Evaluation of Elite Heat and Drought Tolerant Wheat (*Triticum aestivum*) Genotypes Based on Drought Tolerance and Water-Use Efficiency Parameters

by

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Abstract

Drought stress is one of the most important limiting factors to sustainable and profitable wheat production in sub-Saharan Africa (SSA), including South Africa. Use of drought adapted genetic resources is regarded to be the most economic and environmentally friendly approach to mitigating the adverse effects of heat and drought stress. Therefore, there is need to select desirable wheat genotypes with enhanced water-use efficiency and drought tolerance parameters to boost wheat production in water-limited environments. Genotypes with enhanced drought-tolerance and water-use efficiency can be developed targeting yield-related agronomic and physiological traits which are well-correlated with grain yield potential. Therefore, the objectives of this study were: 1) to determine drought tolerance of dryland wheat genotypes based on leaf gas exchange and water-use efficiency in order to identify promising genotypes for drought tolerance breeding and 2) to examine associations between morphological and physiological traits of selected wheat genotypes under drought stress in order to identify unique traits that may be used as direct or indirect selection criteria for improving water-use efficiency and drought tolerance in wheat.

In the first study, leaf gas exchange and water use efficiency of ten genetically diverse wheat genotypes were tested under water-stressed and non-stressed conditions. Results showed high significant differences ($P < 0.001$) in water condition x genotypes interaction with regards to net photosynthetic rate (A), the ratio of net CO_2 assimilation rate and intercellular CO_2 concentration (A/C_i), the ratio of intercellular and atmospheric CO_2 (C_i/Ca), intrinsic water-use efficiency (WUE_i), instantaneous water-use efficiency (WUE_{inst}) and water-use efficiency (WUE). This suggests that genotypic variability of wheat exists for these traits. Heat and drought tolerant wheat genotypes such as G339 and G334 were identified and selected for breeding for enhanced drought tolerance possessing suitable physiological traits such as high A, transpiration rate (T), stomatal conductance (gs), A/Ci, WUE_i and WUE_{inst} under drought stress condition.

In the second study, response of wheat genotypes were assessed based on morpho-physiological traits and water use efficiency under water-stressed and non-stressed conditions. Significant differences ($P < 0.05$) were observed among the tested wheat genotypes with regards to the number of productive tillers (NT), number of leaves per

plant (NL), total dry mass (DM), leaf area index (LA), leaf area ratio (LAR), *A, gs, T*, WUE_{Einst}, WUE_i, WUE. Pearson's correlation analysis indicated that NL, NT, plant height (PH), DM, grain yield (GY), *A* were positively and significantly correlated with WUE_{inst}. Instantaneous water use-efficiency positively correlated with NL ($r = 0.76$; $P < 0.001$), NT ($r = 0.67$; $P = 0.03$), PH ($r = 0.72$; $P = 0.01$), DM ($r = 0.81$; $P < 0.001$) and GY ($r = 0.70$; $P = 0.02$) under water stress (WS) condition. Wheat genotypes namely: G339, G343 and G344 which exhibited high NT and DM under WS condition were selected with enhanced water-use efficiency.

Overall, the present study evaluated and selected drought tolerance wheat genotypes that can be used to improve wheat grain yield under water stress conditions. Furthermore, morphological traits (NT and DM) and physiological traits (*A*, *T*, gs, A/C_i and WUE) well-associated with water-use efficiency were detected. These traits can be used as direct and indirect selection criteria in dry land wheat improvement programmes.

Declaration

I, Tshikunde Nkhathutsheleni Maureen declare that:

(i) The research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) This dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii) This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

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

> b) Where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

(v) Where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) This dissertation is primarily a collection of material, prepared by me, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

(vii) This dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

Signed:

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Tshikunde N.M

As the student's supervisors, we agree to the submission of this dissertation:

Prof. Hussein Shimelis (Supervisor)

Dr Alfred O. Odindo (Co-Supervisor)

Acknowledgments

Lastly "If it had not been for the Lord on my side, where would I be"?

Dedication

This work is dedicated to my Family.

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Chapter two

Tshikunde N.M., Odindo A., Shimelis H., Mashilo J. (2018) leaf gas exchange and water-use efficiency of dry-land wheat genotypes under water stressed and non-stressed conditions. Acta Agriculturae Scandinavica, Section B- Soil and Plant Science. 68:738-748.

Chapter three

Tshikunde N.M., Odindo A., Shimelis H., Mashilo J. (2019) Morpho-physiological traits associated with water-use efficiency in selected dry land wheat (*Triticum aestivum* L.) genotypes. Under review in South Africa Journal of Plant and Soil.

Introduction to dissertation

Background

Wheat (*Triticum aestivum* L., 2n=6x=42) is one of the most important cereal crops cultivated globally (Abdullah et al., 2011). It is high in minerals, vitamins, carbohydrates and proteins (FAO 2002). In South Africa, the area under wheat cultivation decreased from 805 000 ha to 450 000 ha during 2001 and 2015 production periods. However, the total production increased by over 80% with an average yield of 3.5 million tons per year during the same period (DAFF, 2015). The decline in planted area is attributed to several factors including erratic and poorly distributed rainfall (Dube et al., 2015). The increase in yield is attributed but not limited to the use of improved agricultural practices and technology such as fertilizer application, improved water conservation strategies, and cultivation of high yielding and drought tolerant wheat genotypes (Dube et al., 2015).

Drought stress is the leading limiting factor to wheat production in sub-Saharan Africa (SSA), including South Africa (Matiu et al., 2017; Parry et al., 2007). International and national wheat research programs have developed wheat genotypes with enhanced drought tolerance. This resulted in improved grain yield potential under water-limited conditions (Foulkes et al., 2007; Nouri-Gambalani et al., 2009; Lopes et al., 2012; Beche et al., 2014; Chen et al., 2015; Zhang et al., 2016; Gao et al., 2017). However, yield gains yet remain very low varying from 0.5 to 1% per year under water-stressed condition (Sharma et al., 2012; Crespo-Herrera et al., 2018). Drought stress may likely escalate due to climate change that will cause even hotter and drier growing conditions (Kiliç and Yağbasanlar, 2010). Breeding drought adapted wheat genotypes is important to improve grain yield potential of wheat under water stress condition (Belagrouz et al., 2018). Furthermore, breeding for drought tolerance can potentially enhance water-use efficiency (WUE) (Zhang et al., 2004) reducing the use of irrigation water. Water use efficiency is described as the ability of the plants to produce biomass or yield per unit water used by the plant (Blum, 2005). It is an essential trait for determining grain yield under water stressed condition (Ehdaie, 1995; Kirda et al., 1999; Zhang et al., 2005; Rebetzke et al., 2002, Franks et al., 2015).

Wheat improvement programmes developed elite genotypes targeting yield-promoting agronomic and physiological traits which enhanced WUE to improve yield potential

(Belagrouz et al., 2018; Sakumona et al., 2014). Physiological traits such as chlorophyll content, osmotic adjustment, canopy temperature, relative water content, carbohydrate content, and morphological traits such as early flowering and maturity, harvest index are reportedly associated with drought tolerance breeding and wheat yield gains (Quin et al., 2013; Richard et al., 2015; Nakhforoosh et al., 2016; Christy et al., 2018; Rashid et al., 2018). As a result, these traits can be simultaneously selected in improvement programmes to design and develop highly-adapted, high-yielding and drought tolerant wheat genotypes with enhanced water-use efficiency to improve yield gains under water-limited environments. The frequent occurrence of drought stress in arid and semi-arid environments suggest the need to develop wheat genotypes that are even more efficient in the utilization of limited resources such as water, nutrients and light energy under dry environments. Such genotypes can be developed targeting yield-influencing agronomic and physiological drought-tolerance enhancing traits. This will likely improve wheat yield potential resulting in food security in SSA and globally.

Rationale of the study

Climate change is resulting in highly variable weather conditions causing prolonged dry spells and erratic rainfall patterns contributing to increased water crisis in South Africa. There is need to improve wheat production under the changing climatic conditions. This can be achieved by identifying and selecting wheat genotypes that use less water, while maintaining relatively good yield performance under water-limited conditions.

Current crop production trends in South Africa show that the total planted area under dry land wheat production has decreased. This is mainly due to poor and erratic rainfall occurring during the summer rainfall season affecting soil moisture availability in winter wheat production (DAFF, 2015). Despite the apparent decline in planted area, wheat yields have increased from about 2.5 to 3.5 million tons for the past 4 years, mainly because of the use of improved technology and agricultural practices and cultivation of locally adapted wheat genotypes. There is potential to increase wheat yields further, and this could be achieved by developing wheat genotypes that utilize limited resources such as water, light and nutrients more efficiently. Genotypes that possess

traits that enable them to optimize available resources for producing higher yields are urgently needed to boost wheat production in the country.

In the past wheat breeding program focused on yield improvement and disease and pest resistance under specific production environments. However, wheat producers require varieties that combine tolerance to biotic and abiotic stress. Drought and heat stress which have become key production constraints in the country necessitates the need to develop drought tolerant wheat genotypes. As a result, elite wheat genotypes where acquired from the International Wheat and Maize Improvement Center (CIMMYT) for abiotic stress tolerance breeding. CIMMYT's elite germplasm need further evaluation to identify and select promising genotypes under the target production environment. This will enable identification and selection of breeding parents possessing yield-influencing agronomic and physiological traits for cultivar development to enhance grain yield potential under low-yielding environments of South Africa.

Aim of the study

The aim of this study was to evaluate and select drought tolerant wheat genotypes possessing key yield-influencing and drought-adaptive agronomic and physiological traits for breeding for high-yield potential, enhanced drought tolerance and water-use efficiency for water-limited wheat producing regions of South Africa.

Specific objectives of the study

1. To determine drought tolerance of dryland wheat genotypes based on leaf gas exchange and water-use efficiency in order to identify promising genotypes for drought tolerance breeding
2. To examine associations between morpho-physiological traits of selected wheat genotypes under drought stress in order to identify unique traits that may be used as direct or indirect selection criterion for improving water-use efficiency and drought tolerance in wheat.

Research hypotheses

The present study was based on the following test hypotheses:

1. Genotypic variation exists among wheat genotypes with respect to drought tolerance and water-use efficiency.
2. Morphological and physiological traits are well-correlated with water-use efficiency useful for selection.

Dissertation Outline

This dissertation is comprised of 3 chapters, which are outlined below. The referencing style used in this dissertation is based on the referencing style of the Journal of Crop Science. The dissertation chapters follow a format of a stand-alone research paper (whether or not the chapter has already been published). This is the dominant dissertation format adopted by the University of KwaZulu-Natal. As such, there is some unavoidable repetition of references and some introductory information between chapters. The research outcomes covered in Chapter two is published in Acta Agriculturae Scandinavica, Section B- Soil and Plant Science Volume 68, No. 8, 2018.

The structure of the dissertation is outlined below:

Chapter	Title
-	Dissertation Introduction
1	Review of Literature
2	Leaf gas exchange and water use efficiency of dry land wheat (*Triticum aestivum* L.) genotypes under water stressed and non-stressed conditions
3	Morpho-physiological traits associated with water-use efficiency in selected dry land wheat (*Triticum aestivum* L.) genotypes
-	An overview of the research findings

References

Abdullah F., Hareri M., Naaesan M., Ammar M.A., Kanbar O.Z. (2011) Effect of drought on different physiological characters and yield components in different varieties of Syrian durum wheat. Journal of Agricultural Science 3:127–133.

Akhkha A., Boutraa T., Alhejely A. (2011) The rate of photosynthesis, chlorophyll content, dark respiration, proline and Abscicic acid (ABA) in wheat (Triticum durum) under water deficit conditions. International Journal of Agriculture and Biology 13:215–221.

Belagrouz A., Chennafi H., Bouzerzour H., Hakimi M., Razem R., Sahraoui A.S. (2018) Relationships among water use efficiency and the physio-agronomic traits in durum wheat (*Triticum durum* desf.) cultivars assessed under rainfed conditions of the eastern high plateaus of Algeria. Agriculture & Forestry 64:159-172.

Blum A. (2005) Drought resistance, water-use efficiency, and yield potential – are they compatible, dissonant, or mutually exclusive. Australian Journal of Agriculture Research 56:1159–1168.

Christy B., Tausz-Posch S., Tausz M., Richards R., Rebetzke G., Condon A., McLean T., Fitzgerald G., Bourgault M., O'Leary G. (2018) Benefits of increasing transpiration efficiency in wheat under elevated CO_2 for rainfed regions. Global Change Biology 24: 1965-1977.

CYMMIT. (2015). Scientists aim to adapt wheat to a warmer climate with less water. http://www.cimmyt.org/scientists-aim-to-adapt-wheat-to-a-warmer-climate-with-less-water/. Accessed: 22 October 2016.

Dube E., Mare-Patose R., Kilian W., Barnard A., Tsilo T.J. (2015) Identifying high-yielding dryland wheat cultivars for the summer rainfall area of South Africa. South African Journal Plant and Soil 33:77–81.

Ehdaie B. (1995) Variation in Water-Use Efficiency and Its Components in Wheat: II. Pot and Field Experiments. Crop Science 35:1617-1626.

Ehdaie B., Waines G.G. (1993) Variation in water-use efficiency and its components in wheat. I. Well-watered pot experiment. Crop Science 33: 294-299

FAO. 2002. Plant production and protection series. No. 30. [Accessed 10 November 2015]. www.fao.org/docrep/006/ y4011e/y4011eoo.htm#.

Farooq M., Hussain M., Siddique K.H.M. (2014) Drought Stress in Wheat during Flowering and Grain-filling Periods. Critical Review in Plant Science 33:331–349.

Franks P.J., Doheny-Adams T.W., Britton-Harper Z.J., Gray J.E. (2015) Increasing water-use efficiency directly through genetic manipulation of stomatal density. New Phytologist 207:188–195.

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 Agrobotanicicluj-Napoca 38:164-170.

Kirda C., Kanber R., Tulucu, K. (1999) Yield response of cotton, maize, soybean, sugar beet, sunflower, and wheat to deficit irrigation. In: Kirda, C., Moutonnet, P., Hera, C., Nielsen, D.R., (Eds.), Crop Yield Response to Deficit Irrigations. Kluwer Academic Publishers, Dordrecht, The Netherlands: 21-38.

Lesk C., Rowhani P., Ramankutty N. (2016) Influence of extreme weather disasters on global crop production. Nature 529:84–87.

Matiu M., Ankerst D.P., Menzel A. (2017) Interactions between temperature and drought in global and regional crop yield variability during 1961–2014. PLoS ONE 12.

Nakhforoosh A., Bodewein T., Fiorani F., Bodner G. (2016) Identification of Water Use Strategies at Early Growth Stages in Durum Wheat from Shoot Phenotyping and Physiological Measurements. Front Plant Science 7: 1155.

Parry M.L., Canziani O.F., Palutikof J.P., Linden P.J.V.D., Hanson C.E. (2007) Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Elsevier 12:171–175.

Quin W., Chi B., Oenema O. (2013) Long-term monitoring of rainfed wheat yield and soil water at the Loess plateau reveals low water use efficiency. PLoS ONE 8.

Rashid M.A., Andersen M.N., Wollenweber B., Zhang X., Olesen J.E. (2018) Acclimation to higher VPD and temperature minimized negative effects on

assimilation and grain yield of wheat. Agricultural and Forest Meteorology 248, 119–129.

Rebetzke G.J., Condon A.G., Richards R.A., Farquhar G.D. (2002) Selection for reduced carbon isotope discrimination increases aerial biomass and grain yield of rainfed bread wheat. Crop Science 42: 739–745.

Richard C., Hickey L.T., Fletcher S., Jennings R., Chenu K., Christopher J.T. (2015) High-throughput phenotyping of seminal root traits in wheat. Plant Methods 11: 13.

Sakumona M., Lungu D., Munyinda K. (2014) Evaluation of Twelve Spring Wheat (Triticum aestivum L.) Genotypes for Water Use Efficiency under Varying Water Regimes. Journal of Applied Environmental and Biological Sciences 4. 63-70.

Siddique K.H.M., Tennant D., Perry M.W., Belford R.K. (1990) Water use and water use efficiency of old and modern wheat cultivars in a Mediterranean-type environment. Australian Journal of Agricultural Research 41: 431-447.

Zhang Z.B., Shan L. (1998) Comparison study on water use efficiency of wheat flag leaf. Chinese Science Bulletin 43:1205–1209.

Zhang X Y, Pei D, Chen S Y. (2004) Root growth and soil water utilization of winter wheat in the North China Plain. Hydrological Processes 18: 2275–2287.

Zhang P., Dreisigacker S., Melchinger A.E., Reif J.C., Kazi A.M., Van Ginkel M. (2005) Quantifying novel sequence variation and selective advantage in synthetic hexaploid wheat and their backcross-derived lines using SSR markers. Molecular Breeding 15:1-10.

CHAPTER 1: Literature Review

Agronomic and physiological traits, and associated quantitative trait loci (QTL) affecting yield response in wheat (Triticum aestivum L.): A review

1.1 Abstract

Enhanced grain yield has been achieved in bread wheat (*Triticum aestivum* L., 2n=6x=42) through development and cultivation of superior genotypes incorporating yield-related agronomic and physiological traits derived from genetically diverse and complementary genetic pool. Despite significant breeding progress, yield levels in wheat have remained relatively low and stagnant under marginal growing environments due to climate change. There is a need for genetic improvement of wheat using yield-promoting morpho-physiological attributes and desired genotypes under the target production environments to meet the demand for food and feed. This review presents breeding progress in wheat for yield gains using agronomic and physiological traits. Further, the paper discusses globally available wheat genetic resources to identify and select promising genotypes possessing useful agronomic and physiological traits to enhance water, nutrient- and radiation-use efficiency to improve grain yield potential and tolerance to abiotic stresses. Finally, the paper highlights quantitative trait loci (QTL) linked to agronomic and physiological traits to aid breeding of high-performing wheat genotypes.

Keywords: Morphological traits, physiological traits, QTL, yield gains, wheat

1.2 Introduction

Wheat (*Triticum aestivum* L., 2n=6x=42) is the world's third important staple food crop after maize (*Zea mays*) and rice (*Oryza sativa*) (Cetin and Akinci, 2015; Dube et al., 2015). The crop is a vital source of proteins, carbohydrates, vitamins (e.g. B1, B2, B3 and E) and mineral elements (e.g. Se, Mn, P and Cu). Wheat is used for food, industrial raw material to prepare alcoholic beverages, starch and straws, and animal feed (Nhemachena and Kirsten, 2017).

Globally, 79% of total wheat production comes from China, United States of America, Turkey, Canada, Australia, India and Argentina (FAOSTAT, 2018) accounting to approximately 751 million tons per annum (FAOSTAT, 2018). Yield gains in wheat are currently estimated at about 0.5 to 1% per year which is below the 2.4% required to satisfy global demand (Sharma et al., 2012; Crespo-Herrera et al., 2018). In order to sustain the fast-growing human population, wheat production must increase by at least 50% by 2030 (Parry et al., 2011). Additionally, the global average wheat yields must increase from 3 to 5 t ha^{-1}, a growth of 1.3% yr^{-1} by 2050 to meet demands (Rosegrant and Agcaoili, 2010). Increased wheat production can be achieved through development and cultivation of genotypes with tolerance to abiotic stress and enhanced nutrient, radiation- and water-use efficiency. Such genotypes can be developed through identification and selection of drought-adaptive and yield-influencing agronomic and physiological traits and associated quantitative trait loci (QTL) (Lopes et al., 2012; Chen et al., 2014; Liu et al., 2015).

Grain yield response in wheat is influenced by several agronomic and physiological traits (Chen et al., 2012; Liu et al., 2015). Agronomic traits such as plant height, harvest index, total biomass, number of productive tillers, grain number per spike, spike length, number of kernels per spike, thousand seed weight, and grain weight per spike; and physiological traits such as canopy temperature, chlorophyll content, photosynthetic rate, water-soluble carbohydrates have contributed to grain yield improvement in wheat (Foulkes et al., 2007; Nouri-Gambalani et al., 2009; Lopes et al., 2012; Beche et al., 2014; Chen et al., 2015; Zhang et al., 2016; Gao et al., 2017). Therefore, there is a need for trait-based breeding using high performing and genetically complementary genotypes to accelerate further grain yield improvement in wheat (Reynolds & Tuberosa 2008; Chen et al., 2012; Bustos et al., 2013; Liu et al., 2015;

Reynolds et al., 2017). The objective of this review is to present breeding progress in wheat for yield gains using agronomic and physiological traits. Globally available wheat genetic resources to aid in the identification and selection of promising genotypes are discussed. In addition, genotypes possessing useful agronomic and physiological traits to enhance water, nutrient- and radiation-use efficiency to improve grain yield potential and tolerance to abiotic stresses are discussed. Finally, the paper highlights quantitative trait loci (QTL) linked to agronomic and physiological traits to aid breeding of high-performing wheat genotypes.

