicates. The results indicated that plant height was not affected ($p > 0.05$) by water stress at tillering and grain filling. Water stress imposed at tillering stage reduced the number of fertile tillers ($p < 0.05$) in susceptible genotypes while at flowering and grain filling stage all genotypes were tolerant ($p > 0.05$). The spike length was affected by water stress in all growth stages ($p < 0.05$). Aboveground biomass was only affected ($p < 0.05$) by water stress imposed at tillering stage and the harvest index was not affected ($p > 0.05$) at all growth stages. Water stress reduced grain yield on the genotypes where stress was imposed at tillering stage ($p < 0.05$) whereas when stress was imposed at flowering and grain filling the grain yield was not reduced ($p > 0.05$). This was linked to the number of fertile tillers after water stress at each growth stage. This study provided sufficient evidence that suggests that most genotypes at flowering and grain filling stage are tolerant to water stress, while, the tillering stage is susceptible. Genotype performance after water stress was imposed at tillering stage indicated that LM83 is tolerant to water stress while LM47, LM79 and LM66 are most vulnerable.

Keywords: Wheat, water stress, tillering, flowering, grain filling, wheat tolerant genotypes.

4.2 Introduction

Water is a scarce resource in South Africa. However, the country still has the potential to increase its wheat production while minimizing water use (Muller et al., 2009; Negassa et al., 2013; Heyns and Malan, 2015). This can be achieved through the implementation of alternative strategies and research priorities that consider the country's changing climatic conditions and working towards saving available water (Negassa et al., 2013). Wheat is one of the major crops produced in South Africa, but its production is threatened by the frequent occurrence of drought period in the country (Macauley, 2015; Daryanto et al., 2016). Water shortage has also threatened the production of irrigated wheat which used to boost the total wheat production of the country during dry periods (Fourie and Botha, 2011; Dube et al., 2016).The Water Research Commission of South Africa (2015, 2016), has reported water use restrictions of up to 60% in some provinces because of the severe drought. Hence, for crop production, yield improvements and effective water conservation strategies, identification of water stress tolerant growth stages is one of the best alternative measures. This will allow for the innovative use of water by saving water at tolerant growth stages while maximizing wheat production (Evans and Sadler, 2008).

Plant height, spike length, the number of fertile tillers, biomass production, grain yield, and harvest index have been reported in many research studies to be affected by water stress (Qadir et al., 1999; Taheri et al., 2011; Valizadeh et al., 2014). These traits have been shown that they are reduced by water stress than when wheat is grown under full irrigation (Sharma, 1993; Boutraa et al., 2010; Ruttanaprasert et al., 2016). However, the response of these traits to water stress varies depending on the genotype, duration of water stress period and the sensitivity of the growth stage (Nezhadahmadi et al., 2013). Therefore, a better understanding of the physiological response of these traits to water stress imposed at different growth stages can aid in the identification of adaptable, water conserving growth stages for irrigated wheat. It is essential that the available water is used economically and efficiently while at the same time not adversely affect wheat production. As part of the global effort, the International Maize and Wheat Improvement Center (CIMMYT) has bred new genotypes which are reported to be highly tolerant to very hot and dry conditions hence requiring less water. However, the survival of these genotypes against water stress at different growth stages still needs to be assessed. Due to the water crisis in South Africa, the severe effect of water stress on wheat yields and the limitation of water use across the country, it is imperative to assist irrigated wheat farmers to conserve water by identifying water stress tolerant growth stages in wheat genotypes. Hence, the objective of this study was to determine the growth stage at which limited water supply would have minimal effect on the growth, development and yield of eight newly developed wheat genotypes.

4.3 Material and methods

4.3.1 Plant materials

Seeds of studied wheat genotypes (Table 1) were obtained and developed at the CIMMTY’s heat and drought nursery. The genotypes were all bred to tolerate very hot and dry conditions.

Table 1. Pedigree/name of genotype planted with their entry code

Pedigree/Name	Entry code
WBLL1//UP2338*2/VIVITSI	LM35
ROLF07*2/6/PVN//CAR422/ANA/5/BOW/CROW//BUC/PVN/3/YR/4/TRAP#1	LM66
FRET2/KUKUNA//FRET2/3/YANAC/4/FRET2/KIRITATI	LM47
PBW343*2/KUKUNA//SRTU/3/PBW343*2/KHVAKI	LM83
Local check	LM79
CROC_1/AE.SQUARROSA (205) //BORL95/3/KENNEDY	LM57
PASTOR/3/VEE#5//DOVE/BUC	LM43
KABY//2*ALUBUC/BAYA	LM98

4.3.2 Site description

This study was conducted in the tunnel at the University of KwaZulu-Natal, Pietermaritzburg, South Africa (29˚ 37' S, 30˚ 24' E). The tunnels day and night temperature were 30° C and 18° C respectively while the relative humidity ranged between 45 – 55%. Temperature and relative humidity were monitored electronically using a HOBO 2K logger (Onset Computer Corporation, Bourne, USA).

4.3.3 Experimental design

The experiment was designed as 8 × 2 × 3 factorial experiment carried out in a randomized complete block design (RCBD) with three replicates. The three factors were; factor A): genotypes on 8 levels, factor B): water treatment on 2 levels and factor C): growth stages on 3 levels. The two levels of water treatment were T_1 - stressed and T_2 - no - stress (control). The three levels of growth stages were tillering, flowering and grain filling and the 8 levels of genotypes are those listed in Table 1. The whole experiment consisted of 144 experimental units. Water was applied using drip irrigation system.

Soil moisture was monitored daily with a Time Domain Reflectometer (Campbell Scientific Inc., USA). The control was irrigated to field capacity and stress treatment was imposed by withholding water at each growth stage (tillering, flowering and grain filling). The Zadoks scale was used as a guide throughout this experiment to accurately determine the beginning and end of each growth stage (Zadoks et al., 1974; ARC-SGI, 2014). The Zadoks scale is a highly descriptive growth scale that is widely used for studies in grain crops (Zadoks et al., 1974). It is mostly recommended due to its simple and effective layout that assist in determining the growth stages of wheat.

4.3.4 Agronomic practices

Planting was done in July 2015 and the soil used in this study was collected at Ukulinga Research Farm, of the University of KwaZulu-Natal, Pietermaritzburg, South Africa (29˚ 40' S, 30˚ 24' E). Prior to planting soil samples were analyzed for soil fertility and soil texture which was classified as clay loam (USDA taxonomic system) with the chemical properties displayed in Table 2. Based on fertility test, 160 kg ha^{-1} N; 20 kg ha^{-1} P and 0 kg ha $^{-1}$ K were applied at planting using urea (46%) and single superphosphate (10.2%) to meet crop nutritional requirement. Weeds were removed by hand every week.