1.3 Global wheat production and yield gains

India, Russia, China and Kazakhstan are currently the leading wheat producers with approximately 30, 27, 24 and 12 million hectares devoted to wheat production, respectively. In terms of total production, China is the world's leading wheat producer with approximately 131 million tons per year (FAOSTAT, 2018). India is the second largest wheat producer followed by Russia, Canada, Argentina, Ukraine and Turkey (FAOSTAT, 2018). Among African countries, Ethiopia Algeria, Egypt, Kenya, Morocco and South Africa have the largest area devoted to wheat production with total production above 1 million tons per year. Variable wheat grain yield response per unit area are reported from New Zealand with 9 tons/ha, Saudi Arabia (6 tons/ha), Zambia (6.6 tons/ha), Egypt (6.5 tons/ha) and China (5.4 tons/ha) in 2016 (FAOSTAT, 2018). The world average wheat yield is 2.9 tons/ha (FAOSTAT, 2018). Worldwide, about 33% countries achieved yield levels ≤ 2 tons/ha, while 21% countries had ≥ 3 tons/ha and 22% had yield levels ≥ 5 tons/ha (FAOSTAT, 2018). Differences in yield levels is attributed to variable climatic conditions, agronomic practices and genetic potential of cultivars.

Wheat yield gains across the major wheat producing countries are presented in Table 1.1. Genetic gains estimated through yield trials are variable among modern varieties released at various time periods compared with checks (Graybosch and Peterson, 2010; Lopes et al., 2012; Sharma et al., 2012). This variation is mainly influenced by yield-related agronomic and physiological traits (De Vita et al., 2007; Beche et al., 2014). The highest yield gains were reported in China (123 kg ha^{-1} yr^{-1}), Chile (246 kg ha^{-1} yr^{-1}), France (123 kg ha^{-1} yr^{-1}) and Mexico (41.77 kg ha^{-1} yr^{-1}), whereas relatively lower genetic progress were reported in Spain (24 kg ha^{-1} yr^{-1}), Australia

(25 kg ha^{-1} yr^{-1}) and Siberia (15.3 kg ha^{-1} yr^{-1}). Annual yield gains in Egypt, India, and Pakistan were estimated at 27.4 kg ha^{-1} yr^{-1} (0.55%), 21.4 kg ha^{-1} yr^{-1} (0.62%), 111.6 kg ha^{-1} yr^{-1} (1.13%), 32.5 kg ha^{-1} yr^{-1} (0.83%), and 18.5 kg ha^{-1} yr^{-1} (0.5%), respectively (Sharma et al., 2012). Genetic gains among CIMMYT's spring bread wheat in the Elite Spring Wheat Yield Trial (ESWYT) in the past 15 years (i.e. 1995–2009) in 69 countries showed an annual gain of 27.8 kg ha^{-1} (0.65%) (Sharma et al., 2012). The rate of genetic progress in certain instances (e.g. CIMMYT spring wheat improvement programme in Mexico) has been relatively slow and has not reached maximum threshold levels (Aisawi et al., 2018). Countries such as the USA, Chile, France and Brazil, had reportedly reached maximum limits (Brisson et al., 2010; Graybosch and Peterson, 2010; Matus et al., 2012; Beche et al., 2014).

Genetic progress is relatively lower under low-yielding environments compared to high-yielding environments (Lopes et al., 2012; Joudi et al., 2014; Keser et al., 2017; Crespo-Herera et al., 2018b). Therefore, targeted breeding for low-yielding environments (e.g. under drought stressed and high pest and disease pressure environments) is crucial to improve grain yield. Differences in rates of genetic progress across different breeding programmes suggested that newly developed and high-yielding genotypes possess different genetic and adaptation mechanisms to reach their yield potentials (Gummadov et al., 2015).

Genetic gains in grain yield have been attributed to development and deployment of high-yielding wheat genotypes with improved agronomic and physiological traits related with high yield potential (De Vita et al., 2007; Manes et al., 2012; Lopes et al., 2012; Aisawi et al., 2015; Zhang et al., 2016; Gao et al., 2017). For example, in Mexico genetic gains in grain yield were associated with fewer days to heading, cooler and reduced canopy temperatures at grain filling, increased stay-green and thousand kernel weight (Lopes et al., 2012). Similarly, significant yield increases in China resulted from increased grain number per spike, thousand kernel weight, harvest index and plant height (Zhang et al., 2016). Genetic gains among CIMMYT's spring wheat cultivars developed between 1966 and 2009 in Mexico was associated with increased above-ground dry matter and increased seed weight (Lopes et al., 2012; Aisawi et al., 2015). Further improvement in wheat genetic gains is likely to be realized through breeding for important yield-related agronomic and physiological traits

Table 1. 1: Global yield gains in wheat from 1874 to 2014.

Country	Years	Yield change (tons/ha)	Mean yield increment (kg ha−1yr-1)	Genetic gain (% yr^{-1})	Reference
Canada	1885-2008		8	----------	Kamran et al., (2013)
Canada	2005-2014	2.7 -3.1	35.7	----------	Perez-Lara et al., (2016)
China	1981-2008	----------	51.3	0.6	Zheng et al., (2011)
China	1962-2006	----------	62 kg	0.85	Xiao et al., (2012)
China	1960-2000	----------	32.07 to 72.11	0.48 to 1.23	Zhou et al., (2007a)
China	1949-2000	----------	13.96	0.31	Zhou et al., (2007b)
China	1940-2010	----------	22.8	0.48	Sun et al., (2014)
China	1975-2007	----------	103.5	1.09	Zhang et al., (2016)
China	1950-2012	5-8.5	57.5	----------	Gao et al., (2017)
				0.7	
China	1945- 2010	6.08 - 7.37	66	----------	Wu et al., (2016)
Mexico	1977-2008	----------	3.5 g m^{-2} yr^{-1}	0.7	Lopes et al., (2012)
Mexico	1977-2008	----------	6.4 g m^{-2} yr^{-1} (HYE)	0.9	Lopes et al., (2012)
Mexico	1977-2008	----------	3.0 g m^{-2} yr^{-1} (IME)	0.7	Lopes et al., (2012)
Mexico	1977-2008	----------	1.0 g m^{-2} yr^{-1} (LYE)	0.5	Lopes et al., (2012)
Mexico	1994-2010	1.76 to 2.88 (LYE)	31	0.5	Manes et al., (2012)
Mexico	1994-2010	3.78 to 6.02 (HYE)	----------	1	Manes et al., (2012)
Mexico	1961-2005	1 - 2.5	41.77	----------	Ortiz et al., (2008)
Mexico	2013–2014	3.53 to 6.0	57.71 (HYE)	----------	Ortiz et al., (2008)
Mexico	1966-2009	----------	30 (HYE)	0.59	Aisawi et al., (2015)
Mexico	2002–2003	0.15 to 3.5	38.13 (LYE)	----------	Crespo-Herrera et al., (2018)
USA	1950-2009	----------	37	0.98	Green et al., (2012)

Table 1.1: (Continued).

Country	Years	Yield change (tons/ha)	Mean yield increment (kg ha−1yr-1)	Genetic gain (% yr^{-1})	Reference
USA	1959-2008	----------	----------	1.1	Graybosch and Peterson (2010)
USA	1874-2000	----------	10.4	0.48	Fufa et al., (2005)
USA	1971-2008	----------	14.6	0.93	Battenfield et al., (2013)
USA	1968-2002	----------	30.4	1.3	Underdahl et al., (2008)
Brazil	1940-2009	0.17 - 2.14	29	0.92	Beche et al., (2014)
Brazil	1999–2009	----------	16	0.45	Beche et al., (2014)
Brazil	1998-2014	----------	34.8	1	Bornhofen et al., (2018)
United Kingdom	1972-1995	----------	0.12 Mg ha^{-1}yr^{-1}	----------	Shearman et al., (2005)
United Kingdom	1982-2007	----------	74	----------	Mackay et al., (2011)
Spain	1988–2000	----------	24	----------	Royo et al., (2008)
Spain	1980-2009	----------	24	0.44	Chairi et al., (2018)
Italy	1900-1990	----------	19.9	----------	DeVitta et al., (2007)
Italy	1950-2000	----------	25.6	----------	Giunta et al., (2007)
Australia	1958-2007	4.1 - 6.1	25 .0	----------	Sadras and Lawson (2011)
	1901-2014	----------	26.0 (LYE)	0.4	Flohr et al., (2018)
Australia	1958-2011	----------	21	----------	Kitonyo et al., (2017)
France	1950-1996	----------	123	----------	Brisson et al., (2010)
Siberia	1900-2000	2.18 - 3.71	15.3	0.7	Morgounov et al., (2010)
Argentina	1940-1999	----------	51	1.17	Lo Valvo et al., (2018)
Argentina	1999–2011	----------	14	0.18	Lo Valvo et al., (2018)
Iran	1930-2006	----------	31	----------	Joudi et al., (2014)
Iran	1930-2006	----------	20	----------	Joudi et al., (2014)
Chile	1965-2001	----------	246	2.6	Matus et al., (2012)
France	1970–2010	0.065 - 0.137	0.114	----------	Oury et al., (2012)

Table 1.1: (Continued).

Country	Years	Yield change (tons/ha)	Mean yield increment (kg ha−1yr-1)	Genetic gain (% yr^{-1})	Reference
Turkey	1931- 2006	2.9 – 3.8	12.5	0.5	Keser et al., (2017)
Turkey	1931- 2006	0.6 – 1.8	6.1 (LYE)	0.66	Keser et al., (2017)
Turkey	1931- 2006	4.0 -5.1	18.0 (LYE)	0.49	Keser et al., (2017)
Turkey	1963- 2004	4.1 – 5.5	58.0 (HYE)	1.37	Gummadov et al., (2015)

LYE = Low-yielding environment, IME = Intermediate-yielding environment, HYE = High-yielding environment

1.4 Use of agronomic traits in phenotyping wheat

Grain yield in wheat is influenced by several agronomic traits (Chen et al., 2012; Liu et al., 2015) which have been widely explored in wheat improvement programmes to accelerate cultivar development. Due to their high heritability and correlation with grain yield, agronomic traits can be used as indirect selection criteria during breeding and cultivar development (Table 1.2) (Chen et al., 2012; Abdolshahi et al., 2015; Liu et al., 2015; Gao et al., 2017). Moreover, it has been suggested that genetic progress in yield can be achieved if several traits conferring better agronomic and physiological performance with biotic and abiotic stress tolerance are simultaneously selected and introgressed in a single variety (Lopes et al., 2012). Some important agronomic traits that have been exploited in wheat improvement programmes to aid cultivar development and increase grain yield potential and genetic gains are discussed below.

1.4.1 Early flowering and maturity

Breeding novel wheat genotypes with early flowering and maturity is an important objective in wheat breeding programmes (Chen et al., 2016; Mondal et al., 2016; Ochagavía et al., 2018). The focus is developing early maturing wheat genotypes as an adaptive mechanism for environments experiencing terminal heat and drought stress (Motzo and Giunta, 2007; Mondal et al., 2016). Understanding the genetic factors controlling flowering time is essential to manipulate phenological development processes to improve yield potential in wheat (Royo et al., 2018). Most modern wheat genotypes incorporated vernalization and photo-period insensitive genes to promote early flowering and maturity (Chen et al., 2016). Genes conditioning vernalization namely *Vrn-A1, Vrn-B1* and *Vrn-D1* regulate flowering and maturity in wheat (Iwaki et al., 2002). The effect of *Vrn* loci on heading and maturity and grain yield potential are ranked as follows: *Vrn-A1 < Vrn-B1 < Vrn- D1* (Zheng et al., 2013; Zhang et al., 2014; Ogbonnaya et al., 2017) (with singular or combined effect). This resulted in increased days to heading and grain yield under optimal environments, but decreased grain yield under heat prone environments (Zhang et al., 2008; Kamran et al., 2013; Ogbonnaya et al., 2017).

Wheat breeders have developed genotypes combining vernalization to promote early maturity and improve grain yield potential. Canadian spring wheat cultivars possessed *Vrn-A1* gene at a frequency of 94 % (Chen et al., 2016). In Mexico, *Vrn-D1* was

identified in 66% of wheat cultivars, while *Vrn-A1*, *Vrn-B1*, and *Vrn4* were present in 41, 39, and 8% of the cultivars, respectively, either singly or in combination (van Beem et al., 2005). *Vrn-D1* allele showed the highest frequency (64%) among Chinese wheat cultivars followed by *Vrn-A1* (Zhang et al., 2008). This indicated successful breeding using vernalization genes in wheat improvement is variable across different breeding programmes. Breeding strategies to replace the winter-type alleles, especially *Vrn-A1* and *Vrn-D1* loci associated with late heading times (Zhang et al., 2008), has been recommended to develop early-flowering cultivars for water-limited environments. Zhang et al., (2014) reported that the genotypes possessing the *Vrn-A1avrn-B1Vrn-D1a* loci would result in reduced time to anthesis and improve grain yield potential and kernel number in water-stressed environments. Contrastingly, incorporation of *Vrn-D1* is recommended in spring wheat to increase grain yield and improve adaptation to late drought and heat stress tolerance.

Photoperiod sensitive genes namely: *Ppd-D1a, Ppd-B1* and *Ppd-A1* control photoperiod sensitivity impacting on flowering and maturation times in wheat (Langer et al., 2014). The effect of selected photoperiod genes on key agronomic traits in wheat are presented in Table 1.2. Early flowering wheat genotypes with photo-period insensitivity produce high biomass and grain yield, whereas photo-period sensitivity alleles *Ppd-A1b* and *Ppd-B1b* resulted in lower yields (Royo et al., 2018). Conversely, late flowering response was induced by photo-period sensitivity due to the presence of alleles *Ppd-A1b* and *Ppd-B1b.* This produced high dry matter with little advantage in terms of grain yield potential (Royo et al., 2018). Early maturity achieved through early flowering and maturity resulted in positive genetic gains (DeVitta et al., 2007; Motzo and Giunta, 2007; Morgounov et al., 2010; Kamran et al., 2013; Liu et al., 2018). In some cases, yield increase was not associated with earlier flowering in wheat (Chairi et al., 2018; Flohr et al., 2018). The limited genetic gains incorporating early maturity may be due to reduced time available for assimilate partitioning required for high grain yield development (Royo et al., 2007) partly explained by the negative association (Figure 1.1) between kernel weight per spike and heading date (Zhou et al., 2007a).

The combination of *Ppd-D1* and dwarfing gene *Rht5* were reported to have negligible effect on plant growth, flowering time, spike development, and grain yield in wheat. This suggests that exploiting photoperiod-insensitive and dwarfing genes may improve

grain yield by balancing flowering time and yield components (Chen et al., 2018; Ochagavía et al., 2018). Chen et al., (2018) reported that *Ppd-D1* and *Rht5* can shorten the duration of the reproductive phase and facilitate early flowering. *Ppd-D1* can also reduce plant height, whereas the combination of *Ppd-D1* and *Rht5* resulted in shorter plants with increased lodging resistance (Table 1.3). Furthermore, *Ppd-D1* can increase grain number from 6 to 10%, 1000-grain weight (13 to 22%), grain yield (23 and 40%) and harvest index (31 and 50%) from tall and dwarf genotypes, respectively. Canadian spring wheat carrying dominant allele of *Vrn-B1*, photo-period insensitive allele of *Ppd-D1* and height reducing allele *Rht-1* produced shorter plants and higher grain yield (Chen et al., 2016). In some breeding programmes, the photo-period sensitive gene *Ppd-D1b* is being replaced with the photo insensitive gene to develop early maturing genotypes (Kamran et al., 2013). *Vrn-B1* can also act additively with a region on chromosome 2B near the *Ppd-B1* locus, indicating that a shorter vernalization requirement combined with the *Ppd-B1b* allele for photoperiod sensitivity may play a key role in wheat adaptation to varied environmental conditions (Addison et al., 2016). Early-maturing, high-yielding, heat-tolerant wheat genotypes with excellent adaptation to diverse environments that incorporated vernalization, photo-period and dwarfing genes have been developed by CIMMYT and other breeding programmes globally for further breeding (Chen et al., 2016; Mondal et al., 2016; Royo et al., 2018). Negative and significant correlations (Figure 1.1) exists between days to flowering and grain yield potential suggesting that breeding for high yielding and early-maturing wheat genotypes can further be achieved by manipulating wheat phenology (Kamran et al., 2013; Bennani et al., 2016; Mondal et al., 2016). However, such genotypes should have faster growth rates and accumulate enough biomass production in shorter times to increase grain yield potential. Molecular markers linked to vernalization and photo-period genes useful for marker-assisted breeding have been identified in wheat (Chen et al., 2016; Igbal et al., 2007).

1.4.2 Plant height

Breeding novel wheat genotypes with reduced plant height has increased genetic gains in wheat and significantly contributed to increased wheat productivity globally (Beche et al., 2014; Gummadov et al., 2015; Würschum et al., 2015; Zhang et al., 2016). Many wheat improvement programmes have developed wheat genotypes incorporating the dwarfing/height reducing genes namely: *Rht1 (Rht-B1b)*, *Rht2 (Rht-*

D1b), Rht-D1c and *Rht8* (Zheng et al., 2011; Chairi et al., 2018; Green et al., 2012; Lopes et al., 2012; Joudi et al., 2014; Zhang et al., 2016). The genes reduce coleoptile and internode length and plant height (Rebetzke et al., 2011; 2012) resulting in increased grain yield (Grover et al., 2018) by increasing assimilate partitioning to the ear. This resulted in higher harvest index and lodging resistance (Divashuk et al., 2013). Breeding progress to improve lodging resistance and grain yield in wheat resulted in plant height reduction from 130 to 60 cm in China (Gao et al., 2017), 110 to 95 cm in the UK (Berry et al., 2015), 120 to 57 cm in Italy (De Vita et al., 2007), 130 to 60 cm in Brazil (Beche et al., 2014) and from 125 to 65 cm in Spain (Royo et al., 2007) when replacing old by recent and short plant height wheat cultivars. In the USA the genetic progress of breeding for reduced plant height varied from -0.32 to -0.33% yr^{-1} and -0.37 to -0.43% yr^{-1} across varied environments (Graybosch and Peterson, 2010). Zhou et al., (2007a) and Beche et al., (2014) reported a reduction in plant height by -0.69% and -0.74% yr^{-1} among Chinese and Brazilian wheat genotypes, respectively.

To date approximately 24 height reducing genes are reported including *Rht-B1b, Rht-B1c, Rht-B1d, Rht-B1e, Rht-B1f, Rht-B1 g, Rht-D1b, Rht-D1c, Rht-D1d, Rht4, Rht5, Rht7, Rht8, Rht9, Rht12, Rht13, Rht14, Rht16, Rht18*, and *Rht21*). These genes regulate plant height in wheat (McIntosh et al., 2013). The effect of selected height reducing genes on selected agronomic traits are summarized in Table 1.2. However, only a few dwarfing genes have been widely utilized for improving yield in wheat (Chen et al., 2015). Knowledge regarding the function of other dwarfing genes is important for breeding (Zhang et al., 2006). Further, opportunities exist for integrating commonly used height reducing genes (i.e. *Rht1, Rht2, Rht8*) with other dwarfing (GAR) genes such as *Rht4, Rht5, Rht11, Rht12 and Rht24* to improve yield and lodging resistance (Ellis et al., 2005; Rebetzke et al., 2012; Chen et al., 2018; Mo et al., 2018). Combination of *Rht-B1e* with *Rht8* or *Rht-B1b* with *Rht8* reportedly improved grain yield potential (Divashuk et al., 2013). Wheat genotypes with either *Rht-B1b* + *Rht8c* or *Rht-D1b* + *Rht8c* exhibits higher grain yield, spike number, kernel number, thousand grain weight, above-ground biomass, harvest index, stem water-soluble carbohydrates, chlorophyll content and reduced plant height (Gao et al., 2017). The combination of *Rht4+Rht8* dwarfing genes has no effect on leaf length, leaf width and flag leaf area but resulted in reduced grain number per spike and increased 1000–

kernel weight, above-ground biomass and grain yield in wheat (Du et al., 2018). These suggested that combinations of *Rht4* and *Rht8* could reduce plant height to desirable levels, while improving grain yield and yield-related traits in wheat (Du et al., 2018). Similarly, combinations of dwarfing genes *Rht4* and *Rht-B1b* reduce plant height and increase grain yield due to increased grain number, greater spike number and higher harvest index in wheat (Liu et al., 2017) suggesting *Rht4* can be successfully combined with *Rht-B1b* in wheat improvement to accelerate yield gains (Liu et al., 2017). Similarly, *Rht5/Rht8* improved heading date and maturity in wheat (Daoura et al., 2014) useful for breeding and cultivar development (Table 1.3). Tian et al. (2019) showed that a combination of diverse height reducing genes have already been incorporated in elite Chinese wheat genotypes. For instance, combinations of *Rht24+Rht1, Rht24+Rht2, Rht24+Rht8, Rht1+Rht8, Rht2+Rht8, Rht24+Rht1+Rht8, Rht24+Rht2+Rht8* occurred at frequencies of 86, 117, 137, 56, 77, 47 and 70%, respectively in Chinese wheat genotypes.