Table 2. Chemical properties of the soil collected at Ukulinga Farm

P	**K**	**Ca**	**Mg**	**Zn**	**Mn**	**Cu**	**pH**	**Org. C**	**Total N.**
mg/L							Water %		
18	188	1300	314	5	73	15.1	4.64	2.1	0.19

4.3.5 Data collection

Plant height, the number of fertile tillers and spike length were determined during the day at each growth stage after the water stress period was imposed. Three plants of each genotype were randomly selected for measurement and a measuring tape was placed from the base of the plant up to the end of the spike to measure plant height. The number of fertile tillers was counted in each genotype under each treatment and growth stage (Boutraa et al., 2010; Grenzdorffer, 2014). The spike length was measured at maturity for all growth stages and under both treatments with a ruler. Grain yield was determined by harvesting spikes from all genotypes, in all growth stages, treatments and replicate and placed in brown paper bags. The thrashing was done by hand and the grains were placed back in bags for weighing.

The grain yield was weighed and reported in t ha^{-1}. Aboveground biomass was determined after removing the spikes, the whole plant excluding the roots was harvested in each genotype for all growth stages under both treatments. The samples were then placed in brown paper bags and transferred to the laboratory. The samples were oven dried at 70 $^{\circ}$C for 48 hours to remove moisture and the aboveground biomass was weighed and reported in t ha^{-1} (Brisson et al. 2001). The harvest index (HI) is the ratio of grain yield to total aboveground biomass and it indicates the ability of the crop to allocate biomass (Asseng et al., 2001; Wnuk et al., 2013). The HI was determined using Eq. 1.

$$HI(\%) = \frac{GY\ (t\ ha^{-1})}{Biomass\ (t\ ha^{-1})} \times 100\% \qquad 1$$

Where: *HI* = Harvest index, *GY* = Grain yield.

4.3.6 Statistical analysis

The data from this study was subjected to the analysis of variance (ANOVA) using GenStat® 18th Edition (VSN International, Hemel Hempstead, UK). The means were separated with the Tukey's test at 5% probability level. Correlation analysis of the measured traits was done with the principal component analysis (PCA) based on correlation matrix and biplots were plotted for both water stress and well-irrigated conditions.

4.4 Results and discussion

4.4.1 Plant height

Plant height was not affected ($p > 0.05$) by water stress at tillering and flowering stage but the effect was significant ($p < 0.05$) when water stress was imposed at grain filling stage (Fig. 1). These results suggest that the grain filling stage is the most water stress sensitive growth stage in the studied genotypes while the tillering and flowering growth stages are tolerant to water stress.

Genotype performance after water stress was imposed at grain filling indicated that only genotype LM66 (90.67cm plant height after water stress), LM83 (87.33 cm plant height after water stress) and LM79 (88.67 cm plant height after water stress) were susceptible to water stress with 11%, 12% and 11% reduction in plant height respectively (Fig. 1). The rest of the genotypes were not affected by water stress imposed at grain filling (Fig. 1). The overall findings suggest that plant height reduced by water stress maybe due to the difference in the genetic character of the different genotypes which determines the sensitivity of the growth stages to water stress and in turn influences plant growth.

These results contradict those of Khakwani et al. (2012) and Maqbool et al. (2015) who found that plant height is mostly affected when water stress is imposed at flowering stage. But, they are explained by the conclusion made by these authors that plant height is only reduced when water stress is imposed at water stress sensitive growth stages while the is no effect in plant height when water stress is imposed at tolerant growth stages. Sarvestani et al. (2008) who agree with the results of this study indicated that the plant height was significantly affected by water stress at grain filling stage. However, these authors also discovered that plant height is affected by water stress in wheat genotypes with a water stress sensitive flowering growth stage. This, therefore, may imply that in the studied genotypes the flowering and tillering growth stage are water stress tolerant while the grain filling stage is a sensitive growth stage to water stress.

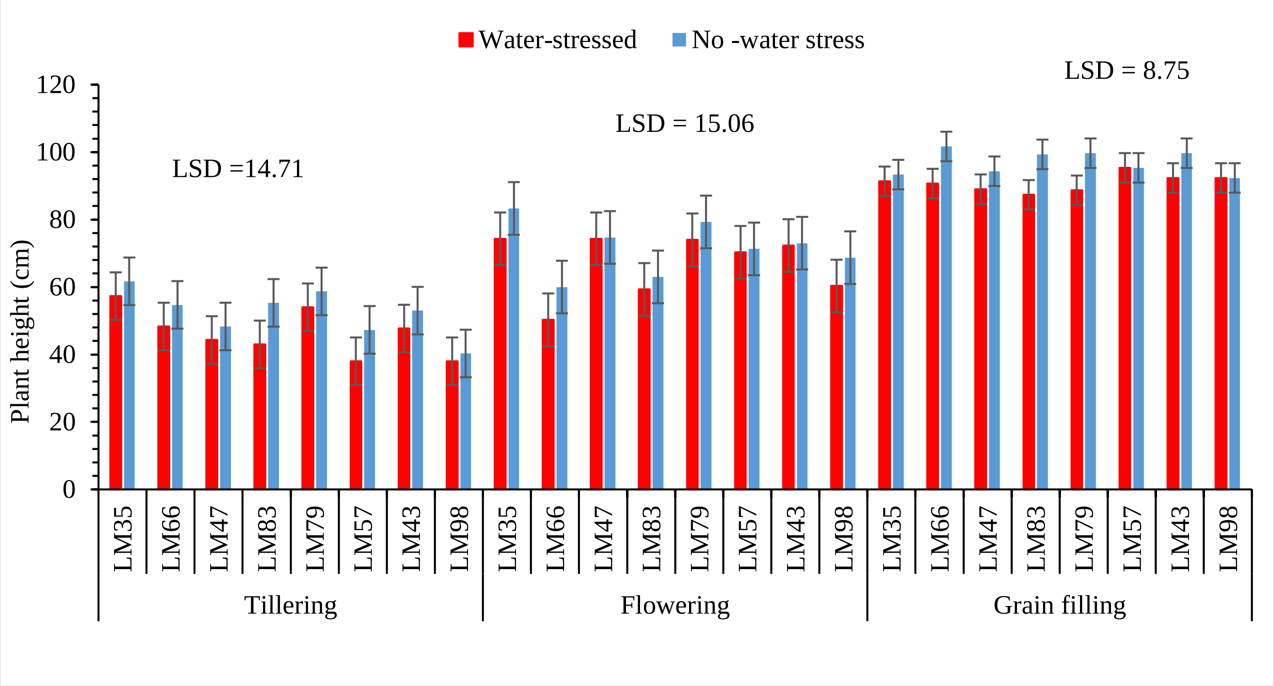

Figure 1. Effect of water stress imposed at different growth stages on the plant height of wheat genotypes.