A dwarfing gene *Rht5* has been shown to reduce plant height by approximately 40% without affecting coleoptile length and seedling vigour (Chen et al., 2018). However, *Rht5* can reduce spike length by approximately 16.7 and 22.6%, grain number by 11.5 and 14.5%, 1000-grain weight by 18.4 and 24.1% and grain yield by 21.5 and 35.1% and delayed ear emergence and anthesis time, thus hindering effective utilization in wheat improvement (Chen et al., 2018). Therefore, genes promoting plant development and flowering times need to be incorporated with *Rht5* dwarf lines to exploit their potential in wheat breeding programmes. The combination of *Rht5* with other dwarfing genes to improve genetic gains in grain yield remains unexplored and un-investigated (Chen et al., 2018). Recently, a dwarfing gene *Rht25*, with *Rht25a* representing the height-increasing allele and *Rht25b* designated the dwarfing allele were identified in wheat (Mo et al., 2018). The average dwarfing effect of *Rht25b* was found to be approximately half of the effect observed for *Rht-B1b* and *Rht-D1b*, and the effect greater in the presence of height-increasing *Rht-B1a* and *Rht-D1a* alleles than in the presence of the dwarfing alleles (Mo et al., 2018). *Rht25b* is gibberellin acid sensitive gene and shows significant pleiotropic effects on coleoptile length, heading date, spike length, spikelet number, spikelet density and grain weight (Mo et al., 2018). Therefore, *Rht25* represents may serve as an alternative dwarfing gene to improve wheat yield potential across diverse environments (Mo et al., 2018).

Some studies suggested that wheat plant height has reached its theoretical limit at about 70 to 80 cm, suggesting that limited progress will be achieved through further reduction in plant height (Shearman et al., 2005). As a result, plant height cannot be decreased any further to avoid risking reductions in biomass and grain yield (Berry et al., 2015). Therefore, strategic breeding that combines both plant height and grain yield to maximise yield potential and lodging resistance has been suggested (Gao et al., 2017). GAR dwarfing genes, such as *Rht4, Rht5, Rht8, Rht11, Rht12, Rht13, Rht24 and Rht25* have the potential to reduce plant height further (Rebetzke et al., 2012; Chen et al., 2018; Mo et al., 2018). These genes (i.e. *Rht4, Rht5, Rht8, Rht11, Rht12, Rht13, Rht24 and Rht25*) have negligible effects on biomass production, whereas some (i.e. *Rht4, Rht12*; *Rht13; Rht24*) can increase above-ground biomass, kernel weight, and grain yield (Rebetzke et al., 2012; Yang et al., 2015; Würschum et al. 2017b; Tian et al., 2019). The *Rht24b* allele is already used in combination with the two *Rht-1b* semi-dwarfing genes in wheat breeding (Würschum et al., 2017b; Tian et al., 2019). As a result, *Rht24* utilization has increased in European countries, China and the USA, indicating that wheat breeders have actively selected for this locus for cultivar development to improve lodging resistance and grain yield potential (Würschum et al., 2017b; Tian et al., 2019). *Rht24* occurs at a frequency of about 84.2% than other important dwarfing alleles in elite wheat varieties in China and usually couples with *Rht2* or *Rht8* (Tian et al., 2019). Similarly, Würschum et al., (2017b) also showed that *Rht24* occurred at high frequency of approximately 67% compared with GR genes and *Rht8* in >1000 wheat varieties originating mainly from Europe. However, while transferring height reducing genes to well-adapted wheat genotypes, attention should be directed to selection of the most suitable adapted parents as the effect of the gene vary with different genetic backgrounds (Yang et al., 2015). Additionally, very limited information is available detailing the effect of dwarfing genes on wheat physiological processes which may limit effective breeding targeting such traits.

1.4.3 Harvest index

Harvest index (HI) has accelerated breeding for improved grain yield potential in wheat. For example, HI in wheat improved from approximately 0.25 to 0.44 (Gao et al., 2017) and 0.26 – 0.55 (Zhang et al., 2016) in China, 0.42 – 0.46 in the USA (Green et al., 2012), 0.26 – 0.42 in Spain (Royo et al., 2007), 0.21– 0.43 in Australia (Flohr et

al., 2018), 0.41– 0.43 in Italy (Giunta et al., 2007) and 0.28 – 0.36 in Turkey (Gummadov et al., 2015). Additionally, gains in HI increased at an average of 0.51 and 0.63% yr^{-1} in China (Zhou et al., 2007a; Gao et al., 2017), 0.19% yr^{-1} in Italy (Giunta et al., 2007) and 0.002 % yr^{-1} in Australia (Flohr et al., 2018). Despite significant improvement in HI, the trait has remained at approximately 0.55 which is below a theoretical limit of 0.62 (Gaju et al., 2009). In China, HI of some widely cultivated cultivars released between 1945 and 2010 have reportedly reached their theoretical maximum limit suggesting future gains in yield may depend on achieving greater harvest biomass production, while maintaining harvest index (Shearman et al., 2005). A linear and positive relationship was observed between HI with grain yield (Figure 1.1) over time suggesting that HI can improve yield gains even further (Zheng et al., 2011).

1.4.4 Biomass production

Increased biomass has resulted in grain yield improvement in wheat. The increase in biomass has been largely attributed to higher photosynthetic rate, stomatal conductance, leaf chlorophyll content and improved radiation-use efficiency (Bustos et al., 2011). It has been suggested that further improvements in grain yield can be achieved by increasing photosynthetic capacity by optimizing biomass production while maintaining lodging resistance (Beche et al., 2014). Several studies showed that biomass contributed significantly to increased grain yield (Shearman et al., 2005; Xiao et al., 2012; Bustos et al., 2013; Aisawi et al., 2015; Gao et al., 2017), whereas other studies indicated very little contribution of this trait (Royo et al., 2007; Tian et al., 2011; Zheng et al., 2011; Sun et al., 2014; Zhang et al., 2016). In China, Gao et al. (2017) reported that biomass accumulation significantly increased by 0.39% yr^{-1} or 62.6 kg ha^{-1} yr^{-1}, among new Chinese wheat cultivars. Reynolds et al., (2017) reported that crossing complementary genotypes exhibiting high biomass and HI may improve yield gains in wheat than crossing only high yielding genotypes. Zheng et al., (2011) also reported that further increases in above-ground biomass and HI may continue to contribute to grain yield improvement in genotypes within optimum plant height. However, the negative relationship between plant height with HI and biomass may offset such gains (Figure 1.1). In some instances, positive association has been reported (Aisawi et al., 2018) which further suggests manipulation of this trait can improve genetic gains in grain yield even further.

1.4.5 Kernel weight

Grain yield improvement has been significantly (Figure 1.1) associated with increased total kernel weight (TKW) (Zhou et al., 2007a; Morgounov et al., 2010; Tian et al., 2011; Zheng et al., 2011; Lopes et al., 2012; Aisawi et al., 2015). On the contrary, non-significant contribution of TKW were reported (Shearman et al., 2005; Royo et al., 2007; Acreche et al., 2008; Brisson et al., 2010; Xiao et al., 2012) especially under heat stress condition limiting the selection response for this trait under low-yielding environments (Sharma et al., 2008; Lopes et al., 2012). Improvement in TKW ranged from 39 to 55 g (Gao et al., 2017) and 29 to 49 g (Zhang et al., 2016) among old landrace varieties and newly-developed Chinese wheat genotypes. Similarly, Giunta et al. (2007) reported TKW of 33 mg to 54–55 mg in old cultivars and 41 mg to 57 mg in modern wheat cultivars. In the USA, Underdahl et al., (2008) also reported improvement in TKW ranging between 20.4 to 33.6 g for old (i.e. 1973) and newly released (i.e. 2004) cultivars, respectively. Additionally, Gao et al., (2017) and Underdahl et al., (2008) reported genetic gains of 0.35% yr^{-1} (0.18 g yr^{-1}) and 0.3% yr^{-1} for TKW among Chinese and American wheat genotypes, respectively. Similarly, Beche et al., (2014) reported increased TKW of 0.03 g yr^{-1} among Brazilian wheat genotypes.

TKW is reportedly linear with moderate to high correlation with grain yield (Morgounov et al., 2010; Zheng et al., 2011; Qin et al., 2015; Gao et al., 2017) (Table 1.3) suggesting selection of heavier grains could be highly effective for improving wheat yield gains. As a result, increasing grain weight potential at specific positions within the spikelet has been suggested (Calderini and Reynolds, 2000), rather than breeding for higher TKW. Breeding for high grain number and TKW in the same genotype has been reported to be difficult due to trade-offs. Gaju et al., (2009) suggested trade-off can be minimized by selecting genotypes with higher number of spikelets per spike. These authors showed that genotypes with high spikelet number resulted in spikes with higher grain number and heavier TKW. An alternative approach involving crossing of suitable genotypes possessing contrasting grain number and grain weight to combine both traits in the progeny has also been proposed by Bustos et al., (2013). These authors reported an increase in grain yield combining both traits confirming the possibility that crossing genotypes expressing high grain number with those

expressing high TKW (and with similar yield and biomass) might be a useful strategy to increase yield potential in wheat.

1.4.6 Number of grains per spike

The number of grains per spike has been identified as an important trait for improving grain yield (Yu et al., 2014; Alonso et al., 2018; Würschum et al., 2018; Liu et al., 2018). Yield gains resulting from improvement in grain number has been reported (Tian et al., 2011; Flohr et al., 2018; Liu et al., 2018) suggesting successful selection in wheat breeding (Xiao et al., 2012; Aisawi et al., 2015); whereas in some instances it was not associated with genetic progress in grain yield (Zhou et al., 2007a; Xiao et al., 2012; Gao et al., 2017). Chairi et al. (2018) reported grain yield increases of 24 kg ha^{-1} y^{-1} (0.44% yr^{-1}) between 1980 and 2003 attributed to high number of kernels spike^{-1} (0.24 kernels spike^{-1} yr^{-1}) in Spain. Similarly, Joudi et al. (2014) reported improvement of grain number per spike of 35 grains m^{-2} yr^{-1} through breeding and selection spanning over 50 years in Iran. Grain number among Brazilian wheat genotypes was increased by 77.89 grains yr^{-1}. In China grain number per spike varied between 26 for landraces developed in 1941 to 38 for improved wheat genotypes released between 2007 and 2011 (Zhang et al., 2016). Among wheat cultivars developed in the USA, number of grains per spike varied between 25 and 38 for old and modern cultivars (Green et al., 2012).

Although indirect selection for genotypes with a higher grain number has been effective, the negative correlation between the number of grains per spike and thousand kernel weight (Figure 1.1) suggests that further increases in number of grains would be partially offset by reductions in grain weight (Sadras and Lawson, 2011; Bustos et al., 2013). Therefore, an increase in the number of spikelets can be selected concurrently with increased spike length, to offset an increase in spike compactness (Würschum et al., 2018). The relationship between grain yield and grain number is reportedly curvilinear in some instances (Table 1.3) suggesting that the strategy for increasing grain yield through higher grain number could be less efficient (Sadras and Lawson, 2011; Calderini et al., 2012; Bustos et al., 2013). On the contrary, the linear relationship reported between grain number per spike and grain yield suggest the likelihood of this trait in improving grain yield potential in some instances (Tian et al., 2011; Qin et al., 2015).

1.4.7 Spike fertility

Spike fertility (SF) is a grain yield component that influence the increase in the number of kernels per spike (Reynolds et al., 2017; Würschum et al., 2018). For instance, selection for spike fertility either solely or in combination result in higher grain yield, than selecting for high yield alone (Alonso et al., 2018). Increase in the number of kernels per spike were attributed to increased SF (Würschum et al., 2018). In addition, SF is a highly heritable trait, controlled by several genes with additive effects (Alonso et al., 2018). Number of kernels per spike and spikelet fertility are significantly and positively correlated but negatively correlated with kernel weight (Würschum et al., 2018). These suggested increase in either number of kernels per spike and spikelet fertility will likely reduce TKW and grain yield potential in wheat. This effect suggests that improvement in grain yield can be achieved through an integrated approach targeting several yield-component traits (Würschum et al., 2018). Novel wheat genotypes possessing large spikes (e.g. high assimilate partitioning to spike, long rachis, high spikelet number per spike, high fertile florets per spikelet) are maintained by CIMMYT useful of breeding (Gaju et al., 2009). In China, wheat genotype Zhongmai 895 released in 2012 with a yield potential at 8906 kg ha^{-1} was derived from 'Zhoumai 16' x 'Liken 4'. Zhoumai 16 was developed from 'Yumai 21' x 'Zhou 8425B' whereby Zhou 8425B is characterized by large spikes and high TKW (Gao et al., 2017), demonstrating the feasibility of incorporating large spikes in wheat improvement programmes.

Other spike characteristics useful for breeding include spike length (SL), number of spikelets per spike (SPS) and spike compactness (SC) (Chairi et al., 2018; Würschum et al., 2018). The number of kernels per spike is positively and moderately correlated with SPS and SL (Figure 1.1) whereas SL is positively correlated to SC (Würschum et al., 2018). Spike characteristics are highly heritable traits with heritability values of 0.90 for SL, 0.92 for SPS, 0.93 and 0.67 for SC (Würschum et al., 2018). De Vita et al. (2007) reported that SL and SPS did not improve grain yield potential of durum wheat cultivars released in Italy between 1900 and 1990. The contribution of other spike traits as selection criterion for advancing grain yield genetic gains in wheat are yet to be explored.

1.4.8 Number of productive tillers

Number of productive tillers defined as the number of tillers that produce spikes and seeds, is a key agronomic trait that affect biomass production and grain yield potential in wheat. Wheat genotypes with reduced tillering capacity are more productive than free-tillering genotypes under drought stressed conditions (Narouka et al., 2011; Wang et al., 2016) due to reduced sterile spikelets (Gaju et al., 2014). Contrastingly, Sadras and Rebetzke (2013) reported that lines possessing the free-tillering allele showed increased tiller production which was related to increased grain yield potential under high-yielding environments. Several tiller inhibition genes (Duggan et al., 2005; Mitchell et al., 2012; Wang et al., 2018) and tiller promoting genes (Naruoka et al., 2011) have been identified in wheat useful for improving wheat grain yield. *Tin1* tiller inhibition gene can increase grain number per spike (Duggan et al., 2005; Gaju et al., 2014) and harvest index from 0.31 to 0.35 (Motzo et al., 2004). Therefore, the introgression of the *Tin1* gene into modern wheat germplasm may offer opportunities to increase grain number per spike, grain m^{-2}, harvest index and ultimately grain yield improvement in wheat (Gaju et al., 2014) to improve grain yield.

1.4.9 Leaf morphology and its component traits

Optimal flag leaf morphology can improve light absorption, which improves photosynthesis and grain yield potential (Liu et al., 2018a). Leaf traits such as flag leaf angle (FLAN), flag leaf width (FLW), flag leaf length (FLL), the ratio of length/width of flag leaf (FLR) and flag leaf area (FLA) may be useful for improving grain yield in wheat. FLL, FLW and FLA are reported correlated with some important agronomic traits (Liu et al., 2018 a, b). Additionally, FLL, FLW and FLA have been reported to be significantly and positively correlated to spike length, grain weight per spike and grain number per spike (Figure 1.1) (Fan et al., 2015; Liu et al., 2018a;b Wu et al., 2018; Zhao et al., 2018) indicating leaf traits influence yield-related traits (Liu et al., 2018a). Wheat genotypes with relatively larger flag leaf size tends to produce more kernel number per spike and higher kernel number per spike (Zhao et al., 2018), suggesting appropriate flag leaf size could promote development of high grain yield potential. FLA is reportedly the most yield contributing trait, followed by FLW and FLL (Fan et al., 2015). In the USA, Balota et al., (2018) reported that yield gains in soft red winter wheat developed between 1919 to 2009 were associated with reduced leaf area

suggesting yield increases were achieved through selection of smaller leaf size. Broad-sense heritability for FLAN, FLW, FLL, FLR and FLA are reportedly higher (> 70%), indicating that flag leaf traits could be targeted for breeding and cultivar development (Wu et al., 2016; Liu et al., 2018a).
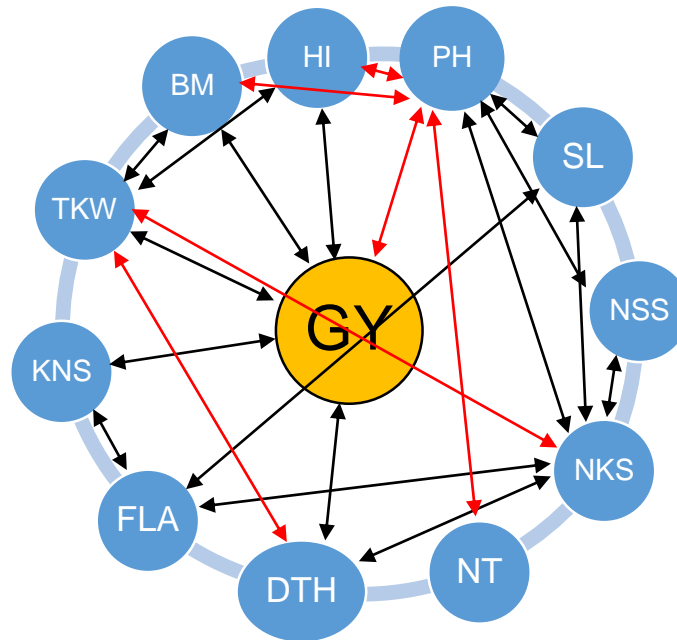
Figure 1. 1: Associations between grain yield and its components in wheat. Black and red arrows indicate positive and negative correlations,, respectively (Giunta et al., 2007; Zhou et al., 2007a; Zheng et al., 2011; Rebetzke et al., 2012b; Beche et al., 2014; Wu et al., 2016; Gao et al., 2017; Alonso et al., 2018; Dauora et al., 2018; Ogbonnaya et al., 2018; Würschum et al., 2018. PH = plant height, SL = spike length, NSS= number of spikelets per spike, NKS = number of kernels per spike, NT = number of tillers, DTH= Days to heading, FLA = flag leaf area

Table 1. 2: Increased (+), reduced (-), no change (#) or not yet known (?) effect of selected height reducing, photoperiod and vernalization genes on key agronomic traits in wheat.

Gene name	DH	PH	HI	BM	FLL	FLW	FLA	TKW	GNPS	GY	References
Plant height											
Rht4	?	-	?	-	+	+	+	-	-	-	Du et al., (2018)
Rth4	?	-	#	?	?	?	?	-	+	#	Liu et al., (2017)
Rht4	?	-	+	+	?	?	?	?	+	+	Rebetze et al., (2012a)
Rht5	?	+	-	?	?	?	?	-	-	-	Chen et al., (2018)
Rht5	-	-	?	?	?	?	?	?	-	?	Daoura et al., (2014)
Rht5	+	-	?	?	?	?	?	?	-	?	Rebetze et al., (2012a)
Rht8	?	-	+	-	?	?	?	+	?	-	Wang et al., (2015)
Rht8	?	-	?	?	?	?	?	?	?	?	Rebetze et al., (2012a)
Rht8c	?	-	?	?	?	?	?	?	+	?	Rebetze et al., (2012b)
Rht12	?	-	+	+	?	?	?	?	+	+	Rebetze et al., (2012a)
Rht13	?	-	#	-	?	?	?	#	#	-	Wang et al., (2015)
Rht13	?	-	?	?	-	?	?	?	?	?	Wang et al., (2014)
Rht13	?	-	+	?	?	?	?	?	+	?	Rebetze et al., (2011)
Rht13	?	-	+	+	?	?	?	?	+	+	Rebetze et al., (2012a)
Rht18	?	-	+	-	?	?	?	-	+	-	Yang et al., (2015)
RhtB1b	?	-	?	?	?	?	?	?	+	?	Rebetze et al., (2012a)
RhtB1b	?	-	+	-	?	?	?	-	+	+	Liu et al., (2017)
RhtD1b	?	-	?	?	?	?	?	#	?	?	Wang et al., (2014)
Rht-B1b	?	-	?	?	?	?	?	?	+	?	Rebetze et al., (2012b)
Rht-D1b	?	-	+	+	?	?	?	?	?	+	Rebetze et al., (2012b)
Vernalization											
Vrn-B1	#	#	#	#	#	#	#	#	#	#	Chen et al., (2018)
Photoperiod											
Ppd-D1	-	-	+	?	?	?	?	+	+	+	Chen et al., (2018)
Ppd-A1	+	?	+	?	?	?	?	?	?	+	Royo et al., (2018)
Ppd-A1b	+	?	?	+	?	?	?	?	?	#	Royo et al., (2018)
Ppd-B1b	+	?	?	+	?	?	?	?	?	#	Royo et al., (2018)
Combinations											
Ppd-A1b+ Ppd-B1b	-	?	+	?	?	?	?	?	?	+	Royo et al., (2018)
Ppd-D1+Rht5	-	#	#	#	#	#	#	#	#	#	Chen et al., (2018)
Rth4+ RhtB1b	?	-	+	?	?	?	?	?	+	+	Liu et al., (2017)
Rht4+Rht8	?	-	?	+	#	#	#	+	-	+	Du et al., (2018)
Rht13+ Rht8	?	?	?	?	?	?	?	?	-	?	Wang et al., (2015)
Rht13+ RhtD1b	?	-	?	?	?	?	?	#	?	?	Wang et al., (2014)
Rht8c+Rht-B1b	?	-	+	+	?	?	?	?	?	+	Rebetze et al., (2012b)
Rht8c+Rht-D1b	?	-	+	+	?	?	?	?	?	+	Rebetze et al., (2012b)

DH = Days to heading, PH = plant height, HI = Harvest index, BM = Biomass, FLL = Flag leaf length, FLW = Flag leaf width, TKW = Thousand kernel weight, GNPS = Grain number per spike, GY = Grain yield.