4.4.2. Number of fertile tillers

A higher number of fertile tillers were recorded in none stressed treatment (Fig. 2). While, when the stress was induced, the number of fertile tillers were found to have been reduced after water stress at tillering stage for genotype LM79 (1.67 fertile tillers after water stress), LM66 (2.00 fertile tillers after water stress), LM43 (1.67 fertile tillers after water stress), LM47 (2.33 fertile tillers after water stress), LM57 (2.33 fertile tillers after water stress) and LM35 (2.33 fertile tillers after water stress) (Fig. 2). The number fertile tillers of these genotypes were reduced by 61%, 60%, 58%, 53%, 46% and 42%, respectively. However, water stress induced at flowering and grain filling had no effect ($p > 0.05$) on the number of fertile tillers (Fig. 2).

Genotypes with better-adapted growth stages are represented by a higher number of fertile tillers after water stress at each growth stage whereas few fertile tillers denote susceptibility (Akram, 2011) (Fig.2). These results are in line with those of Maqbool et al. (2015) who found that water stress imposed at tillering stage reduces the number of fertile tillers in wheat genotypes (Faisalabad-2008, Lasani-2008 and Kohistan-97). However, the findings of this study contradict some of the findings by these authors as water stress at flowering and grain filling stage were found to have no effect on the number of fertile tillers. Whereas, Maqbool et al. (2015) indicated that water stress at flowering and grain filling reduced the number of fertile tillers. This suggests that the studied genotypes have a flowering and grain filling growth stage that can allow the crop to resume normal plant functioning though subjected to water stress.

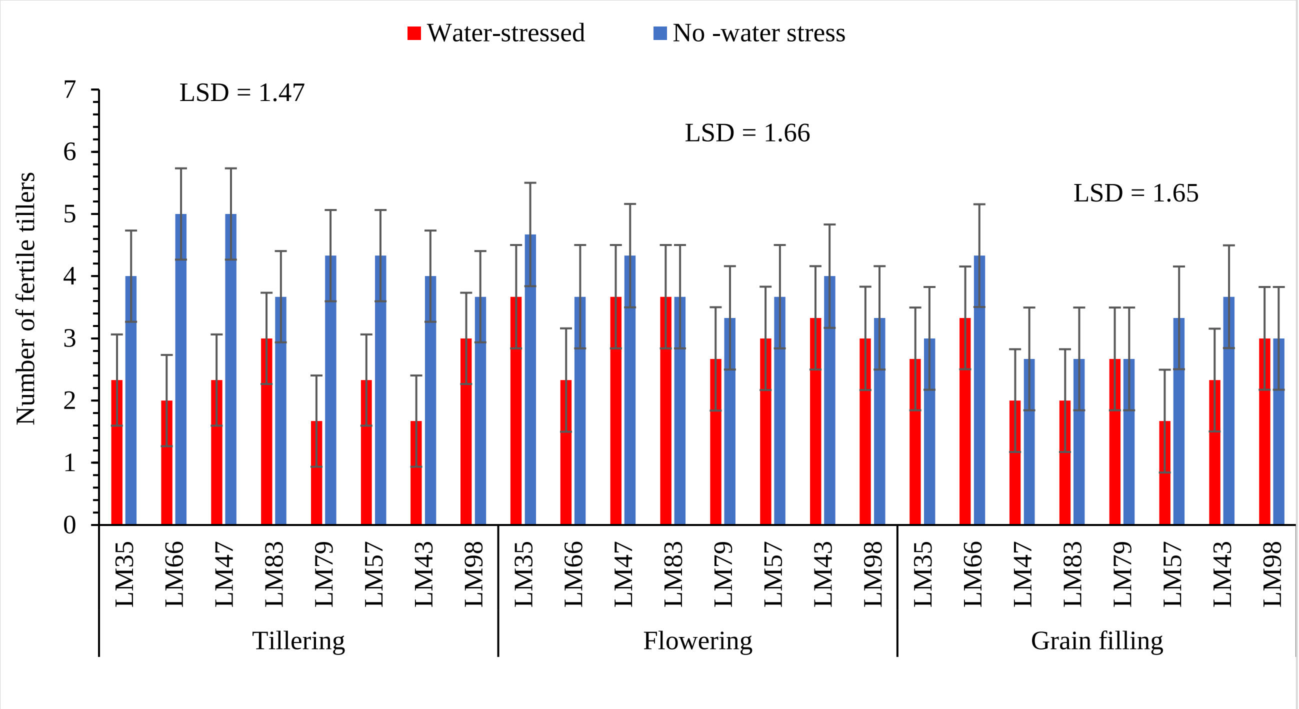

Figure 2. Effect of water stress imposed at different growth stages on the number of fertile tillers

4.4.3 Spike length

Higher spike length was attained in wheat genotypes under none stressed treatments in all growth stages (Fig. 3). When water stress was imposed at the flowering stage only genotype LM43 (20% spike length reduction) was affected ($p < 0.05$) (Fig. 3). Water stress at tillering stage had an effect on genotype LM35 (31% spike length reduction) and LM79 (38% spike length reduction). Only genotype LM47 (38% spike length reduction) was affected ($p < 0.05$) by water stress at grain filling stage (Fig. 3). This suggests that the genotypes that had their spike length reduced after water stress was imposed at each growth stage are susceptible and the rest of the genotypes are tolerant.

These results agree with those of Sokoto and Singh (2013) who reported that water stress at flowering, tillering and grain filling stage resulted in shorter spike length in genotypes (that are sensitive to water stress at each growth stage. However, they oppose those of Vafa et al. (2014) who showed that water stress during flowering and grain filling stage does not affect spike length. As this study was able to discover genotypes that are sensitive to water stress at grain filling and flowering growth stage (Fig. 3). The different responses of these genotypes to water stress at each growth stage can be associated with their genetic make-up which influences their defense mechanisms to water stress.

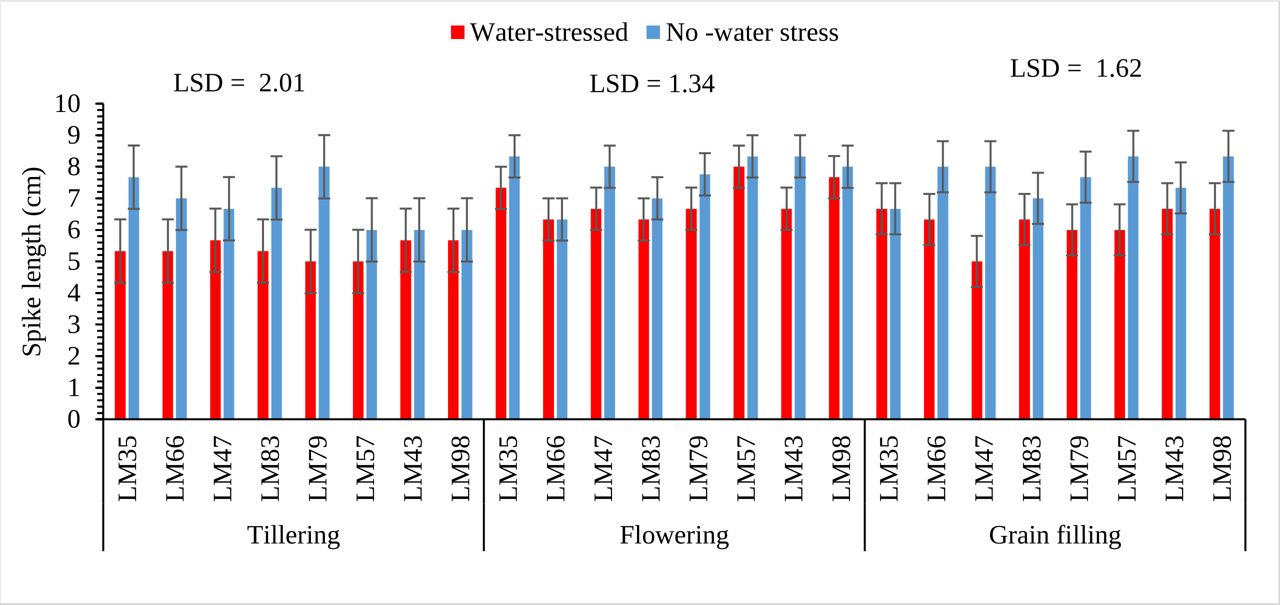

Figure 3. Spike length of wheat genotypes after water stress imposed at different growth stages

4.4.4 Aboveground biomass

The aboveground biomass of the studied genotypes was affected ($p < 0.05$) by water stress imposed at the tillering stage, however, at flowering and grain filling stage the aboveground biomass was not affected ($p > 0.05$) by water stress (Table 3). Growth stages of wheat genotypes that have the crop’s aboveground biomass not affected by water stress are referred to as water stress tolerant growth stages (Castro-Nova et al., 2012; Boudjabi et al., 2015).

Evaluation of genotype performance indicated that all genotypes except LM83 were affected but water stress at tillering (Table 3). Genotype LM66, LM79, LM35, LM43, LM57, LM98 and LM47 produced an aboveground biomass of 1.03 t ha^{-1}, 1.22 t ha^{-1}, 2.26 t ha^{-1}, 2.58 t ha^{-1}, 1.87 t ha^{-1}, 1.62 t ha^{-1} and 2.07 t ha^{-1} correspondingly after water stress at tillering stage. Compared to when these genotypes are subjected to full irrigation the aboveground biomass was reduced by 68%, 77%, 48%, 45%, 60%, 45% and 67% when subjected to water stress at tillering (Table 3). The variability of aboveground biomass in these genotypes after water stress could be due to the level of water stress susceptibility of each growth stage in each genotype. This reaction is governed by the genetic makeup of each genotype. These results agree with part of the results by Gonfa et al. (2011) where water stress was found to have no effect on the aboveground biomass at flowering and grain filling stage. However, in this study, the tillering stage was found to be affected by water stress whereas these authors found that it was not affected. The results of the current study suggest that the tested genotypes are sensitive to water stress at tillering stage.

Table 3. Effect of water stress imposed at different growth stages on the above ground biomass of wheat.

	Above ground biomass (t ha^{-1})					
	Tillering		**Flowering**		**Grain filling**	
Genotype	**T_1**	**T_2**	**T_1**	**T_2**	**T_1**	**T_2**
LM35	2.26^{abc}	4.36^{abcd}	0.33^{a}	0.51^{a}	0.17^{a}	0.28^{a}
LM66	1.03^{a}	3.23^{abcd}	0.24^{a}	0.32^{a}	0.23^{a}	0.29^{a}
LM47	2.07^{abc}	6.35^{d}	0.23^{a}	0.45^{a}	0.20^{a}	0.23^{a}
LM83	4.01^{abcd}	4.12^{abcd}	0.27^{a}	0.36^{a}	0.21^{a}	0.26^{a}
LM79	1.22^{a}	5.29^{cd}	0.22^{a}	0.31^{a}	0.20^{a}	0.31^{a}
LM57	1.87^{abc}	4.70^{bcd}	0.26^{a}	0.34^{a}	0.24^{a}	0.29^{a}
LM43	2.58^{abc}	4.72^{bcd}	0.38^{a}	0.49^{a}	0.14^{a}	0.33^{a}
LM98	1.62^{ab}	2.92^{abcd}	0.14^{a}	0.18^{a}	0.21^{a}	0.32^{a}
p-value	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
LSD	1.89	1.89	0.25	0.25	0.22	0.22
SE ±	0.65	0.65	0.09	0.09	0.07	0.07
CV (%)	34.50	34.50	47.90	47.90	51.50	51.50

Means in a column and treatment group followed by the same letter are not significantly different from each other using Tukey's test at 5% probability level; T_1, stress; T_2, no stress (control); CV, Coefficient of Variation; LSD, Least Significant Difference; SE, standard error; P-value= Significance of interaction between treatment x genotype x growth stage.