1.5 Phenotyping based on physiological traits in wheat

Knowledge of physiological traits associated with genetic gains in yield is important for breeding (Aisawi et al., 2015; Beche et al., 2014; Zhang et al., 2016). It has been reported that breeding wheat genotypes incorporating physiological traits can improve grain yield genetic gains by approximately 50% (Flood et al., 2011; Reynolds et al., 2011). Physiological traits that have contributed to grain yield improvement in wheat are discussed below.

1.5.1 Canopy temperature

Canopy temperature (CT) has significantly played a key role in improving yield potential in wheat (Lopes et al., 2012; Gao et al., 2017). Cooler plant canopy during mid-grain filling is linked to higher drought tolerance and yield under water-limited condition (Thapa et al., 2018). Breeding genotypes with reduced CT resulted in lowering these traits from 30 to 29° C in CIMMYT spring bread wheat programme spanning over 30 years (Lopes et al., 2012). Similarly, genetic gains in CT increased by 0.12% yr^{-1} among Chinese wheat cultivars (Gao et al., 2017). Further, a significant negative linear relationship existed between CT and grain yield (Figure 1.2) with year of cultivar release (Lopes et al., 2012; Thapa et al., 2018) indicating possibilities to reduce CT further to increase grain yield in wheat (Lopes et al., 2012).

1.5.2 Chlorophyll content

Chlorophyll content is useful trait for breeding for high grain yield potential in wheat. Several reports have shown some breeding progress incorporating this trait with new wheat cultivars showing slightly high chlorophyll content than old cultivars (Beche et al., 2014). Increased post-anthesis chlorophyll content is positively and moderately correlated with harvest index, leaf canopy temperature, water soluble carbohydrates and grain yield (Figure 1.2) (Lopes et al., 2012; Gao et al., 2017). The stay-green trait which is related to retention of chlorophyll content has been identified as a key target trait for improving light interception and utilization and can contribute to increased wheat yield (Cossani and Reynolds, 2012). Similarly, Lopes and Reynolds (2012) showed that stay-green was correlated with yield under heat stress and heat combined with drought in spring wheat. Therefore, selection for stay-green trait in promising wheat genotypes will likely increase the rate of genetic progress for adaptation of

wheat under both well-watered and water-limited environments (Christopher et al., 2018). The linear association reported between stay-green trait and grain yield improvement suggest the latter can be targeted for cultivar development (Lopes et al., 2012; Beche et al., 2014).

1.5.3 Enhanced photosynthetic capacity

Understanding changes in photosynthetic capacity among elite wheat genotypes is important for improving yield gains (Zheng et al., 2011; Parry et al., 2011; Reynolds et al., 2012). In China, changes in leaf photosynthesis were not associated with improved grain yield in 70 years of wheat improvement (Chen and Hao 2015). The lack of correlation between genetic changes in photosynthetic rate and yield increase suggested that leaf photosynthesis does not limit/improve grain yield development or that cultivar development has not specifically targeted improved photosynthetic capacity. As result, determinants of sink strength should be targeted for increasing yield rather than selection for higher photosynthetic rates under drought stress condition (Chen and Hao, 2015).

Conversely, genetic gains in rates of post-anthesis net photosynthesis were closely and positively correlated with grain yield (Figure 1.2) (Zheng et al., 2011; Beche et al., 2014). Fischer et al. (1998) also reported that wheat yield gains were associated with higher stomatal conductance and increased photosynthetic rate. Other photosynthesis-related traits such transpiration rate, stomatal conductance or water-use efficiency (WUE) were reported non-influential on yield development (Chen and Hao, 2015) whereas other studies (i.e. Sayre et al., 1997; Beche et al., 2014) reported improved genetic gains. CIMMYT's heat and drought tolerant wheat genotypes showed genetic gains in yield with correlation to physiological traits (Lopes et al., 2012). Positive relationships have been reported between photosynthetic rate and chlorophyll content (Figure 1.2) suggesting increased chlorophyll content improves photosynthetic efficiency (Zhang et al., 2009).

Positive correlations have been reported between photosynthetic rate with stomatal conductance (Figure 1.2) and biomass production (Beche et al., 2014) suggesting enhanced stomatal conductance and photosynthetic rate increases the rate of biomass accumulation (Parry et al., 2011). To improve photosynthetic efficiency, crosses can be conducted between adapted wheat cultivars with those exhibiting high-

photosynthetic rates. Higher yield levels can be achieved by integrating photosynthesis related traits (e.g. stomatal conductance and transpiration rate) with yield-related agronomic traits (Zhang et al., 2016) to develop genotypes with higher yield potential (Rebetzke et al., 2013).

1.5.4 Water soluble carbohydrates

Water-soluble carbohydrate (WSC) significantly improved yield gains in wheat (Shearman et al., 2005; Foulkes et al., 2007; Gao et al., 2017). In addition, significant correlations with grain yield have been reported between radiation-use efficiency and WSC in wheat (Figure 1.2) (Shearman et al., 2005; Folkes et al., 2007), which suggested that genetic gains in wheat yield is driven by improved growth rate due to increased accumulation of WSC (Shearman et al., 2005). Gao et al. (2017) reported genetic gains in WSC of 0.81% yr^{-1} among Chinese wheat genotypes, respectively. Genotypes with high WSC are commonly shorter, flower and mature earlier, and produce significantly fewer tillers than those with low WSC (Rebetzke et al., 2008), suggesting cultivar development targeting incorporation of plant height (e.g. *Rht-B1*) and/or anthesis date genes (e.g. *Ppd1*) resulted in improvement of WSC. In addition, wheat genotypes with high WSC produce more fertile tillers, reduced days to anthesis, increased biomass, grain number and grain yield than genotypes with low WSC (Rebetzke et al., 2008). Grain weight is high in genotypes with high WSC during early grain filling stages, indicating that more available assimilates contribute to higher grain weight potential (Dreccer et al., 2009). Further, WSC accumulation ability and remobilization efficiency in drought tolerant cultivars is much higher than those in sensitive wheat genotypes (Hou et al., 2018) suggesting increased WSC enhances drought tolerance in wheat (Hou et al., 2018). It has been suggested that cultivar development may have targeted improvement of photosynthetic efficiency which has driven increases in number of grains and a larger source for grain filling through increases in stem WSC (Shearman et al., 2005). Moderate to high heritability of WSC (Ruuska et al., 2006; Rebetzke et al., 2008) suggest breeding for either high or low concentration of WSC is possible.
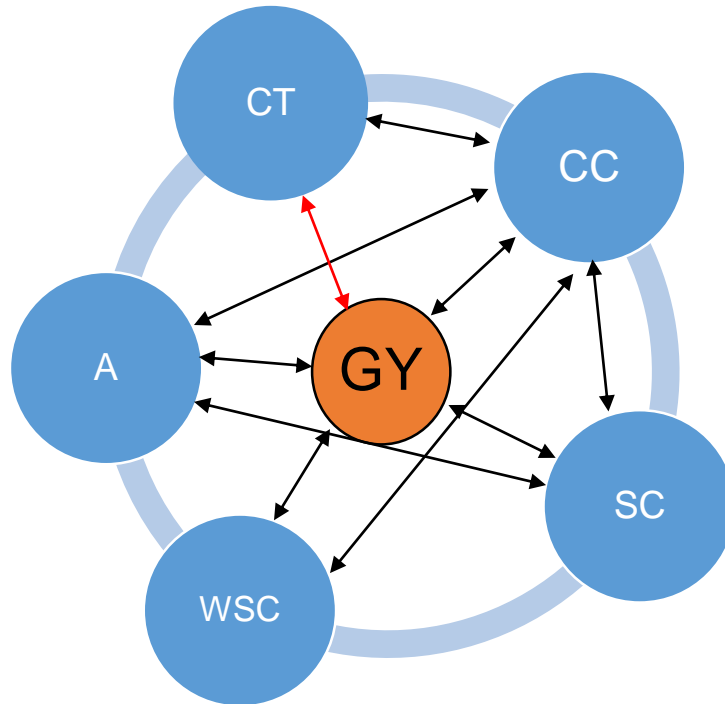
Figure 1. 2: Associations between grain yield and physiological traits and between physiological traits in wheat. Black and red arrows indicate positive and negative correlations, respectively (Tian et al., 2011; Chen et al., 2012; Lopes et al., 2012; Beche et al., 2014; Gao et al., 2017). A = Photysnthetic rate, CT = canopy temperature, CC = chlorophyll content, SC = stomatal conductance, WSC = water-soluble carbohydrates.

1.6 Water use efficiency

Water use efficiency (WUE) refers to the ratio of grain yield or biomass per unit water used by the plant during crop growth (Blum 2005). It is considered an important physiological trait for improving grain yield in wheat under water stress conditions (Deng et al., 2006; Silva et al., 2013; Varga et al., 2015). It is also essential for determining the level of drought tolerance in crops (Blum 2009; Lawlor and Tezara 2009). However, Araus et al. (2003), reported negative correlation between WUE and drought tolerance under water scarce conditions. Two major components of WUE at the leaf level are recognised: Instantaneous water-use efficiency (WUEinst) and intrinsic water-use efficiency (WUEi). WUE$_{inst}$ is defined as the ratio of photosynthetic rate and transpiration rate, whereas, WUE$_i$ is defined as the ratio of photosynthetic rate and stomatal conductance (Ehleringer et al., 1993; Tambussi et al., 2007). WUE$_{inst}$ and WUE$_i$ are considered important short-term physiological measurements of water-use efficiency (Ehleringer et al., 1993).

WUE at the leaf level can be enhanced by the reduction of stomatal conductance per the amount of CO_2 assimilated or by improving the assimilation rate at a given stomatal conductance (Sonja et al., 2018). Under both circumstances, intercellular CO_2 concentration (C_i) is lowered and consequently leading to an increased stomatal CO_2 gradient (Long and Bernacchi, 2003; Williams et al.,2004). An increase in WUE at the leaf level has been observed in several crops including wheat when plants reduce stomatal conductance (Sonja et al., 2018). However, a reduced stomatal conductance has been reported to reduce the transpiration rate and further lower the photosynthetic capacity of the plants, which may result in low grain yield (Flexas et al., 2016; Flexas et al., 2004). A couple of studies in various crops under water stressed conditions, reported an improved WUE due to a reduced stomatal conductance (Van den Boogaard et al. 1997; Condon et al., 2002). Therefore, a reduced stomatal conductance may be a useful physiological attribute for the evaluation of breeding material for improved WUE under stress conditions, however, this may lead to substantial lower grain yield.

1.7   Wheat genetic resources for improving wheat grain yield genetic gains

Exploration of wheat genetic resources is useful to identify sources of variation for agronomic and physiological traits and discovery of new alleles for improving grain yield potential (Zhang et al., 2016; Reynolds et al., 2017; Liu et al., 2018). Wheat genetic resources including landrace varieties, synthetic cultivars and wild relatives including *Triticum tauschii* L. and wild emmer wheat (*T. dicoccoides* Korn (2n = 28, AABB) possess useful source of alleles for enhancing drought tolerance and improving yield and its component traits (Gororo et al., 2002; Moeller et al., 2014; Cossani and Reynolds, 2015; Mohammadi et al., 2015; Gaju et al., 2016; Merchuk-Ovnat et al., 2016; Pinto et al., 2017; Reynolds et al., 2017; Liu et al., 2018). In China, about 48 improved wheat genotypes released between 2011 and 2016 were developed using synthetic wheat (Liu et al., 2018). Reynolds et al. (2015) identified synthetic wheat genotypes with high biomass and yield expression, and physiological traits such as higher leaf photosynthetic rate (Del Blanco et al., 2000) and lower leaf transpiration rates (Pinto et al., 2017).

Modern high-yielding cultivars that incorporated genes from synthetic-wheat tend to have higher gas exchange rates compared to older cultivars (De Vita et al., 2007; Sadras and Lawson, 2011; Xiao et al., 2012; Beche et al., 2014). Cossani and Reynolds, (2015) identified a set of advanced wheat lines derived from synthetic

hexaploid wheat with high levels of heat tolerance incorporating several drought adaptive mechanisms such as such as higher crop growth rate, increased water-soluble carbohydrates storage in stems, cooler canopy temperature, and spectral indices which are related to pigment composition, photo-protective mechanisms, and increased radiation use efficiency. These traits result in increased number of grains, growth of taller stems with a greater water-soluble carbohydrates storage capacity significantly related to increased kernel weight (Cossani and Reynolds, 2015). Tetraploid wheat (*T. turgidum* L.) is also identified as a useful genetic resource for wheat breeding possessing functional genes that surpass the early maturity effect caused by the early flowering allele *Ppd-A1a* found in *T. turgidum* L. ssp. turgidum conv. pyramidale) (Nishimura et al., 2018). Wild emmer wheat is also considered a promising source of useful genes for improving stress resistance, grain protein quality and quantity and micronutrient concentrations in domesticated wheat (Xie and Nevo, 2008; Merchuk-Ovnat et al., 2016).

Wheat genotypes with drought and heat tolerance that incorporated genes from landraces and synthetic wheat have been developed for cultivation in arid and semi-arid environments to boost grain yield potential (Manes et al., 2012; Crossani and Reynolds, 2015; Mondal et al., 2016; Pinto et al., 2017; Crespo-Herrera et al., 2018). Further, molecular and physiological characterization of wheat genetic resources is useful to increase the probability of achieving cumulative gene action to improve yield gains (Ortiz et al., 2008; Reynolds et al., 2017).

1.8 Quantitative trait loci (QTLs) associated with agronomic and physiological traits in wheat

Quantitative trait loci (QTL) mapping of agronomic and physiological traits is important for marker-assisted breeding in wheat (Huang et al., 2003; Faji et al., 2018; Liu et al., 2018a). Agronomic and physiological traits are controlled by several QTL (Tables 1.3 & 1.4). Several multiple QTLs linked to agronomic traits have also been identified such as *QTLs QTn.ipk-5D*, *QTn.ipk-2D*, *QTn.ipk-3B* and *QTn.ipk-1B* which are associated with productive tiller number (Huang et al., 2003). QTL *QFlt.dms-2D*, *QFlt.dms-5B, QFlt.dms-2D*, *QFlt.dms-7A* and *QFlt.dms-6B.2* are linked to days to flowering; whereas, QTLs *QMat.dms-2D*, *QMat.dms-2D*, *QMat.dms-7A.2* and *QMat.dms-4A.1* are associated with days to maturity (Perez-Lara et al., 2016). About 40 QTL's

associated with kernel morphological traits such as kernel length, kernel width, kernel thickness, kernel length/width ratio, kernel length/thickness ratio and kernel width/thickness ratio have been recently mapped in wheat (Chen et al., 2019). New QTLs linked to flag leaf length, flag leaf width and flag leaf area were recently identified and mapped in wheat (Liu et al., 2018). The identified QTLs can be transferred to new or well-adapted cultivars to improve yield in wheat (Zhang et al., 2018).

Maqsood et al., (2017) identified QTL linked to relative water content, cell wall membrane thermo-stability and photosynthetic rate. Christopher et al. (2018) identified several QTLs associated with the stay-green trait in wheat. QTLs for photosynthetic rate were identified on chromosomes 2A, 7A, 6A and 7D (Ilyae et al., 2014; Malik and Malik, 2015). In certain instances, genetic regions linked to physiological traits (e.g. stay-green) were co-located with QTL for yield –related traits yield (Acuna-Galindo et al., 2014). Genomic regions have also been reported for grain yield, thousand kernel weight, biomass and days to heading which suggested that a group of linked and (or) co-located QTL affected phenological and yield-related traits (Tahmasebi et al., 2017). QTL involved in days to heading and thousand grain weight suggested that early maturity would favour the post-anthesis grain-filling periods and increased grain size and grain yield (Tahmasebi et al., 2017). QTL for chlorophyll content, water-use efficiency, photosynthetic rate and internal CO_2 concentration were co-located with QTL for grain yield and/or yield components (Xu et al., 2017). QTL which simultaneously control flag leaf traits and yield related traits have also been identified on chromosomes 1B, 2D, 4A, 4D, 4B, 5A. 5B, 6B, 6D, and 7D in wheat (Fan et al. 2015; Wu et al., 2016). Such pleiotropic effects are useful to understand relationships among QTLs and pyramiding favourable alleles in different genetic loci (Hai et al. 2008). Marker-assisted recurrent selection involving pyramiding of important QTL can improve grain yield potential in wheat (Gahlau et al., 2017). Generally, QTL mapped for physiological traits are limited in wheat, only few identified for chlorophyll content, normalized difference in vegetation index (NDVI), and canopy temperature (Table 1.4). Though heritability of physiogical traits is relatively low (Chen et al., 2012; Ogbonnaya et al., 2017), their incorporation in breeding programmes may be useful for cultivar development (Lopes et al., 2012). Therefore, to accelerate breeding aimed at improving grain yield genetic gains in wheat, it is important to dissect genomic regions influencing physiological traits and design associated markers for strategic breeding.

Table 1. 3: Key agronomic traits and their quantitative trait loci (QTLs) in wheat.

Trait	QTL name	Location on chromosomes	References
Days to flowering & maturity	*QEps.dms-1B1*	1B	Kamran et al., (2013)
	QEps.dms-1B2	1B	Kamran et al., (2013)
	QEps.dms-5B1	5B	Kamran et al., (2013)
	wPt-741686	7A	Ogbonnaya et al., (2017)
Days to flowering	*QFlt.dms-4A1*	4A	Kamran et al., (2013)
	D993093	5A	Würschum et al., (2017a)
	S3064789	6A	Würschum et al., (2017a)
	D1239681	6D	Würschum et al., (2017a)
	D1093788	7D	Würschum et al., (2017a)
	wPt-2822	6A	Ogbonnaya et al., (2017)
Plant height	*P3176.1*	2D	Wu et al., (2012)
	Xgwm132	6B	Wu et al., (2012)
	Rht24	6A	Würschum et al., (2017b)
	qRht.3A	3A	Würschum et al., (2017b)
	qRht.2D	2D	Würschum et al., (2017b)
	Ppd-D1	2D	Würschum et al., (2017b)
Harvest index	*qHI-2B*	2B	Ehdaie et al., (2016)
	qHI-2D	2D	Ehdaie et al., (2016)

Table 1.3: (Continued)

Trait	QTL name	Location on chromosomes	References
Biomass	*qPBio-7D*	7D	Ehdaie et al., (2016)
	qPBio-2D	2D	Ehdaie et al., (2016)
	qPBio-3A	3A	Ehdaie et al., (2016)
	qPBio-6B2	6B	Ehdaie et al., (2016)
Tillering (low)	*Qltn.sicau-2D*	2D	Wang et al., (2016)
	Qltn.sicau-2B	2B	Wang et al., (2016)
	Qltn.sicau-5A	5A	Wang et al., (2016)
Tillering (High)	*QTn.mst-6B*	6B	Naruoka et al., (2011)
Grain/kernel number per spike	*Xcwm145*	4A	Wu et al., (2012)
	Xgwm219	6B	Wu et al., (2012)
	S3222159	2A	Würschum et al., (2018)
	S1290099	2A	Würschum et al., (2018)
	D1280633	7A	Würschum et al., (2018)
	D1056474	3B	Würschum et al., (2018)
	wPt-730427	2D	Ogbonnaya et al., (2017)
Spike length	*Xbcd1150–Xbarc61*	1A	Yu et al., (2014)
	Xmwg912–Xbarc80	1B	Yu et al., (2014)
	D3027644	2A	Yu et al., (2014)
	S1006957 (Rht24)	6A	Würschum et al., (2018)

Table 1.3: (Continued)

Trait	QTL name	Location on chromosomes	References
Spike length	*D1128060*	3B	Würschum et al., (2018)
	D1109894	6B	Würschum et al., (2018)
	wPt-2872	1A	Ogbonnaya et al., (2017)
Spike fertility	*Xwmc524*	5A	Wu et al., (2012)
	P3474.2	6A	Wu et al., (2012)
	Rht-B1	4B	Würschum et al., (2018)
Spikelets per spike	*D1108041*	2A	Würschum et al., (2018)
	D1128642	7A	Würschum et al., (2018)
	D1082846	7A	Würschum et al., (2018)
	S2255090	7A	Würschum et al., (2018)
	Ppd-D1	2D	Würschum et al., (2018)
	D1208470	5D	Würschum et al., (2018)
Spike compactness	*D1221700*	4A	Würschum et al., (2018)
	S1089640	5A	Würschum et al., (2018)
	D1109152 (Rht24)	6A	Würschum et al., (2018)
	D2254379	7A	Würschum et al., (2018)
	D1100166	2D	Würschum et al., (2018)

Trait	QTL name	Location on chromosomes	References
Grain weight per spike	wPt-6709	1A	Ogbonnaya et al., (2017)
	wPt-6502	4A	Ogbonnaya et al., (2017)
Thousand grain weight	P5322	2B	Wu et al., (2012)
	P2076	3B	Wu et al., (2012)
	IACX987	2A	Sukumaran et al., (2018)
	BobWhite_c30995_403	2B	Sukumaran et al., (2018)
	D3956560	2A	Würschum et al., (2018)
	D1296988	3D	Würschum et al., (2018)
	wPt-2315	1B	Ogbonnaya et al., (2017)
	wPt-0153	2D	Ogbonnaya et al., (2017)
Grain yield	wPt-6832	1B	Ogbonnaya et al., (2017)
	wPt-7883	2B	Ogbonnaya et al., (2017)
	wPt-664276	6B	Ogbonnaya et al., (2017)
	qGY-2B	2B	Ehdaie et al., (2016)
Flag leaf length	*QFLL-2B & QFLL-3A*	4B & 5A	Liu et al., (2018)
	qFll-1B.1	*1B.1*	Fan et al., (2015)
	qFll-2B.2	2B.2	Fan et al., (2015)
Flag leaf width	*QFLW-4B.1*	4B	Liu et al., (2018)
	qFlw-4B.3	4B	Fan et al., (2015)
	qFlw-6B.2	6B	Fan et al., (2015)
Flag leaf area	*QFLA-5A.1*	5A.1	Liu et al., (2018)
	qFla-1B.2	*1B.2*	Fan et al., (2015)
	qFla-5B	*5B*	Fan et al., (2015)
	qFLA-3A	3A	Ehdaie et al., (2016)
Flag leaf angle	*QFLL-4B.1*	4B.1	Liu et al., (2018)

Table 1. 4: Quantitative trait loci (QTLs) of some physiological traits in wheat.

Trait	QTL name	Chromosome location of QTL	References
Canopy temperature	*4A-wmc048d*	4A	Lopes et al., (2013)
	6A-gwm617b	7D-b	Lopes et al., (2013)
Stay-green	*QSG.qgw-3B.1*	3A.1	Christopher et al., (2018)
	QSG.qgw–7B	7B	Christopher et al., (2018)
Chlorophyll content	*QCH-ds*	4A, 3B, 6B, 7D	Barakat et al., (2015)
	QChlc.cgb-7A	7A	Yang et al., (2007b)
	QChlc.cgb-5A-1	5A	Yang et al., (2007b)
Photosynthetic capacity	*QFv/Fm.cgb-3B-1*	3B	Yang et al., (2007b)
	QFv/Fm.cgb-3B-2	3B	Yang et al., (2007b)
	QFv/Fm.cgb-6A	6A	Yang et al., (2007b)
	QFv/Fm.cgb-7D-1	7D	Yang et al., (2007b)
Water-soluble carbohydrates	*QSwscf.cgb-1A.1*	1A	Yang et al., (2007a)
	QSwscf.cgb-4B.1	4B	Yang et al., (2007a)
	QSwscf.cgb-4B.1	4B	Yang et al., (2007a)
	QSwscg.cgb-4A	4A	Yang et al., (2007a)
	QSwscm.cgb-1A.1	1A	Yang et al., (2007a)
Normalized vegetation index	*QNDVI-A.caas-4AL*	4AL	Gao et al., (2015)
	QNDVI-A.caas-3AL	3AL	Gao et al., (2015)
	QNDVI-A.caas-1BS	1BS	Gao et al., (2015)

1.9   Conclusion and future prospects

Genetic improvement can be achieved by either direct selection for primary traits such as grain yield or indirectly through selection of secondary traits related to higher grain yield potential. Breeding high-yielding genotypes incorporating yield-promoting agronomic and physiological traits has accelerated yield gains in wheat. As a result, further grain yield improvement will likely be achieved through in/direct selection targeting yield-related agronomic and physiological attributes. Furthermore, QTL associated with agronomic and physiological traits linked to grain yield are useful for marker-assisted selection of high-performing wheat genotypes.

References

Abdolshahi A., Nazari M., Safarian A., Sadathossini T.S., Salarpour M., Amiri H. (2015) Integrated selection criteria for drought tolerance in wheat (*Triticum aestivum* L.) breeding programs using discriminant analysis. Field Crops Research 174: 20–29.

Acuna-Galindo M.A., Mason R.E., Subrahmanyam N.K., Hays D. (2014) Meta-analysis of wheat QTL regions associated with adaptation to drought and heat stress. Crop Science 55:477–492.

Addison C., Mason R., Brown-Guedira G., Guedira M., Hao Y., G. Miller R., Subramanian N., Lozada D.N., Acuna A., N. Arguello M., W. Johnson J., M. H. Ibrahim A., Sutton R., Harrison S. (2016) QTL and major genes influencing grain yield potential in soft red winter wheat adapted to the Southern United States. Euphytica 209: 665–677.

Aisawi K.A.B., Reynolds M.P., Singh R.P., Foulkes M.J. (2015.) The Physiological Basis of the Genetic Progress in Yield Potential of CIMMYT Spring Wheat Cultivars from 1966 to 2009. Crop Science 55:1749-1764.

Alonso M.P., Mirabella N.E., Panelo J.S. (2018) Selection for high spike fertility index increases genetic progress in grain yield and stability in bread wheat. Euphytica 214:112.

Acreche M.M., Briceno-Felix G., Sanchez J., Slafer G.A. (2008) Physiological bases of genetic gains in Mediterranean bread wheat yield in Spain. European Journal of Agronomy 28:162–170.

Araus J.L., Villegas D., Aparicio N., García del Moral L.F., El Hani S., Rharrabti Y., Ferrio J.P., Royo C. (2003): Environmental factors determining carbon isotope discrimination and yield in durum wheat under Mediterranean conditions. Crop Science, 43: 170–180.

Battenfield S.D., Klatt A.R., Raun W. R. (2013) Genetic Yield Potential Improvement of Semi-dwarf Winter Wheat in the Great Plains. Crop Science. 53:946-955.

Beche E., Benin D., Da Silva C.L., Munaro L.B., Marchese J.A. (2014) Genetic gain in yield and changes associated with physiological traits in Brazilian wheat during the 20th century. Europian Journal of Agronomy 61:49-59.

Berry P.M., Kendall S., Rutterford Z. (2015) Historical analysis of the effects of breeding on the height of winter wheat (*Triticum aestivum*) and consequences for lodging. Euphytica 203:375.

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.

Blum A. (2009) Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. Field Crops Research 112:119–123.

Brisson N., Gate P., Gouache D., Charmet G., Oury F., Huard F. (2010) Why are wheat yields stagnating in Europe? A comprehensive data analysis for France. Field Crops Research 119:201–212.

Bornhofen, E., M. H. Todeschini, M. G. Stoco, A. Madureira, V. S. Marchioro, L. Storck, and G. Benin. 2018. Wheat Yield Improvements in Brazil: Roles of Genetics and Environment. Crop Science 58:1082-1093.

Bustos D.V., Hasan A.K., Reynolds M.P., Calderini D.F. (2013) Combining high grain number and weight through a DH-population to improve grain yield potential of wheat in high-yielding environments. Field Crops Research 145:106–115.

Calderini D., Dreccer M., Slafer G. (1997) Consequences of breeding on biomass, radiation interception and radiation-use efficiency in wheat.

Chairi F., Vergara-Diaz O., Vatter T., Aparicio N., Nieto-Taladriz M.T., Kefauver S.C., Bort J., Serret M.D., Araus J.L. (2018) Post-green revolution genetic advance in durum wheat: The case of Spain. Field Crops Research 228:158-169.

Chen H., Moakhar N.P., Iqbal M. (2016) Genetic variation for flowering time and height reducing genes and important traits in western Canadian spring wheat. Euphytica 208: 277.

Chen L., Yang Y., Cui C., Lu S., Lu Q., Du Y., Su R., Chai Y., Li H., Chen F., Yu F., Hu Y.G. (2018) Effects of Vrn-B1 and Ppd-D1 on developmental and agronomic traits in Rht5 dwarf plants of bread wheat. Field Crops Research 219:24-32.

Chen W., Sun D., Yan X. (2019) QTL analysis of wheat kernel traits, and genetic effects of qKW-6A on kernel width et al. Euphytica 215: 11.

Chen X., Hao M.D. (2015) Low contribution of photosynthesis and water-use efficiency to improvement of grain yield in Chinese wheat. Photosynthetica 53.

Chen X., Min D., Yasir T.A., Hu Y.G. (2012) Evaluation of 14 morphological, yield-related and physiological traits as indicators of drought tolerance in Chinese winter bread wheat revealed by analysis of the membership function value of drought tolerance (MFVD). Field Crops Research 137:195-201.

Christopher M., Chenu K., Jennings R., Fletcher S., Butler D., Borrell A., Christopher J. (2018) QTL for stay-green traits in wheat in well-watered and water-limited environments. Field Crops Research 17:32-44.

Condon A.G., Richards R.A., Rebetzke G.J and Farquhar G.D. (2004) Breeding for high water use efficiency. Journal of Experimental Botany. 55: 2447-2460.

Cossani C.M., Reynolds M.P. (2012) Physiological traits for improving heat tolerance in wheat. Plant Physiology 160:1710–1718.

Cossani C.M., Reynolds M.P. (2015) Heat Stress Adaptation in Elite Lines Derived from Synthetic Hexaploid Wheat. Crop Science 55:2719-2735.

Crespo-Herrera L.A., Crossa J., Huerta-Espino J., Vargas M., Mondal S., Velu G., Payne T.S., Braun H., Singh R.P. (2018) Genetic Gains for Grain Yield in CIMMYT's Semi-Arid Wheat Yield Trials Grown in Suboptimal Environments. Crop Science 58:1890-189.

Daoura B.G., Chen L., Du Y., Hu Y.G. (2014) Genetic effects of dwarfing gene Rht-5 on agronomic traits in common wheat (*Triticum aestivum* L.) and QTL analysis on its linked traits. Field Crops Research 156:22-29.

Deng X.P., Shan L., Zhang H.P., Turner N.C. (2006) Improving agricultural water use efficiency in arid and semiarid areas of China. Agriculture Water Management 80:23–40.

De Vita P., Li Destri Nicosia O., Nigro F., Platani C., Riefolo C., Di Fonzo N., Cattivelli L. (2007) Breeding progress in morpho-physiological, agronomical and qualitative traits of durum wheat cultivars released in Italy during the 20th century. European Journal of Agronomy 26:39–53.

Del Blanco I.A., Rajaram S., Kronstad W.E., Reynolds M.P. (2000) Physiological performance of synthetic hexaploid wheat-derived populations. Crop science 40:1257-1263.

Divashuk M.G., Bespalova L.A., Vasilyev A.V. (2013) Reduced height genes and their importance in winter wheat cultivars grown in southern Russia. Euphytica 190:137.

Dreccer M.F., van Herwaarden A.F., Chapman S.C. (2009) Grain number and grainweight in wheat lines contrasting for stem water soluble carbohydrate concentration. Field Crops Research 112:43–54.

Du Y., Chen L., Wang Y., Yang Z., Saeed I., Daoura B.D., Hu Y.G. (2018) The combination of dwarfing genes Rht4 and Rht8 reduced plant height, improved yield traits of rainfed bread wheat (*Triticum aestivum* L.) Field Crops Research 215:149-155.

Dube E., Mare-Patose R., Kilian W., Barnard A., Tsilo T.J. (2015) Identifying high-yielding dryland wheat cultivars for the summer rainfall area of South Africa. South African Journal of Plant and Soil 33:77-81.

Duggan B.L., Richards R.A., van Herwaarden A.F., Fettell N.A. (2005) Agronomic evaluation of a tiller inhibition gene (tin) in wheat. I. effect on yield, yield components, and grain protein. Australian Journal of Agriculture Research 56:169–178.

Ehdaie B., Mohammadi S.A., Nouraein M. (2016) QTLs for root traits at mid-tillering and for root and shoot traits at maturity in a RIL population of spring bread wheat grown under well-watered conditions. Euphytica 211:17.

Ehleringer J.R., Hall A.E., Farguhar G.D. (1993) Introduction: water use in relation to productivity. In: Ehleringer J.R., Hall A.E., Farguhar G.D, editors. Stable isoptopes and plant carbon water relations. San Diego: Academic Press:3–7.

Ellis M.H., Rebetzke G.J., Azanza F., Richards R.A., Spielmeyer W. (2005) Molecular mapping of gibberellin-responsive dwarfing genes in bread wheat. Theoretical Applied Genetics 111:423–430.

Faji L. (2018) Genome-wide linkage mapping of yield-related traits in three Chinese bread wheat populations using high-density SNP markers. Theoretical and Applied Genetics 131:1903.

Fan X., Cui F., Zhao C.H., Zhang W., Yang L.J., Zhao X.Q., Han J., Su Q.N., Ji J., Zhao Z.W., Tong Y.P., Li J.M. (2015) QTL for flag leaf size and their influence on yield-related traits in wheat (*Triticum aestivum* L.). Molecular Breedind 35:1-16.

FAOSTAT, 2018. http: www.fao.org/faostat/en/#data/QC. (Accessed 10 September 2018).

Ferrante A., Savin R., Slafer G.A. (2012) Differences in yield physiology between modern, well adapted durum wheat cultivars grown under contrasting conditions. Field Crops Research 136:52-64.

Fischer R.A., Rees D., Sayre K.D., Condon G.A., Larque S. (1998) Wheat yield progress associated with higher stomatal conductance and photosynthetic rate and cooler canopies. Crop Science 38:1467-1475.

Flexas J., Díaz-Espejo A., Conesa M.A., Coopman R.E., Douthe C., Gago J., Gallé A., Galmés J., Medrano H., Ribas-Carbo M. (2016) Mesophyll conductance to CO2 and Rubisco as targets for improving intrinsic water use efficiency in C3 plants. Plant Cell Environment 39:965–982.

Flexas J., Bota J., Loreto F., Cornic G., Sharkey T.D. (2004) Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. Plant Biology 6:269–279.

Flohr B.M., Hunt J.R., Kirkegaard J.A., Evans J.R., Swan A., Rheinheimer B. (2018) Genetic gains in NSW wheat cultivars from 1901 to 2014 as revealed from synchronous flowering during the optimum period. European Journal of Agronomy 98:1-13.

Foulkes M.J., Reynolds M.P., Sylvester-Bradley R. (2009) Genetic improvement of grain crops: yield potential. In: Sadras, V., Calderini, D. (Eds.), Crop Physiology: Applications for Genetic Improvement and Agronomy Academic Press:581.

Foulkes M.J., Slafer G.A., Davies W.J., Berry P.M., Sylvester Bradley R., Martre P., Calderini D.F., Griffiths S., Reynolds M.P. (2011) Raising yield potential of wheat III. Optimizing partitioning to grain while maintaining lodging resistance. Journal of Experimental Botany 62:469–486.

Fufa H., Baenziger P.S., Beecher B.S., Graybosch R.A., Eskridge K.M., Nelson L.A. (2005) Genetic improvement trends in agronomic performances and end-use quality characteristics among hard winter wheat cultivars in Nebraska. Euphytica 144:187–198.

Gahlaut V., Jaiswal V., Tyagi B.S., Singh G., Sareen S., Balyan H.S. (2017) QTL mapping for nine drought-responsive agronomic traits in bread wheat under irrigated and rain-fed environments. PLoS ONE 12.

Gaju O., Reynolds M.P., Sparkes D.L., Foulkes M.J. (2009) Relationships between large-spike phenotype, grain number, and yield potential in spring wheat. Crop science 49:961–973.

Gaju O., DeSilva J., Carvalho P., Hawkesford M.J., Griffiths S., Greenland A., Foulkes M.J. (2016) Leaf photosynthesis and associations with grain yield, biomass and nitrogen-use efficiency in landraces, synthetic-derived lines and cultivars in wheat. Field Crops Research 193:1-15.

Gaju O., Allard V., Martre P., Le Gouis J., Moreau D., Bogard M., Hubbart S., Foulkes M.J. (2014) Nitrogen partitioning and remobilization in relation to leaf senescence, grain yield and grain nitrogen concentration in wheat cultivars. Field Crops Research 155:213-223.

Gao F., Wen W., Liu J., Rasheed A., Yin G., Xia X., Wu X., He Z. (2015) Genome-Wide Linkage Mapping of QTL for Yield Components, Plant Height and Yield-Related Physiological Traits in the Chinese Wheat. Frontiers in Plant Science 6:1099.

Gao F., Ma D., Yin G., Rasheed A., Dong Y., Xiao Y., Xia X., Wu X., He Z. (2017) Genetic Progress in Grain Yield and Physiological Traits in Chinese Wheat Cultivars of Southern Yellow and Huai Valley since 1950. Crop Science 57:760-773.

Gororo N.N., Eagles H.A., Eastwood R.F., Nicolas M.E., Flood R.G. (2002) Use of Triticum tauschii to improve yield of wheat in low-yielding environments. Euphytica 123:241–254.

Gomez D., Vanzetti L., Helguera M., Lombardo L., Fraschina J., Miralles D.J. (2014) Effect of Vrn-1, Ppd-1 genes and earliness per se on heading time in Argentinean bread wheat cultivars. Field Crops Research 158:73-81.

Graybosch R.A., Peterson C.J. (2010) Genetic improvement in winter wheat yields in the Great Plains of North America 1959–2008. Crop Science 50:1882–1890.

Green A.J., Berger G., Griffey C.A., Pitman R., Thomason W., Balota M. (2012) Genetic yield improvement in soft red winter wheat in theEastern United States from 1919 to 2009. Crop Science 52:2097–2108.

Grover G., Sharma A., Gill H.S., Srivastava P., Bains N.S. (2018) Rht8 gene as an alternate dwarfing gene in elite Indian spring wheat cultivars. PLoS ONE 13:1-11.

Gummadov N., Keser M., Akin B., Cakmak M., Mert Z., Taner S., Ozturk I., Topal A., Yazar S., Morgounov A. (2015) Genetic gains in wheat in Turkey: winter wheat for irrigated conditions. Crop Journal 3:507-516.

Giunta F., Motzo R., Pruneddu G. (2007) Trends since 1900 in the yield potential of Italian-bred durum wheat cultivars. European Journal of Agronomy 27:12-24.

Guzmán C., Autrique, E., Mondal S., Huerta-Espino J., Singh R.P., Vargas M., Crossa J., Amaya A., Peña, R.J. (2017) Genetic improvement of grain quality traits for

CIMMYT semi-dwarf spring bread wheat varieties developed during 1965–2015: 50 years of breeding, Field Crops Research 210:192-196.

Hai L., Guo H., Wagner C., Xiao S., Friedt W. (2008) Genomic regions for yield and yield parameters in Chinese winter wheat (*Triticum aestivum* L.) genotypes tested under varying environments correspond to QTL in widely different wheat materials. Plant Science 175:226–232.

Hernández-Espinosa N., Mondal S., Autrique E., Gonzalez-Santoyo H., Cross J., Huerta-Espino J., Singh R.P., Guzmán C. (2018a) Milling, processing and end-use quality traits of CIMMYT spring bread wheat germplasm under drought and heat stress. Field Crops Research 215:104-112.

Hou J., Huang X., Sun W., Du C., Wang C., Xie Y., Ma Y., Ma D. (2018) Accumulation of water-soluble carbohydrates and gene expression in wheat stems correlates with drought resistance. Journal of Plant Physiology 231:182-191.

Huang X., Q., Ster H.C., Ganal M.W., Roder M.S. (2003) Advanced backcross QTL analysis for the identification of quantitative trait loci alleles from wild relatives of wheat (*Triticum aestivum* L.). Theoretical Applied Genetics 106:1379–1389.