4.4.5 Grain yield

The results of grain yield indicated that water stress imposed at flowering and grain filling stage did not ($p > 0.05$) cause a decline in the yield production of the studied genotypes (Table 4). However, these genotypes were susceptible ($p < 0.05$) to water stress imposed at tillering stage and their grain yield were reduced than the control (Table 4).

High yielding genotypes are associated with high level of tolerance, hence, considered as better performers under water-limited conditions (Kilic and Yagbasanlar, 2010). In this study, the most susceptible genotypes to water stress at tillering stage were genotype LM47, LM79 and LM66 with 79%, 82% and 63% grain yield reduction correspondingly. The rest of the genotypes were slightly affected by water stress imposed at tillering stage with genotype LM83 (3.57 t ha^{-1}) obtaining the highest grain yield after water stress compared to other genotypes

(Table 4). These results contradict those of Mirzae et al. (2011) and Ciadir et al. (1999) who indicated that water stress at tillering, flowering and grain filling result in the reduction of grain yield. However, these results are supported by those of Barnard (2012) who indicated that water stress at tillering will lead to less number of tillers which will affect grain yield. The intrinsic paradox in assuming that more fertile tillers after water stress are associated with higher grain yield is also denoted in the results of other studies (Ahmad et al., 2003; Moayedi et al., 2010; Khan et al., 2011). As the yield performance of these genotypes can be linked with the production of fertile tillers after water stress at tillering stage (Table 4) (Fig. 2). The rest of the genotypes showed tolerance at the tillering stage and this is signified by the same letters after the means for both well-watered and water stressed conditions.

Table 4. Grain yield of wheat genotypes after water stress was imposed at different growth stages

	Grain Yield (t ha^{-1})					
	Tillering		**Flowering**		**Grain filling**	
Genotype	**T_1**	**T_2**	**T_1**	**T_2**	**T_1**	**T_2**
LM35	1.83^{ab}	2.59^{abc}	1.17^{a}	2.18^{a}	0.81^{a}	1.44^{a}
LM66	0.97^{a}	1.54^{ab}	0.77^{a}	1.10^{a}	0.96^{a}	1.02^{a}
LM47	1.15^{a}	5.45^{c}	1.02^{a}	3.10^{a}	1.05^{a}	1.31^{a}
LM83	3.57^{abc}	5.41^{c}	2.07^{a}	2.29^{a}	0.79^{a}	1.07^{a}
LM79	0.97^{a}	5.26^{c}	0.92^{a}	2.97^{a}	0.79^{a}	1.12^{a}
LM57	1.62^{ab}	4.61^{bc}	1.34^{a}	2.01^{a}	1.45^{a}	1.93^{a}
LM43	1.58^{ab}	4.82^{bc}	2.21^{a}	2.67^{a}	1.76^{a}	1.99^{a}
LM98	1.52^{ab}	2.72^{abc}	0.96^{a}	0.87^{a}	1.25^{a}	1.59^{a}
p-value	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
LSD	1.82	1.82	1.47	1.47	1.43	1.43
SE ±	0.63	0.63	0.51	0.51	0.49	0.49
CV (%)	38.30	38.30	46.40	46.40	67.40	67.40

Means in a column and treatment group followed by the same letter are not significantly different from each other using Turkey's at 5% probability level; T_1, stress; T_2, no stress (control); CV, Coefficient of Variation; LSD, Least Significant Difference; SE, standard error; P-value= Significance of interaction between treatment x genotype x growth stage.

4.4.6 Harvest index

Results of this study revealed that there was no significant ($p > 0.05$) effect of water stress on the harvest index in all the genotypes at all growth stages (Table 5). These results suggest that water stress at tillering, flowering and grain filling did not affect the crop's efficiency in converting assimilates to grain. The Tukey's test also indicated a high level of water stress tolerance in these genotypes at all growth stages (Table 5). These results contradict those Sokoto and Singh (2013) who indicated that the harvest index is reduced when water stress is imposed at tillering, flowering and grain filling. However, they are justified by the conclusion made by these authors that the harvest index can only be reduced if the tested genotypes are very sensitive to water stress. The results suggest that the studied genotypes have genes that can tolerate prolonged water stress hence the stress imposed did not have a significant effect.

Table 5. Effect of water stress imposed at different growth stages on the harvest index of wheat genotypes

	Harvest index ($t\ ha^{-1}$)					
	Tillering		**Flowering**		**Grain filling**	
Genotype	**T_1**	**T_2**	**T_1**	**T_2**	**T_1**	**T_2**
LM35	0.44^{a}	1.16^{a}	2.51^{a}	6.46^{a}	4.43^{a}	5.22^{a}
LM66	0.48^{a}	1.12^{a}	3.36^{a}	3.75^{a}	3.33^{a}	4.28^{a}
LM47	0.51^{a}	0.85^{a}	4.24^{a}	6.81^{a}	5.39^{a}	6.38^{a}
LM83	0.86^{a}	1.36^{a}	6.47^{a}	7.84^{a}	3.22^{a}	4.93^{a}
LM79	0.75^{a}	1.01^{a}	4.30^{a}	13.87^{a}	2.47^{a}	5.30^{a}
LM57	0.81^{a}	1.02^{a}	5.22^{a}	6.35^{a}	6.46^{a}	8.41^{a}
LM43	0.52^{a}	1.02^{a}	4.97^{a}	15.03^{a}	5.74^{a}	13.87^{a}
LM98	1.06^{a}	5.57^{a}	5.25^{a}	6.36^{a}	7.68^{a}	8.79^{a}
p-value	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
LSD	3.66	3.66	9.17	9.17	8.86	8.86
SE ±	1.27	1.27	3.17	3.17	3.07	3.07
CV (%)	189.60	189.60	85.60	85.60	88.70	88.70

Means in a column and treatment group followed by the same letter are not significantly different from each other using Turkey's at 5% probability level; T_1*, stress;* T_2*, no stress (control); CV, Coefficient of Variation; LSD, Least Significant Difference; SE, standard error; P-value= Significance of interaction between treatment x genotype x growth stage.*

4.4.7 Correlation analysis of the measured traits

The PC-1 and PC-2 axes assist in distinguishing the indices between the different traits at the different growth stages and treatment group. In this study, the axes revealed 98.95% total variability (Fig. 4). The relationship between these traits is shown by how the traits are grouped in the biplot diagram and the direction of the arrows (Fig.4). Positively correlated traits are grouped together on one side of the origin and their arrows are facing the same direction (Fig. 4). While traits negatively correlated traits have their arrows facing the opposing direction (Fig.4).