Joudi M., Ahmadi A., Mohammadi V. (2014) Genetic changes in agronomic and phenologic traits of Iranian wheat cultivars grown in different environmental conditions. Euphytica 196:237.

Kamran A., Iqbal M., Navabi A. (2013) Earliness per se QTLs and their interaction with the photoperiod insensitive allele Ppd D1a in the Cutler × AC Barrie spring wheat population. Theoretical Applied Genetics 126:1965.

Kamran, A., H. S. Randhawa, C. Pozniak, and D. Spaner. 2013. Phenotypic Effects of the Flowering Gene Complex in Canadian Spring Wheat Germplasm. Crop Science 53:84-94.

Keser M., Gummadov N., Akin B., Belen S., Mert Z., Taner S., Topal A., Yazar S., Morgounov A., Sharma R.C., Ozdemir F. (2017) Genetic gains in wheat in Turkey: Winter wheat for dryland conditions. The Crop Journal 5: 533-540.

Langer S.M., Longin C.F.H., Würschum T. (2014) Flowering time control in European winter wheat. Frontiers in Plant Science 5:537.

Lanning S., Martin J., Stougaard R., Guillen-Portal F., Blake N., Sherman J. (2012) Evaluation of near-isogenic lines for three height-reducing genes in hard red spring wheat. Crop Science 52:1145–1152.

Lawlor D.W., Tezara W. (2009) Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes. Annals of Botany 103:561–579.

Liu H., Searle I.R., Mather D.E., Able A.J., Able J.A. (2015) Morphological, physiological and yield responses of durum wheat to pre-anthesis water-deficit stress are genotype-dependent. Crop & Pasture Science 66:1024–1038.

Liu K., Xu H., Liu G. (2018a) QTL mapping of flag leaf-related traits in wheat (*Triticum aestivum* L.). Theoretical Applied Genetics 131:839.

Liu Y., Zhang J., Hu Y.G., Chen J. (2017) Dwarfing genes Rht4 and Rht-B1b affect plant height and key agronomic traits in common wheat under two water regimes. Field Crops Research 204:242-248.

Liu Y.X., Tao Y., Wang Z.Q., Guo Q.L., Wu F.K., Yang X.L., Deng M., Ma J., Chen G.D., Wei Y.M., Zheng Y.L. (2018) Identification of QTL for flag leaf length in common wheat and their pleiotropic effects. Molecular Breeding 38:11.

Liu D., Zhang L., Hao M., Ning S., Yuan Z., Dai S., Huang L., Wu B., Yan Z., Lan X., Zheng Y. (2018) Wheat breeding in the hometown of Chinese Spring, The Crop Journal 6:82-90.

Lo Valvo P.J., Mirallesa D.J., Serragoa R.A. (2018) Genetic progress in Argentine bread wheat varieties released between 1918 and 2011: Changes in physiological and numerical yield components. Field Crops Research 221:314-321.

Long, S.P., Bernacchi, C.J. (2003) Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. Journal of Experimental Botany 54:2393–2401.

Lopes M.S., Reynolds M.P. (2010) Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. Functional Plant Biology 37:147–156.

Lopes M.S., Reynolds M.P. (2012) Stay-green in spring wheat can be determined by spectral reflectance measurements (normalized difference vegetation index) independently from phenology. Journal of Experimental Botany 63:3789–3798.

Lopes M.S., Reynolds M.P., McIntyre C.L. (2013) QTL for yield and associated traits in the Seri/Babax population grown across several environments in Mexico, in the West Asia, North Africa, and South Asia regions. Theoretical Applied Genetics 126:971.

Lopes M.S., Reynolds M.P., Manes Y., Singh R.P., Crossa J., Braun H.J. (2012) Genetic Yield Gains and Changes in Associated Traits of CIMMYT Spring Bread Wheat in a "Historic" Set Representing 30 Years of Breeding. Crop Science 52:1123-1131.

Lozada D.N., Mason R.E., Babar M.A. (2017) Association mapping reveals loci associated with multiple traits that affect grain yield and adaptation in soft winter wheat. Euphytica 213:222.

Mackay I.J., Horwell A., Garner J., White J., McKee J., Philipott H. (2011) Reanalyses of the historical series of UK variety trials to quantify the contributions of genetic and environmental factors to trends and variability in yield over time. Theoretical and Applied Genetics 122:225–238.

Manès Y., Gomez H., Puhl L., Reynolds M., Braun H., Trethowan R. (2012) Genetic yield gains of the CIMMYT international semi-arid wheat yield trials from 1994 to 2010. Crop Science 52:1543–1552.

Maqsood R.M., Amjid M.S., Saleem M.A., Shabbir G., Khaliq I. (2017) Identification of genomic regions conferring drought tolerance in bread wheat using ISSR markers. Pakistan Journal of Botany 49:1821-1827.

McIntosh R.A., Yamazaki Y., Dubcovsky J., Rogers W.J., Morris C., Appels R., Xia X. (2013) Catalogue of gene symbols for wheat. In: Proceedings of 12th

International wheat genetics Symposium, Yokohama, Japan. http://www.shigen.nig.ac.jp/wheat/.

Merchuk-Ovnat L., Fahima T., Krugman T., Saranga Y. (2016) Ancestral QTL alleles from wild emmer wheat improve grain yield, biomass and photosynthesis across enviroinments in modern wheat. Plant Science 251:23-34.

Mitchell J.H., Chapman S.C., Rebetzke G.J., Bonnett D.G., Fukai S. (2012) Evaluation of a reduced-tillering (tin) gene in wheat lines grown across different production environments. Crop Pasture Science 63:128–141.

Mo Y. (2018) Identification and characterization of Rht25, a locus on chromosome arm 6AS affecting wheat plant height, heading time, and spike development. Theoretical and Applied Genetics 131.

Moeller C., Evers J.B., Rebetzke G. (2014) Canopy architectural and physiological characterization of near-isogenic wheat lines differing in the tiller inhibition gene tin. Frontiers in Plant Science 5:617.

Mohammadi M., Blake T.K., Budde A.D., Chao S., Hayes P.M., Horsley R.D. (2015) A genome-wide association study of malting quality across eight U.S. barley breeding programs. Theoretical and Applied Genetics 128:705–721.

Mondal S., Singh R., P.,, Mason E.R., Huerta-Espino J., Autrique E., Joshi A.K. (2016) Grain yield, adaptation and progress in breeding for early-maturing and heat-tolerant wheat lines in South Asia. Field Crops Research 192:78-85.

Morgounova A., Zykinb V., Belanb I., Roseevab L., Zelenskiyc Y., Gomez-Becerrad H.F., Budakd H., Bekes F. (2010) Genetic gains for grain yield in high latitude spring wheat grown in Western Siberia in 1900–2008. Field Crops Research 117:101–112.

Motzo R., Giunta F., Deidda M. (2004) Expression of a tiller inhibitor gene in the progenies of interspecific crosses *Triticum aestivum* L. × T. *turgidum* subsp. durum. Field Crop Research 85:15-20.

Motzo R., Giunta F. (2007) The effect of breeding on the phenology of Italian durum wheats: From and races to modern cultivars. European Journal of Agronomy. 26:462-470.

Naruoka Y., Talbert L.E., Lanning S.P., Blake N.K., Martin J.M., Sherman J.D. (2011) Identification of quantitative trait loci for productive tiller number and its relationship to agronomic traits in spring wheat. Theoretical and Applied Genetics 123:1043–1053.

Nhemachena C.R., Kirsten J. (2017) A historical assessment of sources and uses of wheat varietal innovations in South Africa. South African Journal of Science 113:1-8.

Nishimura K., Moriyama R., Katsura K. (2018) The early flowering trait of an emmer wheat accession (*Triticum turgidum* L. ssp. *dicoccum*) is associated with the cis-element of the Vrn-A3 locus. Theoretical and Applied Genetics 131:2037.

Nouri-Ganbalani A., Nouri-Ganbalani G., Hassanpanah D. (2009) Effects of drought stress condition on the yield and yield components of advanced wheat genotypes in Ardabil. Iranian Journal of Food. Agriculture and Environment 7:228-234.

Ochagavía H., Prieto P., Savin R., Griffiths S., Slafer G. (2018) Dynamics of leaf and spikelet primordia initiation in wheat as affected by Ppd-1a alleles under field conditions. Journal of Experimental Botany 69:2621-2631.

Ogbonnaya F.C., Rasheed A., Okechukwu E.C. (2017) Genome wide association study for agronomic and physiological traits in spring wheat evaluated in a range of heat prone environments. Theoretical and Applied Genetics 130:1819.

Ortiz R., Braun H., Crossa J. (2008) Wheat genetic resources enhancement by the International Maize and Wheat Improvement Center (CIMMYT). Genetic Resource Crop Evolution 55:1095.

Parry M.A.J., Hawkesford M.J. (2010b) Genetic approaches to reduce greenhouse gas emissions: increasing carbon capture and decreasing environmental impact. CABI International, Wallingford, U.K.:139–150.

Parry M.A.J., Hawkesford M.J. (2012) An integrated approach to crop genetic improvement. Journal of Integrated Plant Biology 54:250–259.

Parry M.A.J., Reynolds M., Salvucci M.E., Raines C., Andralojc P.J., Zhu X.G. (2011) Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency. Journal of Experimental Botany 62:45-467.

Pearce S., Saville R., Vaughan S.P., Chandler P.M., Wilhelm E.P., Sparks C.A., Al-Kaff N., Korolev A., Boulton M.I., Phillips A.L. (2011) Molecular characterization of Rht-1 dwarfing genes in hexaploid wheat. Plant Physiology 157:1820–1831.

Perez-Lara E., Semagn K., Chen H., Iqbal M., N'Diaye A., Kamran A., Navabi A., Pozniak C., Spane D. (2016) QTLs Associated with Agronomic Traits in the Cutler × AC Barrie Spring Wheat Mapping Population Using Single Nucleotide Polymorphic Markers. PLoS ONE 11:1-19.

Pinto R.S., Molero G., Reynolds M.P., Ibrahim A. (2017) Identification of heat tolerant wheat lines showing genetic variation in leaf respiration and other physiological traits. Euphytica 213:76.

Pinto R.S., Reynolds M.P., Mathews K.L., McIntyre C.L., Olivares-Villegas J.J., Chapman S.C. (2010) Heat and drought adaptive QTL in a wheat population designed to minimize confounding agronomic effects. Theoretical and Applied Genetics 121:1001–1021.

Qin X., Zhang F., Liu C., Yu H., Cao B., Tian S., Liao Y., Siddique K.H.M. (2015) Wheat yield improvements in China: Past trends and future directions. Field Crops Research 177:117-124.

Rebetzke G.J., Bruce S., Kirkegaard J.A. (2005) Genotypic increases in coleoptile length improves emergence and early vigour with crop residues. Plant and Soil 270:87-100.

Rebetzke G.J., Ellis M.H., Bonnett D.G., Condon A.G., Falk D., Richards R.A. (2011) The Rht13 dwarfing gene reduces peduncle length and plant height to increase grain number and yield of wheat. Field Crops Research 124:323–331.

Rebetzke G.J., Ellis M.H., Bonnett D.G., Mickelson B., Condon A.G., Richards R.A. (2012a) Height reduction and agronomic performance for selected gibberellin-responsive dwarfing genes in bread wheat (*Triticum aestivum* L.). Field Crops Research 126:87–96.

Rebetzke G.J., Bonnett D.G., Ellis M.H. (2012b) Combining gibberellic acid-sensitive and insensitive dwarfing genes in breeding of higher-yielding, sesqui-dwarf wheats. Field Crops Research127:17-25.

Rebetzke G.J., Biddulph B., Chenu K., Deery D., Mayer J., Moeller C. (2013) Development of a multisite, managed environment facility for targeted trait and germplasm evaluation. Functional Plant Biology 40:1-13.

Rebetzke G.J., van Herwaarden A.F., Jenkins C., Weiss M., Lewis D., Ruuska S., Tabe L., Fettell N.L., Richards R.A. (2008) Quantitative trait loci for water-soluble carbohydrates and associations with agronomic traits in wheat. Australian Journal of Agricultural Research 59:891–905.

Reynolds M., Tuberosa R. (2008) Translational research impacting on crop productivity in drought-prone environments. Current Opinion in Plant Biology 11:171-179.

Reynolds M., Mujeeb-Kazi A., Sawkins M. (2005) Prospects for utilising plant-adaptive mechanisms to improve wheat and other crops in drought- and salinity-prone environments. Annals of Applied Biology. 146: 239–259.

Reynolds M., Manes Y., Izanloo A., Langridge P. (2009) Phenotyping approaches for physiological breeding and gene discovery in wheat. Annals of Applied Biology 155:309-320.

Reynolds M., Foulkes J., Furbank R., Griffiths S., King J., Murchie E., Parry M., Slaffer G. (2012) Achieving yield gains in wheat. Plant, Cell & Environment 35:1799-1823.

Reynolds M.P., Braun H.P., Cavalieri A.J., Chapotin S., Davies. W.J., Ellul. P., Feuillet C., Govaerts B., Kropff M.J., Lucas H., Nelson J., Powell W., Quilligan E., Rosegrant M.W., Singh R.P., Sonder K., Tang H., Visscher S., Wang R. (2017) Improving global integration of crop research. Science 357:359–360.

Rosegrant, M.W., M. Agcaoili. 2010. Global food demand, supply, and price prospects to 2010. International Food Policy Research Institute, Washington, DC.

Royo C., Ammar K., Alfaro C., Dreisigacker S., García del Moral L.F., Villegas D. (2018) Effect of Ppd-1 photoperiod sensitivity genes on dry matter production and allocation in durum wheat. Field Crops Research 221:358-367.

Royo C., Álvaro F., Martos V., Ramdani A., Isidro J., Villegas D., Garcia Del Moral L.F. (2007) Genetic changes in durum wheat yield components and associated traits in Italian and Spanish varieties during the 20th century. Euphytica 155:259–270.

Ruuska S.A., Rebetzke G.J., van Herwaarden A.F., Richards R.A., Fettell N.A., Tabe L., Jenkins C.L.D. (2006) Genotypic variation in water-soluble carbohydrate accumulation in wheat. Functional Plant Biology 33:799-809.

Sadras V.O., Lawson C. (2011) Genetic gain in yield and associated changes in phenotype, trait plasticity and competitive ability of South Australian wheat varieties released between 1958 and 2007. Crop Pasture Science 62:533–549.

Sayre K.D., Rajaram S., Fischer R.A. (1997) Yield potential progress in short wheats in North West Mexico. Crop Science 37:36–42.

Sehgal D., Vikram P., Sansaloni C.P., Ortiz C., Pierre C.S., Payne T., Ellis M., Amri A., Petroli C.D., Wenzl P., Singh S. (2015) Exploring and Mobilizing the Gene Bank Biodiversity for Wheat Improvement. PLoS ONE 10:1-18.

Sharma R.C., Crossa J., Velu G., Huerta-Espino J., Vargas M., Payne T.S., Singh R.P. (2012) Genetic Gains for Grain Yield in CIMMYT Spring Bread Wheat across International Environments. Crop Science 52:1522-1533.

Shearman V.J., Sylvester-Bradley R., Scott R.K., Foulkes M.J. (2005) Physiological processes associated with wheat yield progress in the UK. Crop Science 45:175–185.

Silva M.A., Jifon J.L., Santos C.M., Jadoskki C.J., Silva J.A.G. (2013) Photosynthetic capacity and water use efficiency in sugarcane genotypes subjected to water deficit during early growth phase. Brazilian Archives of Biology and Technology 56:735–748.

Sonja B., Zhenyu Y., Viktoriya A., Chris-Carolina S., Erwin G. (2018) Generating plants with improved water use efficiency. Agronomy 8:1-13.

Subira J., Álvaro A., García del Moral L.F., Royo C. (2015) Breeding effects on the cultivar × environment interaction of durum wheat yield. European Journal of Agronomy 68:78-88.

Sukumaran S., Lopes M., Dreisigacker S. (2018) Genetic analysis of multi-environmental spring wheat trials identifies genomic regions for locus-specific trade-offs for grain weight and grain number. Theoretical and Applied Genetics 131:985.

Sun Y., Wang X., Wang N., Chen Y., Zhang S. (2014) Changes in the yield and associated photosynthetic traits of dry-land winter wheat (*Triticum aestivum* L.) from the 1940 to the 2010 in Shaanxi Province of China. Field Crops Research 167:1-10.

Tahmasebi S., Heidari B., Pakniyat H., McIntyre C.L. (2016) Mapping QTLs associated with agronomic and physiological traits under terminal drought and heat stress conditions in wheat (*Triticum aestivum* L.). Genome 59:1-20.

Tausz-Posch S., Dempsey R.W., Seneweera S., Norton R.M., Fitzgerald G., Tausz M. (2015) Does a freely tillering wheat cultivar benefit more from elevated CO2 than a restricted tillering cultivar in a water-limited environment? European Journal of Agronomy 64:21-28.

Tambussi E.A., Bort J., Araus J.L. (2007) Water use efficiency in C4 cereal under Mediterranean conditions: a review of some physiological aspects. CIHEAM (Options Mediterranean's: Series B 57:189–203.

Telfer P., Edwards J., Bennett D., Ganesalingam D., Able J., Kuchel H. (2018) A field and controlled environment evaluation of wheat (*Triticum aestivum*) adaptation to heat stress. Field Crops Research 229:55-65.

Thapa S., Jessup K.E., Pradhan G.P., Rudd J.C., Liu S., Mahan J.R., Devkota R.N., Baker J.A., Xue Q. (2018) Canopy temperature depression at grain filling correlates to winter wheat yield in the U.S. Southern High Plains. Field Crops Research 217:11-19.

Tian Z., Jing Q., Dai T., Jiang D., Cao W. (2011) Effects of genetic improvements on grain yield and agronomic traits of winter wheat in the Yangtze River Basin of China. Field Crops Research 124:417–425.

Tian X., Zhu Z., Xie L., Xu D., Li J., Fu C., Chen X., Wang D., Xia X., He Z., Cao S. 2019. Preliminary Exploration of the Source, Spread, and Distribution of Rht24 Reducing Height in Bread Wheat. Crop Science 59:19-24.

Trethowan R.M., Mujeeb-Kazi A. (2008) Novel Germplasm Resources for Improving Environmental Stress Tolerance of Hexaploid Wheat. Crop Science 48:1255-1265.

Trethowan R.M., Van Ginkel M., Rajaram S. (2002) Progress in breeding for yield and adaptation in global drought affected environments. Crop Science 42:1441–1446.

Underdahl J.L., Mergoum M., Ransom J.K., Schatz B.J. (2008) Agronomic Traits Improvement and Associations in Hard Red Spring Wheat Cultivars Released in North Dakota from 1968 to 2006. Crop Science 48:158-166.

Varga B., Vida G., Varga-László E., Bencze S., Veisz O. (2015) Effect of simulating drought in various phenophases on the water use efficiency of winter wheat. Journal of Agronomy and Crop Science 201:1–9.

Van den Boogaard R., Veneklaas E.J., Lambers H. (1996) The association of biomass allocation with growth and water use efficiency of two *Triticum aestivum* cultivars. Australian Journal of Plant Physiology 23: 751–761.

Van Beem J., Mohler V., Lukman R., van Ginkel M., William M., Crossa J., Worland A.J. (2005) Analysis of Genetic Factors Influencing the Developmental Rate of Globally Important CIMMYT Wheat Cultivars. Crop Science 45:2113-2119.

Vikhe P., Venkatesan S., Chavan A., Tamhankar S., Patil R. (2018) Mapping of dwarfing gene Rht14 in durum wheat and its effect on seedling vigour, internode length and plant height. The Crop Journal: 2214-5141.

Wang Y., Chen L., Du Y., Yang Z., Condon A.G., Hu Y.G. (2014) Genetic effect of dwarfing gene Rht13 compared with *Rht-D1b* on plant height and some

agronomic traits in common wheat (*Triticum aestivum* L.). Field Crops Research.162: 39-47.

Wang Y., Du Y., Yang Z., Chen L., Condon A.G., Hu Y.G. (2015) Comparing the effects of GA-responsive dwarfing genes Rht13 and Rht8 on plant height and some agronomic traits in common wheat. Field Crops Research 179:35-43.

Wang R., Liu Y., Isham K. (2018) QTL identification and KASP marker development for productive tiller and fertile spikelet numbers in two high-yielding hard white spring wheat cultivars. Molecular Breeding 38:1-12.

Wang Z., Liu Y., Shi H. (2016) Identification and validation of novel low-tiller number QTL in common wheat. Theoretical and Applied Genetics 129: 603.

Williams M., Woodward F.I., Baldocchi D.D., Ellsworth, D. (2004) CO2 capture from the leaf to the landscape. In Photosynthetic Adaptation: Chloroplast to Landscape; Smith, W.K., Vogelmann, T.C., Critchley, C., Eds.; Springer: Berlin, Germany, pp. 133–168.

Wu Q., Chen Y., Fu L. (2016) QTL mapping of flag leaf traits in common wheat using an integrated high-density SSR and SNP genetic linkage map. Euphytica 208:337.

Wu W., Li C., Ma B. (2014) Genetic progress in wheat yield and associated traits in China since 1945 and future prospects. Euphytica 196:155.

Wu X., Chang X., Jing R. (2012) Genetic insight into yield-associated traits of wheat grown in multiple rain-fed environments. PLoS ONE 7.

Würschum T., Langer S.M., Longin C.F.H. (2015b) Genetic control of plant height in European winter wheat cultivars. Theoretical and Applied Genetics 128:865–874.

Würschum T., Leiser W.L., Langer S.M. (2018) Phenotypic and genetic analysis of spike and kernel characteristics in wheat reveals long-term genetic trends of grain yield components. Theoretical and Applied Genetics 131:2071.

Würschum T., Leiser W.L., Weissmann S., Maurer H.P. (2017a) Genetic architecture of male fertility restoration of Triticum timopheevii cytoplasm and fine-mapping

of the major restorer locus Rf3 on chromosome 1B. Theoretical and Applied Genetics 130:1253–1266.

Würschum T., Langer S.M., Longin C.F.H., Tucker M.R., Leiser. W.L. (2017b) A modern Green Revolution gene for reduced height in wheat. Plant Journal 92:892–903.

Xiao Y.G., Qian Z., G.,, Wu K., Liu J.J., Xia X.C., Ji W.Q., He Z.H. (2012) genetic gains in grain yield and physiological traits of winter wheat in Shandong Province, China, from 1969 to 2006. Crop Science 52:44–56.

Xie W., Nevo E. (2008) Wild emmer: Genetic resources, gene mapping and potential for wheat improvement.

Xu Y., Li S., Li L. (2017) QTL mapping for yield and photosynthetic related traits under different water regimes in wheat. Molecular Breeding 37:34.

Yang D., Jing R., Chang X., Li W. (2007a) Identification of quantitative trait loci and environmental interactions for accumulation and remobilization of water-soluble carbohydrates in wheat (*Triticum aestivum* L.) stems. Genetics 176:571–584.

Yang D., Jing R., Chang X., Li W. (2007b) Quantitative Trait Loci Mapping for Chlorophyll Fluorescence and Associated Traits in Wheat (*Triticum aestivum*). Journal of Integrative Plant Biology 49:646-654.

Yang Z., Zheng J., Liu C., Wang Y., Condon A.G., Chen Y., Hu Y. (2015) Effects of the GA-responsive dwarfing gene Rht18 from tetraploid wheat on agronomic traits of common wheat. Field Crop Research 183:92-101.

Yu M., Mao S., Chen G. (2014) QTLs for uppermost internode and spike length in two wheat RIL populations and their affect upon plant height at an individual QTL level. Euphytica 200:95.

Zhang X., Yang S., Zhou Y. (2006) Distribution of the Rht-B1b, Rht-D1b and Rht8 reduced height genes in autumn-sown Chinese wheat's detected by molecular markers. Euphytica 152:109.

Zhang X.K., Xiao Y.G., Zhang Y., Xia X.C., Dubcovsky J., He Z.H. (2008) Allelic Variation at the Vernalization Genes Vrn-A1, Vrn-B1, Vrn-D1, and Vrn-B3 in

Chinese Wheat Cultivars and Their Association with Growth Habit. Crop Science 48:458-470.

Zhang K., Zhang Y., Chen G., Tian J. (2009) Genetic analysis of grain yield and leaf Chlorophyll content in common wheat. Cereal Research Communication. 37: 499–511.

Zhang Y., Xu W., Wang W., Dong H., Qi X., Zhao M., Fang Y., Gao Y., Hu L. (2016) Progress in genetic improvement of grain yield and related physiological traits of Chinese wheat in Henan Province. Field Crops Research 199:117-128.

Zhang Y., Li D., Zhang D., Zhao X., Cao X., Dong L., Liu J., Chen K., Zhang H., Gao C., Wang D. (2018) Analysis of the functions of TaGW2 homoeologs in wheat grain weight and protein content traits. The Plant Journal 94:857-866.

Zhang Z., Wang P., Li Y., Ma L., Li L., Yang R., Ma Y., Wang S.a., Wang Q. (2014) Global transcriptome analysis and identification of the flowering regulatory genes expressed in leaves of Lagerstroemia indica. DNA and Cell Biology 33:680-688.

Zhao C., Bao Y., Wang X. (2018) QTL for flag leaf size and their influence on yield-related traits in wheat. Euphytica 214:209.

Zheng B., Biddulph B., Li D., Kuchel H., Chapman S. (2013) Quantification of the effects of VRN1 and Ppd-D1 to predict spring wheat (*Triticum aestivum*) heading time across diverse environments. Journal of Experimental Botany 64:3747–3761.

Zheng T.C., Zhang X.K., Yina G.H., Wanga L.N., Hana Y.L., Chen L., Huanga F., Tanga J.W., Xiad X.C., Hed Z.H. (2011) Genetic gains in grain yield, net photosynthesisand stomatal conductance achieved in Henan Province of China between 1981and 2008. Field Crop Research 122: 225–233.

Zhou Y., Zhu H.Z., Cai S.B. (2007a) Genetic improvement of grain yield and associated traits in the southern China winter wheat region: 1949 to 2000. Euphytica 157:465.

Zhou Y., He Z., Sui X., Xia X., Zhang X., Zhang G. (2007b) Genetic improvement of grain yield and associated traits in the Northern China winter wheat region from 1960 to 2000. Crop Science 47:245–253.

CHAPTER 2

Leaf gas exchange and water-use efficiency of dry-land wheat genotypes under water stressed and non-stressed conditions

2.1 Abstract

The objective of this study was to determine drought tolerance characteristics of dryland wheat genotypes based on leaf gas exchange and water-use efficiency in order to identify promising genotypes for drought tolerance breeding. Physiological responses of ten genetically diverse wheat genotypes were studied under non-stressed (NS) and water stressed (WS) conditions using a 2×10 factorial experiment replicated 3 times. A highly significant water condition $\times$ genotype interaction ($P < 0.001$) was observed for photosynthetic rate (A), ratio of photosynthetic rate and internal CO_2 concentration (A/Ci), ratio of internal and atmospheric CO_2 (Ci/Ca), intrinsic (WUE_i) and instantaneous (WUE_{inst}) water-use efficiency suggesting genotypic variability among wheat genotypes under both test conditions. Principal component analysis (PCA) identified three principal components (PC's) under both test conditions accounting for 84% and 89% of total variation, respectively. Bi-plot analysis identified G339 and G344 as drought tolerant genotypes with higher values of A, T, gs, A/Ci, WUE_i and WUE_{inst} under WS condition. The current study detected significant genetic variation for drought tolerance among the tested wheat genotypes using physiological parameters. Genotypes G339 and G344 were identified to be drought tolerant with efficient A, T, gs, A/Ci and water-use under water stressed condition.

KEYWORDS: Breeding, drought stress, drought tolerance, water-use efficiency, wheat

2.2 Introduction

Wheat (*Triticum aestivum* L., 2n = 6x = 42) is one of the most important cereal crops cultivated globally (Dube et al., 2015) serving as a major staple food for more than one-third of the world's population (Abdullah et al., 2011). It is rich in vitamins and minerals and is a good source of proteins and carbohydrates (FAO 2002). Wheat production in arid and semi-arid areas is affected by drought stress combined with other abiotic stress factors such as heat resulting in considerable yield losses (Akhkha 2011). Drought occurrences are characterised by prolonged dry periods and erratic rainfalls. This phenomenon is likely to increase due to climate change emphasising the urgent need to develop drought tolerant and water-use efficient wheat genotypes to improve wheat production and productivity under water-limited environments.

Plants growing under water limited conditions have developed mechanisms for maintaining plant water balance in order to sustain plant physiological processes (Bartels et al., 1996; Blum 1996). Plants can avoid the effects of drought stress by lowering leaf water potential and reducing transpirational water loss by closing stomatal apertures (Farooq et al., 2009). However, reduced stomatal conductance can lead to reduced photosynthetic rate by limiting CO_2 assimilation rate (Silva and Costa 2009; Jackson et al., 2016; Sourour et al., 2017). Nonetheless, reduced stomatal conductance has been reported to improve water-use efficiency under water limited conditions (Blum 2005; Dong et al., 2008).

Water use efficiency (WUE) at plant level is described as the ability of the plant to produce biomass or yield per unit water used (Blum 2005). WUE is an important physiological trait that can improve crop yields under limited water conditions (Deng et al., 2006; Silva *et al.,* 2013; Varga et al., 2015). This trait is considered an important parameter for measuring the level of drought tolerance in crops (Blum 2009; Lawlor and Tezara 2009). At the leaf level, two WUE components are recognised namely: instantaneous (WUE_{inst}) and intrinsic water-use efficiencies (WUE_i). WUE_{inst} is defined as the ratio of photosynthetic rate and transpiration rate, whereas, WUE_i is defined as the ratio of photosynthetic rate and stomatal conductance (Ehleringer et al., 1993; Tambussi et al., 2007). WUE_{inst} and WUE_i are considered important short-term physiological measurements of water-use efficiency (Ehleringer et al., 1993). In general, leaf gas exchange parameters (e.g. photosynthetic rate, transpiration rate

and stomatal conductance) and WUE are important parameters for measuring drought tolerance in crops (Lawlor and Tezara 2009; Athar and Ashraf 2009; Mashilo et al., 2017).

Improved wheat cultivars with drought and heat tolerance are yet to be released for wider production (Yildirim et al., 2013). Recurrent drought occurrences necessitate the need for development of improved wheat cultivars for cultivation in dry environments to improve productivity. To improve wheat yield levels in dry environments, the International Wheat and Maize Improvement Centre (CIMMYT) is developing candidate genotypes (Lantican et al., 2001; Mwadzingeni et al., 2016). However, the level of drought tolerance characteristics among these genotypes remains largely unknown. Assessing the level of drought tolerance among diverse wheat populations will aid in the identification and selection of promising genotypes for advancing drought tolerance in improvement programmes. The objective of this study was to determine drought tolerance of dryland wheat genotypes based on leaf gas exchange and water-use efficiency in order to identify promising genotypes for drought tolerance breeding.

2.3   Materials and methods

2.3.1   Planting materials

Ten genetically diverse wheat genotypes sourced from the International Maize and Wheat Improvement Center (CYMMIT) were used for the study (Table 2.1). The genotypes were developed for cultivation in dry environments and were selected based on their diverse differential pedigrees. Initially, a set of 100 diverse wheat genotypes were acquired from CYMMIT's drought tolerance nursery, Mexico. Preliminary field evaluations (data not presented) under South African conditions indicated that the 10 genotypes selected for the current study were agronomically suitable with better yield and yield-related traits.

Table 2. 1: Names and pedigree information of 10 wheat genotypes used in the present study.

Genotype CODE	Name/Pedigree
G109	BABAX/3/PRL/SARA//TSI/VEE#5/4/CROC_1/AE.SQUARROSA (224)//2*OPATA
G112	CHIBIA/WEAVER
G115	CROC_1/AE.SQUARROSA (213)//PGO/3/NG8319//SHA4/LIRA
G118	CROC_1/AE.SQUARROSA (224)//OPATA/3/RAC655
G120	CROC_1/AE.SQUARROSA (224)//2*OPATA/3/2*RAC655
G139	SW89.5277/BORL95//SKAUZ
G141	SW94.60002/4/KAUZ*2//DOVE/BUC/3/KAUZ/5/SW91-12331
G339	KABY//2*ALUBUC/BAYA
G343	ALTAR 84/AEGILOPS SQUARROSA (TAUS)//OCI/3/VEE/MJI//2*TUI
G344	MILAN/KAUZ//PRINIA/3/BABAX

2.3.2 Study site

The experiment was conducted at the University of KwaZulu-Natal's Ukulinga Research Farm, Pietermaritzburg, KwaZulu-Natal Province (29°37′00″S30°23′34″E). The soil at the site is characterised by clay loam soils with the following physical properties: Clay % = 28%; Silt %= 48.94% and Coarse sand = 10.35%. The trial was planted under a rain-out shelter using custom-made plastic mulches which allowed the crops to grow under natural conditions excluding rainfall. Wheat was the only crop previously cultivated at the site.

2.3.3 Experimental design and crop establishment

The experiment was laid out as a 2 × 10 factorial treatment structure arranged in a complete randomised block design with the following factors: water condition (non-stressed [NS] and water stressed [WS] and genotypes (10 levels; see Table 2.1) and replicated 3 times. Genotypes were established under both NS and WS conditions. Seven to 10 plants were established in single rows of 1.4 m long for each genotype. Two seeds were planted and later thinned to one two weeks emergence. Intra-row and inter-row spacing were 15 and 40 cm, respectively. The total plot size for NS and WS treatments was 88.34 m2. Water stress was imposed at heading stage by withholding irrigation for 10 days. Plants in the NS treatment were watered daily to maintain soil moisture content close to field capacity (30%) until physiological maturity.

Soil samples were analysed at the KwaZulu-Natal's Department of Agriculture and Rural Development, Research and Technology: Analytical Services Laboratory, South Africa (Table 2.2). Based on soil fertility analysis N, P and K were applied at a rate of 200, 20 and 0 kg/ha, respectively. Weed control was done manually. The experiment was planted on the 11th January 2016 and harvested on the 20th May 2016.

Table 2. 2: Chemical composition of the soil used in the study.

Sample density (g/mL)	N (%)	P (mg/L)	K (mg/L)	Ca mg/L	Mg mg/L	Exch. Acidity (cmol mg/L)	Total cations (cmol/L)	Acid saturation (%)	pH (KCl)	Zn (mg/L)	Mn (mg/L)	Cu/(mg/L)	Organic C (%)
1.08	0.24	127	288	1581.3	318	0.30	11.54	2.66	4	9.73	104.6	14.9	2.23

N = nitrogen, P = phosphorus, K = phosphorus, Ca = calcium, Zn = zinc, Mn = manganese, Cu = copper, C = carbon.

2.4 Data collection

2.4.1 Soil moisture content

Volumetric soil moisture content was measured using a handheld soil moisture Theta probe (Type ML2X attached to HH2 moisture metre, Delta devices, England). The Theta Probe is a frequency domain reflectometry sensor (FDR) that is used to determine volumetric water content by generating an electromagnetic field to the soil. Measurements were made every second day after withholding irrigation in both non-stressed and water stressed treatments. Plants in the WS treatment were re-irrigated after 10 days of water stress and both NS and WS treatments were fully irrigated until physiological maturity stage. Volumetric soil (θ) water content was calculated using the formulae:

$$\theta = \sqrt{\varepsilon} - a0/a1$$

Where $\sqrt{\varepsilon}$ = the square root of the dielectric constant. A third order polynomial of the FDR sensor analog output V (in volts) is used to estimate the square root of the dielectric constant ε (Delta-T Devices, 2013) as $\sqrt{\varepsilon}$: $4.70V3 - 6.40V2 + 6.4V + 1.07$. a0 = $\sqrt{\varepsilon0}$ is the square root of the apparent dielectric constant obtained using the ThetaProbe voltage measured in an air-dry soil. The term a1 = $\sqrt{\varepsilon w}$ is the difference between the square root of the dielectric constant of saturated soil and dry soil divided by the soil water content at saturation.

2.4.2 Leaf gas exchange parameters

The following leaf gas exchange parameters were measured using the LI-COR 6400 XT portable photosynthesis system which was equipped with an LED red/ blue light source (6400-02B) (Licor Biosciences, Inc. Lincoln, Nebraska, USA): stomatal conductance (gs) (mol $H2O m^{-2} s^{-1}$), photosynthetic rate *(A)* (μmol CO2- $m^{-2} s^{-1}$) transpiration rate *(T)* (mmol H_2O $m^{-2} s^{-1}$), intercellular CO_2 concentration *(Ci)* (μmol CO_2 mol^{-1}) and the ratio of intercellular and atmospheric CO_2 *(Ci/ Ca).* The ratio of net CO_2 assimilation rate and intercellular CO_2 concentration *(A/Ci)* was calculated according to Dong et al. (2016). Intrinsic water-use efficiency (WUE$_i$) (μmol (CO_2) m^{-2} (H_2O) was calculated as the ratio of A/gs. Instantaneous water-use efficiency (WUE$_{inst}$) (μmol (CO_2) m^{-2} (H_2O) was calculated as the ratio of A/T (Tambussi et al., 2007).

During measurements, the flow rate of water was maintained at 500 μmol and relative humidity maintained at 43%. The leaf-to-air vapour pressure deficit in the cuvette was maintained at 1.7 kPa to prevent stomatal closure due to the low air humidity effect. Measurements were made after 10 days of withholding irrigation from three randomly selected plants between 11h00 and 14h00 on a clear and cloudless day. Grain yield (in grams) were determined at maturity for all genotypes under NS and WS conditions. Water-use efficiency at the whole-plant level (kg m^{-3}) was calculated as the ratio of grain yield to the water used by the plant according to Chen et al., (2010) as follows:

$$WUE = GY/ET$$

Where:

WUE = water use efficiency

GY = grain yield

ET = evapotranspiration

ET was obtained from the weather station at Ukulinga Research Farm and was calculated based on the water balance equation following the method of Chen et al. (2010) as follows:

$$ET = CR + P + DW - D - R$$

Where:

ET = evapotranspiration

CR = Capillary rise

P = Precipitation

D = Drainage

R = Runoff

DW (mm) = Change in soil moisture content

2.4.3 Data analysis

The data collected was subjected to analysis of variance (ANOVA) using GenStat (Version 17, VSN International, Hempstead, UK). The replications and blocks were treated as fixed factors whereas genotypes, water treatments and their interactions were considered as random factors. Treatment means were separated using the Least

Significant Difference (LSD) at 5% level of significance. Pearson correlation analysis was performed using SPSS 16.0 (SPSS, 2007) to test for associations among the studied parameters. Significance tests of the correlation were determined using the Student t-test. Principal component analysis (PCA) based on the correlation matrix was performed using SPSS 16.0 (SPSS, 2007). Biplot analysis was then used to describe the drought tolerance among wheat genotypes based on the studied traits (Singh and Raja Reddy 2011; Mashilo et al., 2017).

2.5   Results

2.5.1   Soil water content

Volumetric soil moisture content was different between non-stressed and water stressed conditions (Figure 2.1). Soil water content under water stressed condition decreased to about 7–6% after irrigation was withheld for 10 days. Signs of leaf rolling, and leaf wilting were observed in water-stressed plants at maximum stress (10 days). In contrast, soil water content was maintained at field capacity (~25%) under non stressed condition throughout the experiment.
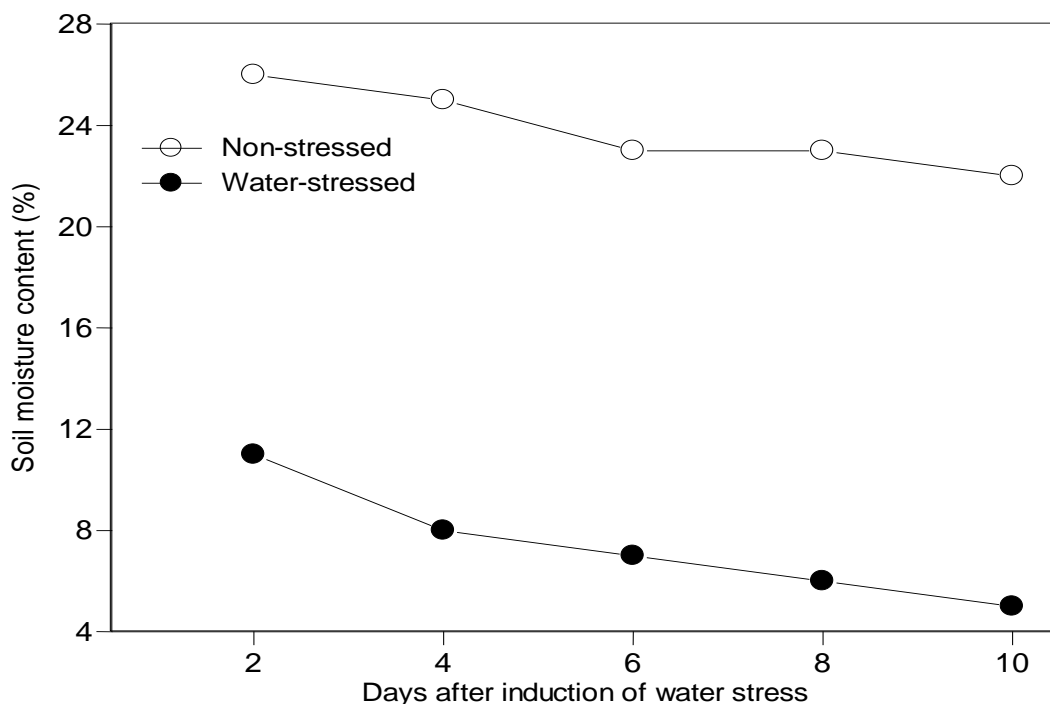
Figure 2. 1: Mean volumetric soil moisture content (%) of wheat genotypes grown under non-stressed and water-stressed conditions.