The correlation analysis of the biplot revealed that the grain yield for each genotype was determined by the spike length and the number of fertile tillers (Fig. 4). These results match those of Okuyama et al. (2005) who also found that the grain yield correlated with the spike length and the number of fertile tillers. The results of this study further showed that the grain yield was not correlated with the plant height, biomass and harvest index. The results for the negative relationship between plant height and grain yield could be due to that, currently, the International Maize and Wheat Improvement Center is developing genotypes with semi-dwarfing genes. This means that the studied genotypes are capable of maximizing wheat yields despite the height. This also justifies the reason why plant height was not affected by water stress at tillering stage but the yield was reduced after water stress at this growth stage. The traits which are positively correlated with grain yield can be used as selection criteria in determining high yielding genotypes under water stress.

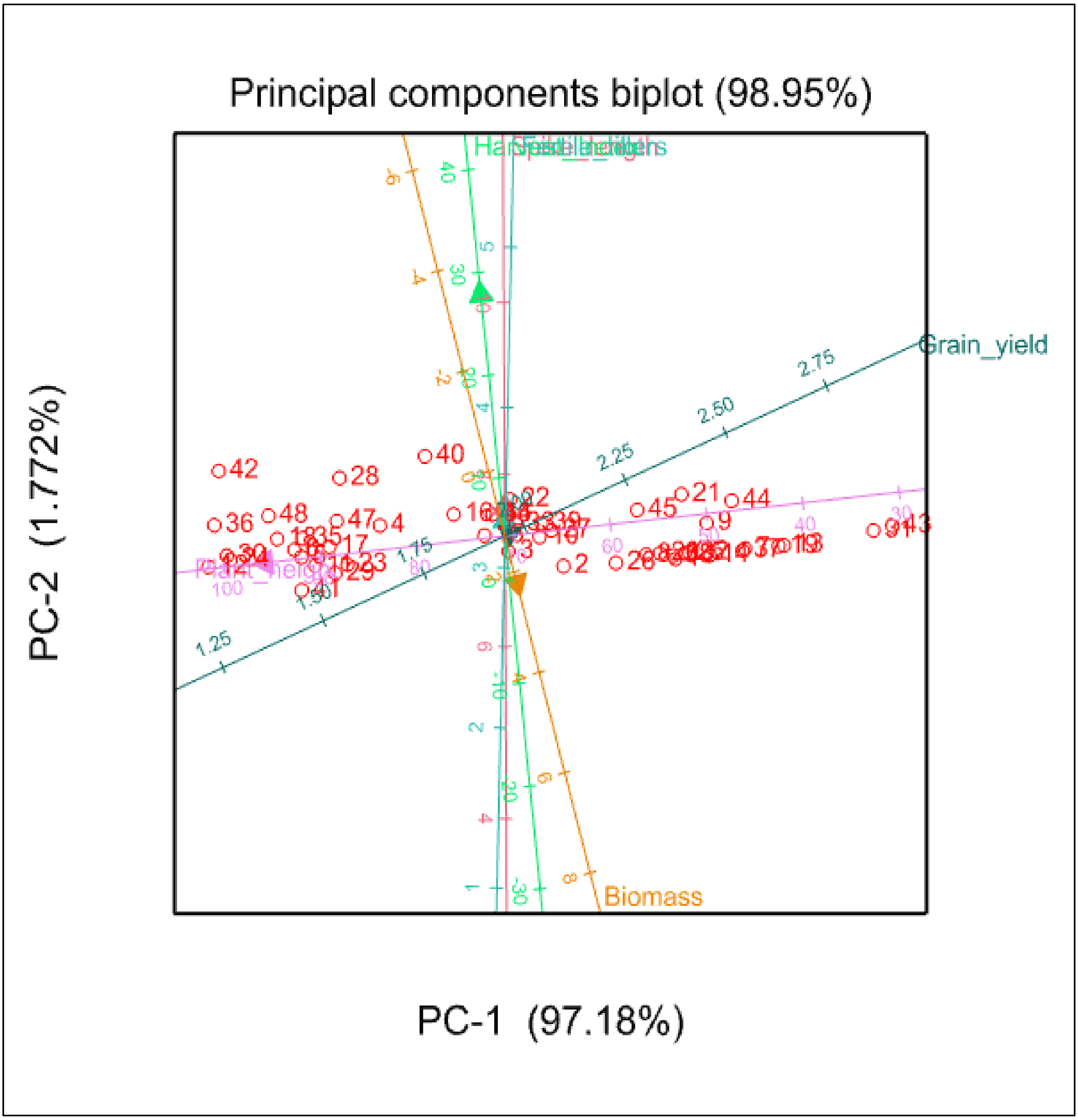

Figure 4. Correlation of yield and yield components under both stress and full irrigation conditions.

4.5 Conclusion

The results of this study have shown that the studied wheat genotypes are tolerant to water stress, particularly, at flowering and grain filling stage with the few exceptions. These stages can, in turn, be used by irrigated wheat farmers to conserve water during the period of water stress and only irrigate at tillering, the most susceptible growth stage to water stress. From the studied genotypes, it was observed that only genotype LM83 is suitable for conserving water at the tillering stage, whereas LM47, LM79 and LM66 are very susceptible to water stress at tillering stage.

References

AHMAD R, QADIR S, AHMAD N, SHAH KH (2003) Yield potential and stability of nine wheat varieties under water stress conditions. *International Journal of Agriculture and Biology* 5:7-8.

AKRAM M (2011) Growth and yield components of wheat under water stress of different growth stages. *Bangladesh Journal of Agricultural Research* 36: 455-468.

ARC-SGI (AGRICULTURAL RESEARCH COUNCIL - SMALL GRAIN INSTITUTE) (2014) *Guidelines for production of small grain in the winter rainfall areas*. Bethlehem, Free State, South Africa.

ASSENG S, TURNER NC, KEATING BA (2001) Analysis of water and nitrogen use efficiency of wheat in a Mediterranean climate. *Journal of Plant and Soil* 233: 127-143. https://doi.org/10.1023/A:1010381602223.

BARNARD A (2012) Physiological changes in the wheat crop. Agricultural Research Council – Small Grain Institute. Bethlehem, Free State.

BOUDJABI S, KRIBAA M, CHENCHOUNI H (2015) Growth, physiology and yield of durum wheat (*Triticum durum*) treated with sewage under water stress conditions. *EXCLI Journal* 14: 320-334. https://doi.org/10.17179/excli2014-715.

BOUTRAA T, AKHKHA A, AL-SHOAIBI AA, ALHEJELI AM (2010) Effect of water stress on growth and water use efficiency (WUE) of some wheat cultivars (*Triticum durum*) grown in Saudi Arabi. *Journal of Taibah University for Science* 3: 39-48. https://doi.org/10.1016/S1658-3655(12)60019-3.

BRISSON N, GUEVARA E, MEIRA S, MATURANO M, COCA G. (2001) Response of five wheat cultivars to early drought in Pampas. *Agronomie, EDP Sciences* 29: 483-495.

CASTRO- NOVA S, ORTIZ- CERESERES OJ, MENDOZA-CASTILLO MC, HUERTA AJ (2012) Biomass production and grain yield of three sorghum lines differing in drought resistance. *Phyton, International Journal of Experimental Botany* 81: 149-156.

CIADIR G, SAEED M, CHEEMA MA (1999) Effect of water stress on growth and yield performance of four wheat cultivars. *Pakistan Journal of Biological Sciences* 2: 236 - 239.

DARYANTO S, WANG L, JACINTHE PA (2016) Global synthesis of drought effects on maize and wheat production. *PLos ONE* 11: 1-15. https://doi.org/10.1371/journal.pone.0156362.

DUBE E, KILIAN W, VAN RENSBURG B, VAN DER MERWE M, DU PLESSIS D, VAN DER MERWE E, HATTING H (2016) *National cultivar evaluation results: Did irrigation wheat beat the heat in 2015?* Agricultural Research Council - Small Grain Institute, Bethlehem, Free State. South Africa.

EVANS RG, SADLER EJ (2008) Methods and technologies to improve efficiency of water use. *Water Resource Research* 44: 3-15. https://doi.org/10.1029/2007WR006200.

FOURIE P, BOTHA P (2011) *The role of irrigation in grain production*. Grain SA, Pretoria, South Africa.

GONFA AB, TESFAYE K, GELETO T (2011) Morphological and physiological attributes associated to drought tolerance of Ethiopian durum wheat genotypes under water deficit. *Journal of Biodiversity and Environmental Sciences* 1: 22-33.

GRENZDORFFER GJ (2014) Crop height determination with UAS point clouds. *The International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences* XL-1: 135-140. https://doi.org/10.5194/isprsarchives-XL-1-135-2014.

HEYNS I, MALAN A (2015) *Breeding for yield: What is the situation in Western Cape?* Agricultural Research Council- Small Grain Institute. Bethlehem, Free State, South Africa.

KHAKWANI AA, DENNET MD, MUNIR M, ABID M (2012) Growth and yield response of wheat varieties to water stress at booting and anthesis stages of development. *Pakistan Journal of Botany* 44: 879-886.

KHAN AS, KHALIL SK, JAN A, KHAN AZ, NAWAB K (2011) Performance of high yielding wheat and barley cultivars under moisture stress, *Pakistan Journal of Botany* 43: 2143-2145.

KILIC H, YAGBASANLAR T (2010) The effect of drought stress on grain yield, yield components and some quality triats of durum wheat (*Triticum turgidum ssp. durum*) cultivars. *Notulae Botanicae Horti Agrobotanici Cju-Napoca* 38: 164-170. http://dx.doi.org/10.15835/nbha 3814 274.

MACAULEY H (2015) Cereal crops: rice, maize, millet, sorghum, wheat. In: *Proceedings of the Abdou Diouf International Conference Center*, 21-23 October 2015. Dakar, Senegal.

MAQBOOL MM, ALI A, HAQ TU, MAJEED MN, LEE DJ (2015) Response of spring wheat (*Triticum aestivum* L.) to induced water stress at critical growth stages. *Sarhad Journal of Agriculture* 31: 53-58.

MIRZAEI A, NASERI R, SOLEIMANI R (2011) Response of different growth stages of wheat to moisture tension in a semiarid land. *World Applied Sciences journal* 12: 83-89.

MOAYDEDI AA, BOYCE AN, BARAKBAH SS (2010) The performance of durum and bread wheat genotypes associated with yield and yield components under different water deficit conditions. *Australian Journal of Basic and Applied Sciences* 4: 106-113.

MULLER M, SCHREINER B, SMITH LAILA VAN KOPPEN B, SALLY H, ALIBER M, COUSINS B, TAPELA B, VAN DER MERWE-BOTHA M, EIMAN K, PIETERSEN K (2009) Water security in South Africa. Development Planning Division. *Working paper Series No.12*.DBSA: Midrand.

NEGASSA A, SHIFERAW B, KOO J, SONDER K, SMALE M, BRAUN HJ, GBEGBELEGBE S GUO Z, HODSON DP, WOOD S AND CO-AUTHORS (2013) *The potential for wheat production analysis of Biophysical suitability and economic profitability*. Mexico, DF (Mexico). CIMMYT. http://repository.cimmyt.org/xmlui/bitstream/handle/10883/4015/97365.pdf. (Assessed 5 March 2017).

NEZHADAHMADI A, PRODHAN ZH, FARUQ G (2013) Drought tolerance in wheat. *The Scientific World Journal:* 1-12.

OKUYAMA LA, FEDERIZZI LC, FERNANDES J, NETO B (2005) Plant traits to complement selection based on yield components in wheat. *Ciência Rural* 35: 1010-1018.

QADIR G, SAEED M, CHEEMA MA (1999) Effect of water stress on growth and yield performance of four wheat cultivars. *Pakistan Journal of Biological Sciences* 2: 236-239.

RUTTANAPRASERT R, JOGLOY S, VORASOOT N, KESMALA T, KANWAR RS, HOLBROOK CC, PATANOTHAI A (2016) Effects on water stress on total biomass, tuber yield, harvest index and water use efficiency in Jerusalem artichoke. *Agricultural Water Management* 166: 130-138. https://doi.org/10.1016/j.agwat.2015.12.022.

SARVESTANI ZT, PIRDASHTI H, SANAVY SAMM, BALOUCHI H (2008) Study of water stress effects in different growth stages on yield and yield components of different rice (*Oryza sativa* L.) cultivars. *Pakistan Journal of Biological Sciences* 10: 1303-1309.

SHARMA RC (1993) Selection for biomass yield in wheat. *Euphtytica*: *International Journal of Plant Breeding* 70: 35-42.

SOKOTO MB, SINGH A (2013) Yield and yield components of bread wheat as influenced by water stress, sowing date and cultivar in Sokoto, Sudan Savannah, Nigeria. *American Journal of Plant Sciences* 4: 122-130. https://doi.org/10.4236/ajps.2013.412A3015.

TAHERI S, SABA J, SHEKARI F, ABDULLAH LT (2011) Effect of drought stress condition on the yield of spring wheat (Triticum aestivum) lines. *African Journal of Biotechnology* 10: 18339-18348. https://doi.org/10.5897/AJB11.352.

VAFA P, NASERI R, MORADI M (2014) The effect of drought stress on grain yield, yield components and protein content of durum wheat cultivars in Ilam province, Iran. *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering* 8: 631-636.

VALIZADEH J, ZIAER SM, MAZLOUMZADEH SM (2014) Assessing climate change impacts on wheat production (a case study). *Journal of the Saudi Society of Agricultural Sciences* 13: 107-115.

WATER RESEARCH COMMISSION (2015) Water restrictions. Water Research Commission, Pretoria, South Africa. URL: http://www.droughtsa.org.za/about-the-drought/water-restrictions.html. (Accessed 20 March 2017).

WATER RESEARCH COMMISSION (2016) Water restrictions. Water Research Commission, Pretoria, South Africa. URL: http://www.droughtsa.org.za/about-the-drought/water-restrictions.html. (Accessed 20 March 2017).

WNUK A, GÓNY AG, BOCIANOWSKI J, KOZAK M (2013) Visualizing harvest index in crops. *Communications in Biometry and Crop Science* 8: 48-59.

ZADOKS JC, CHANG TT, KONZAK CF (1974) A decimal code for the growth stages of cereals. *Weed Research* 14: 415-421.

CHAPTER 5

General discussion, overview of research findings and recommendations

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5.1 Introduction

Wheat is one of an essential crops produced in South Africa (DAFF 2016). However, its production has been threatened by the severe drought conditions occurring in the country (Dube et al. 2016). Consequently, the drought crisis has also threatened the frequent use of irrigation which could have assisted in maintaining higher yields during the drought period (Dube et al. 2016). Therefore, many irrigated wheat farmers in the country fail to maximize yields due to the unavailability of production guidelines or recommendations that could assist them to achieve the targeted yield without irrigating at water stress tolerant growth stages.

Limited research has been conducted in the country regarding this issue (ARC-SGI 2014, 2015). However, the International Maize and Wheat Improvement Center (CIMMYT) has developed new genotypes which are reported to withstand very hot and dry conditions and suitable for the country's climatic conditions. As part of this initiative, this study identified water stress tolerant growth stages of wheat genotypes that will allow farmers to reach their targeted yield with the limited available water.

5.2 Aim and objectives

The overall aim of this study was to determine water stress tolerant growth stages of irrigated wheat genotypes hence investigate the possibility of improving wheat yields during the water shortage period in South Africa. The specific objectives of this study were:

I. To select water stress tolerant irrigated wheat genotypes through evaluating the response of their physiological traits after water stress at tillering, flowering and grain filling. (chapter 3).

II. To determine the growth stage at which limited water supply would have minimal effect on the growth, development and yield of eight newly developed wheat genotypes (chapter 4).

5.3 Overview of research findings

- The comprehensive review of literature gave a clear insight on the effects of water shortage on irrigated wheat at different growth stages. Moreover, it analyzed the traits that assist in determining the level of tolerance of irrigated wheat growth stages and methods of determination. This review discussed in-depth each method together with its success and shortcomings. Furthermore, the responses of wheat genotypes to the different water treatments were also reviewed and the mechanisms behind their response. The justification on the importance of genotype choice was also examined. It was therefore evident from the reviewed literature that significant success has been made in other parts of the world except for South Africa regarding such research and that more in-depth information about water conserving growth stages of irrigated wheat genotypes still needs to be done. The literature review of this study also served as a guide on how to go about implementing such research in South Africa and the important traits that are considered when selecting water stress tolerant growth stages of irrigated wheat hence contributing to the success of this study.

- For the first objective, there was a variation in the measured traits of the studied genotypes. However, the water use efficiency which was the index of the crop's ability to retain water at water limited conditions indicated that most of the studied genotypes still performed better with a few exceptions when stressed at tillering and flowering and more had their water use efficiency reduced when water stress was imposed at grain filling (Wang et al. 2016).
- The response of the water use efficiency in the studied genotypes was correlated with transpiration rate and stomatal conductance.
- In the second objective, results obtained for grain yield were more linked to the number of fertile tillers affected by water stress in each growth stage.
- The grain yield production after water stress at each growth stage was the primary concern (Wang et al., 2016).
- Therefore, findings of the current study suggested that most of the studied genotypes can be subjected to water stress at grain filling and flowering but water stress at tillering stage results in a yield reduction. Genotype performance after water stress at tillering stage suggested that genotype LM83 is the most water stress tolerant genotype whereas, LM47, LM79 and LM66 are more vulnerable to water stress.

5.4 Recommendations

- More in depth research still needs to be done in South Africa regarding water conserving growth stages of irrigated wheat genotypes. This will assist generate information that could save crop failure during the critical water period.
- It is also recommended that trials be planted in different geographical regions to have specific recommendations for each region with regards to its climatic conditions and soil type and genotypes that strive in that area.
- Future genotype evaluation of irrigated wheat performance in South Africa should also consider the crop's physiological responses which can help justify the yield performance of each genotype.

References

ARC-SGI (Agricultural Research Council – Small Grain Institute) (2015) *Guidelines of small grain in the winter rainfall areas*. Bethlehem. Free State.

ARC-SGI (Agricultural Research Council - Small Grain Institute) (2014) *Guidelines for production of small grain in the winter rainfall areas*. Bethlehem. Free State. South Africa.

Dube E, Kilian W, van Rensburg B, van der Merwe M, du Plessis D, van der Merwe E, Hatting H (2016) National cultivar evaluation results: Did irrigation wheat beat the heat in 2015? *Agricultural Research Council- Small Grain Institute*. Bethlehem. Free State. South Africa.

DAFF (Department of Agriculture Forestry and Fisheries) (2016) Trends in the agricultural sector. Pretoria. South Africa.

Wang B, Zhang Y, Hao B, Xu X, Zhao Z, Wang Z, Xue Q (2016) Grain yield and water use efficiency in extremely late-sown winter wheat cultivars under two irrigation regimes in the north China plain. *PLoS One* 11: 1-14.