2.5.2 Effect of genotype, water condition and genotype × water condition interaction on leaf gas exchange variables, water-use efficiency and grain yield

Analysis of variance showing mean squares and significance test among the studied parameters of 10 selected wheat genotypes under non-stressed and water stressed conditions is presented in Table 2.3. Highly significant differences ($P < 0.001$) were observed among the genotypes with regards to *A, gs, T, A/C$_i$, C$_i$/Ca*, WUE$_i$, WUE$_{inst}$ and WUE suggesting genotypic variability among these traits. Highly significant differences ($P < 0.001$) were observed between water conditions with regards to *A, gs, A/C$_i$,* and WUE$_{inst}$. A significant genotype × water conditions interaction ($P < 0.001$) was observed on a number of leaf gas exchange parameters such as *A, A/C$_i$, C$_i$/Ca* and water-use efficiency (WUE$_i$, WUE$_{inst}$ and WUE) suggesting varying responses among the tested wheat genotypes under both non-stressed and water stressed conditions (Table 2.3).

Table 2. 3: Analysis of variance showing mean squares and significance test of leaf gaseous exchange parameters, water-use efficiency and grain yield of the 10 wheat genotypes tested under non-stress and water-stress conditions.

Source of variation	df	A	gs	T	C_i	A/C_i	C_i/C_a	WUE_i	WUE_{inst}	WUE	GY
Genotypes (G)	9	2.13**	0.01**	3.69**	34.22ns	3.49**	0.064*	14.22**	0.017**	0.049**	0.038ns
Water condition (W)	1	9.45**	0.14**	26.04*	60.89ns	1.49**	0.079ns	17.58*	0.050**	23.44**	0.020ns
G x W	9	2.86**	0.007ns	1.90ns	38.52ns	4.75**	0.079**	17.29**	0.022**	0.04**	0.032ns
Residual	36	0.35	0.003	0.62	12.44	5.83	0.013	3.11	0.035	2.15	0.031

A- Photosynthetic rate, gs- stomatal conductance, T-Transpiration, C_i-internal CO_2 concentration, A/C_i- ratio of photosynthetic rate and internal CO_2 concentration, C_i/C_a- ratio of internal CO_2 concentration and atmospheric CO_2, WUE_i- intrinsic water-use efficiency, WUE_{inst}- instantaneous water-use efficiency, WUE-water-use efficiency at whole-plant level, GY- grain yield. * Significant at 0.05 probability level, ** Significant at 0.01 probability level, ns – non-significant, df – degrees of freedom

2.5.3 Effect of water stress on leaf gas exchange parameters, water-use efficiency and grain yield of wheat genotypes

Mean values of gas exchange parameters, water-use efficiency and grain yield of wheat genotypes tested under non-stressed and water stressed conditions are presented in Table 2.4. Significant genotypic differences ($P < 0.001$) was observed with respect to photosynthesis *(A)* under both non-stressed and water stressed conditions. Genotypes G120 and G139 showed significantly higher A (>1.94 µmol CO_2 m^{-2} s^{-1}) under non-stressed condition compared to other genotypes. Under water stressed condition, G344 showed significantly ($P < 0.05$) higher A (2.54 µmol CO2 m^{-2} s^{-1}) compared to other genotypes. A was low for genotypes G112, G118, G120 and G139 which were further reduced by 81, 66, 87 and 85% due to water stress, respectively. Genotypes G339, G118, G139 and G120 showed significantly ($P < 0.05$) higher gs (>0.46 mol H_2Om^{-2} s^{-1}) under non-stressed condition. Non-significant ($P > 0.05$) differences were observed among wheat genotypes with regards to gs under water stressed condition. Non-significant ($P > 0.05$) differences were also observed among genotypes with respect to transpiration rate *(T)* under non-stressed condition. However, highly significant genotypic differences ($P < 0.001$) were observed under water stressed condition. Genotype G343 had a significantly lower T under water stressed condition (8.05 mmol H_2Om^{-2} s^{-1}) and a 31% reduction due to water stress. On the contrary, genotypes G339, G141 and G139 showed significantly higher T values (>10.74 mmol H_2O m^{-2} s^{-1}) under water stressed condition. Non-significant ($P > 0.05$) differences were observed among wheat genotypes under non-stressed condition with respect to internal CO_2 concentration *(C_i)*. However, significant genotypic differences were detected under water stressed condition. Genotype 344 showed significantly lower C_i value (246.2 µmol CO_2-mol^{-1}) compared to genotypes such as G120 and G139 which showed significantly ($P < 0.05$) higher C_i values (>258.1 µmol CO2 mol^{-1}).

Significant ($P < 0.05$) genotypic differences were observed with respect to ratio of photosynthetic rate and internal CO_2 concentration *(A/C_i)* under non-stressed condition. Genotypes G120 and G139 showed higher *A/Ci* values (0.009 and 0.017 µmol mol^{-1}, respectively), whereas G115 showed lower (0.002 µmol mol^{-1}) *A/C_i* value under non-stressed condition. Under water stressed condition, genotype G344 showed significantly higher *A/C_i* value (0.010 µmol mol^{-1}) whereas G112 showed

significantly (P < 0.05) lower A/C_i value (0.001 µmol mol^{-1}). Genotype 139 significantly showed a low ratio of internal CO_2 concentration and atmospheric CO_2 *(C$_i$/Ca)* value (0.89) under non-stressed condition, whereas the rest of the genotypes showed significantly higher *C$_i$/Ca* values (0.92–0.94 respectively). With regards to intrinsic water-use efficiency (WUE$_i$), highly significant differences (P < 0.001) were observed among the tested wheat genotypes under non-stressed condition. Genotypes G344, G112, G120 and G139 recorded significantly higher WUE$_i$ values (>3.38 µmol (CO_2) m^{-2} (H_2O) compared to G339 which recorded the lowest WUE$_i$ value (1.63 µmol (CO_2) m^{-2} (H_2O) under non-stressed condition.

Under water stressed condition, most of the tested wheat genotypes showed significantly (P < 0.05) lower WUE$_i$ values (0.99–1.82 µmol (CO_2) m^{-2} (H_2O) except G344 which had a higher WUE$_i$ value (8.81 µmol (CO_2) m^{-2} (H_2O). Furthermore, water stressed reduced WUE$_i$ of genotypes G112, G139 and G120 by 77, 83 and 60%, respectively. Significant genotypic response with respect to instantaneous water-use efficiency (WUE$_{inst}$) was observed under both non-stressed and water stressed conditions. Genotypes G120 and G139 showed significantly (P < 0.001) higher WUE$_{inst}$ (>0.16 µmol (CO_2) m^{-2} (H_2O) under non-stressed condition compared to other genotypes which showed lower WUE$_{inst}$ values (0.07–0.16 µmol (CO_2) m^{-2} (H_2O). Under water stressed condition, genotype G344 showed significantly (P < 0.05) higher WUEinst value (0.26 µmol (CO_2) m^{-2} (H_2O) than genotypes G115 and G118 which recorded the lowest WUE$_{inst}$ values (0.03 and 0.04 µmol (CO_2) m^{-2} (H_2O), respectively. Water stressed reduced WUE$_{inst}$ of genotypes G112 and G139 by 75 and 82% respectively, whereas the lowest reduction of 40% and 38 were recorded for G109 and G141, respectively. Highly significant (P < 0.001) differences were observed under both non-stressed and water stressed condition with respect to whole-plant water-use efficiency (WUE). Genotype G115 showed significantly (P < 0.001) higher WUE value (0.07 kg$^{-m3)}$) under non-stressed condition. Under water stressed condition, G112 showed a low WUE value of 0.04 kg m3 compared to G115 which showed significantly higher value (0.06 kg$^{-m3)}$) than G112 under water stressed condition. Non-significant differences (P > 0.05) were observed among the tested wheat with respect grain yield (GY) under both test conditions.

Table 2. 4: Leaf gaseous exchange parameters, water-use efficiency and grain yield of wheat genotypes grown under non-stress and water stress conditions.

Genotypes	A			gs			T			C_i		
	NS	WS	R %	NS	WS	R %	NS	WS	R %	NS	WS	R %
G339	0.92ab	1.65ab	44.2	0.56d	0.43	23.2	12.82	11.84f	7.64	258.24	257.7bc	0.20
G115	0.74a	0.42a	44.4	0.35ab	0.39	10.3	10.81	10.74cdef	0.64	255.65	257.5bc	0.71
G118	1.14abc	0.39a	79.8	0.49cd	0.27	45.0	11.74	9.70bc	17.37	255.21	255.9bc	0.24
G109	1.17abc	0.59ab	49.5	0.44c	0.33	25.0	11.12	9.94bcd	10.61	254.30	255.2bc	0.35
G343	1.29abc	0.70ab	46.0	0.46c	0.28	39.1	11.65	8.05a	30.90	254.77	252.2ab	1.00
G141	1.58bc	0.91ab	42.4	0.46c	0.37	20.0	12.13	11.40ef	6.01	249.89	253.7bc	1.50
G344	1.28abc	2.54c	49.6	0.33a	0.28	15.1	9.89	9.83bc	0.60	253.47	246.2a	2.86
G112	1.94cd	0.37a	81.0	0.43bc	0.35	19.0	11.83	9.22b	22.06	251.72	258.1bc	2.47
G120	2.47d	0.33ab	87.0	0.48cd	0.39	18.7	11.74	10.52cde	10.39	253.17	259.4c	2.40
G139	4.47e	0.67ab	85.0	0.47c	0.42	10.6	11.69	11.01def	5.81	248.40	259.1c	4.12
Mean	1.7	0.85	60.8	0.44	0.35	22.60	11.54	10.18	11.20	253.48	255.5	1.58
P- value	<.001	0.023		0.002	0.08		0.056	<.001		0.129	0.014	
LSD	0.81	1.17		0.08842	0.1203		1.51	1.140		6.300	6.073	
SE	0.38	0.561		0.04208	0.057		0.72	0.54		2.99	2.89	

Table 2.4: (Continued)

Genotypes	A/C_i			C_i/C_a			WUE_i		
	NS	WS	R %	NS	WS	R %	NS	WS	R %
G339	0.003ab	0.006bc	50.0	0.94e	0.93b	1.06	1.63a	3.93a	58.52
G115	0.002a	0.001ab	50.0	0.93bcde	0.94b	1.06	2.12ab	1.07a	49.5
G118	0.004ab	0.001a	75.0	0.94de	0.93b	1.06	2.35ab	1.30a	45.0
G109	0.004abc	0.002ab	50.0	0.93cde	0.93b	0.00	2.60abc	1.82a	30.0
G343	0.005abc	0.002ab	60.0	0.94de	0.92b	2.12	2.87abc	2.72a	5.22
G141	0.006bc	0.003ab	50.0	0.93bcd	0.93b	0.00	3.38abcd	2.48a	27.0
G344	0.005abc	0.010c	50.0	0.92bc	0.88a	4.37	3.79bcd	8.81b	57.0
G112	0.007cd	0.001a	86.0	0.92bc	0.94b	2.12	4.36cd	0.99a	77.3
G120	0.009d	0.003ab	67.0	0.92b	0.94b	2.12	5.10d	2.04a	60.0
G139	0.017e	0.002ab	88.2	0.89a	0.94b	5.31	9.42e	1.64a	83.0
Mean	0.006	0.003	62.6	0.92	0.92	1.92	3.76	2.68	49.2
P- value	<.001	0.025		<.001	0.005		<.001	0.017	
LSD	0.003	0.004883		0.014	0.025		1.78	3.87	
SE	0.001	0.002		0.07	0.01		0.85	1.84	

Table 2.4: (Continued)

Genotypes	WUE$_{inst}$			WUE			GY		
	NS	WS	R %	NS	WS	R %	NS	WS	R %
G339	0.07a	0.14a	50.0	0.4a	0.5ab	25.0	0.30	0.27	93.3
G115	0.07a	0.03a	57.1	0.7b	0.6b	14.2	0.37	0.30	91.9
G118	0.09ab	0.04a	56.0	0.5ab	0.5ab	0.0	0.37	0.35	91.9
G109	0.10ab	0.06a	40.0	0.5a	0.5ab	0.0	0.35	0.21	94.3
G343	0.11ab	0.08a	27.3	0.5a	0.5ab	0.0	0.34	0.23	94.1
G141	0.13ab	0.08a	38.4	0.5a	0.5ab	1.0	0.29	0.26	93.1
G344	0.12ab	0.26b	58.3	0.5a	0.6ab	20.0	0.28	0.38	89.3
G112	0.16bc	0.04a	75.0	0.6ab	0.4a	33.3	0.34	0.26	94.1
G120	0.21c	0.07a	67.0	0.5a	0.5ab	0.0	0.30	0.30	90.0
G139	0.38d	0.06a	82.0	0.5a	0.6b	16.7	0.35	0.03	94.2
Mean	0.14	0.08	55.1	0.6	0.6	0.34	0.32	0.02	92.6
P- value	<.001	0.02		<.001	<.001		0.14	0.60	
LSD	0.07	0.123		0.2	0.1		0.07	0.01	
SE	0.03	0.05		0.1	0.07		0.03	0.005	

A- Photosynthetic rate, *gs*- stomatal conductance, *T*-Transpiration, *C$_i$*-internal CO_2 concentration, *A/C$_i$*- ratio of photosynthetic rate and internal CO_2 concentration, *C$_i$/C$_a$*- ratio of internal CO_2 concentration and atmospheric CO_2, WUE$_i$- intrinsic water-use efficiency, WUE$_{inst}$- instantaneous water-use efficiency, WUE-water-use efficiency at whole-plant level, GY- grain yield, R- reduction in %. NS - Non-stress, WS - Water stress. SE - Standard error. Means followed by the same letter (s) are significantly different.

2.5.4   Associations among variables

Correlation coefficients (r) describing the level of associations among the studied traits under non-stressed and water stressed conditions are presented in Table 2.5. Under non-stressed condition (lower diagonal), positive and significant correlations were observed between A with A/C_i (r = 0.74; P = 0.031), WUE$_{inst}$ (r = 0.99; P < 0.001), and WUE$_i$ (r = 0.98; P < 0.001). Stomatal conductance *(gs)* was significantly and positively correlated with *T* (r = 0.79; P = 0.007) under non stressed condition. A/C_i was positively correlated with WUE$_{inst}$ (r = 0.73, P = 0.015) and WUE$_i$ (r = 0.72, P = 0.017) under non-stressed condition. Intrinsic water-use efficiency (WUE$_i$) was positively and significantly correlated with WUE$_{inst}$ (r = 0.99; P < 0.001) under non-stressed condition. Non-significant correlations were observed between WUE and other traits under non-stressed condition. Under water stressed condition (upper diagonal), positive and significant correlations were observed between T and gs (r = 0.91; P < 0.001). A/C_i was significantly and positively correlated with gs (r = 0.76; P = 0.010) and T (r = 0.76; P = 0.010) under water stressed condition. *A* was significantly and negatively correlated with C_i/Ca (r = −0.83; P = 0.003) under water stressed condition. Instantaneous water-use efficiency was positively and significantly correlated with *A* (r = 0.98; P < 0.001) and negatively correlated with C_i/Ca (r = −0.90; P < 0.001) under water stressed condition. WUE$_i$ was positively correlated with *A* (r = 0.96; P < 0.001) and WUE$_{inst}$ (r = 0.99; P < 0.001) and negatively correlated with C_i/Ca (r = −0.94; P < 0.001) under water stressed condition. There were negative and significant correlations between grain yield with A (r = −0.80; P = 0.005), WUE$_{inst}$ (r = −0.76; P < 0.001) and WUE$_i$ (r = −0.72; P = 0.081) under water stressed condition.

Table 2. 5: Pearson's correlation co-efficient (r) describing the association between leaf gaseous exchange parameters, water-use efficiency and grain yield of 10 selected wheat genotypes under non-stress (lower diagonal) and water stress (upper diagonal) conditions.

Parameters	A	gs	C_i	T	A/C_i	C_i/C_a	WUE$_{inst}$	WUE$_i$	WUE	GY
A	1	-0.19ns	0.18ns	-0.32ns	0.20ns	**-0.83****	**0.98****	**0.96****	-0.15ns	**-0.80****
gs	0.40ns	1	0.15ns	**0.91****	**0.76****	0.47ns	-0.27ns	-0.35ns	0.44ns	-0.02ns
C_i	0.38ns	**0.69***	1	0.05ns	0.30ns	-0.13ns	0.14ns	0.13ns	-0.34ns	0.03ns
T	0.16ns	**0.78****	0.36ns	1	**0.76***	0.62ns	-0.41ns	-0.49ns	0.31ns	-0.01ns
A/C_i	**0.74***	0.22ns	0.33ns	-0.03ns	1	0.24ns	0.08ns	-0.00ns	-0.02ns	-0.41ns
C_i/C_a	0.29ns	-0.45ns	-0.09ns	-0.48ns	0.13ns	1	**-0.90****	**-0.94****	0.04ns	0.53ns
WUE$_{inst}$	**0.99****	0.38ns	0.35ns	0.14ns	**0.73***	0.30ns	1	**0.99****	-0.13ns	**-0.76***
WUE$_i$	**0.98****	0.33ns	0.27ns	0.11ns	**0.72***	0.32ns	**0.99****	1	-0.13ns	**-0.72***
WUE	-0.25ns	0.15ns	-0.03ns	0.32ns	-0.52ns	0.15ns	-0.22ns	-0.23ns	1	0.11ns
GY	0.32ns	0.08ns	-0.04ns	0.22ns	0.05ns	0.24ns	0.36ns	0.41ns	**0.50***	1

A- Photosynthetic rate, gs- stomatal conductance, T-Transpiration, C_i-internal CO_2 concentration, A/C_i- ratio of photosynthetic rate and internal CO_2 concentration, C_i/C_a- ratio of internal CO_2 concentration and atmospheric CO_2, WUE$_i$- intrinsic water-use efficiency, WUE$_{inst}$- instantaneous water-use efficiency, WUE-water-use efficiency at whole-plant level, GY- grain yield. * Significant at 0.05 probability level, ** Significant at 0.01 probability level,ns-non-significant. Significant correlation coefficients ($r \geq 0.7$) are boldfaced.

2.5.5 Principal component analysis

Principal component analysis (PCA) showing eigenvectors, eigenvalues, and percent variance of the studied parameters of selected 10 wheat genotypes under non-stressed and water stressed conditions is presented in Table 2.6. Under non-stressed condition, PCA revealed three principal components (PC's) which accounted for 84% of the total variation. *A, A/C$_i$*, WUE$_{inst}$ and WUE$_i$ were positively correlated with PC1 which accounted for 43% of the total variation. Stomatal conductance *(gs)* and *T* where positively correlated with PC2; whereas *C$_i$/Ca* was negatively correlated with PC2 which accounted for 24% of the total variation. WUE and GY were positively correlated with PC3 which accounted for 18% of the total variation. Under water stressed condition, PCA revealed three PC's which accounted for 89% of the total variation. A, WUE$_i$ and WUE$_{inst}$ were positively correlated with PC1; whereas *C$_i$/Ca* was negatively correlated with PC1 which accounted for 49% of the total variation*. T, gs* and *A/C$_i$* where positively correlated with PC2 which accounted for 27.1% of the total variation. *C$_i$* was positively correlated with PC3; whereas WUE was negatively correlated with PC3 which accounted for 14% of the total variation. Principal component bi-plots based on PCA showing percent explaining variance of PC1 vs PC2, PC1 vs PC3 and PC2 vs PC3 superimposed with the studied parameters were constructed to visualise the relationship among the selected wheat genotypes to identify drought tolerant genotypes (Figure 2.2). Under non-stressed condition, genotypes G120 and G139 was differentiated by high values of *A, gs, T, C$_i$, A/C$_i$*, WUE$_i$, WUE$_{inst}$ (Table 2.8). Under water stressed condition, genotypes G339 and G334 were differentiated from the other genotypes by high values of *A, T, gs, A/C$_i$*, WUE$_i$ and WUE$_{inst}$ (Table 2.7). Genotypes G120, G141 and G339 were grouped together based on the high values of *T, gs* and *A/C$_i$.*

Table 2. 6: Principle component analysis showing eigenvectors, eigenvalues, and percent variance of leaf gas exchange, water-use efficiency and grain yield of selected 10 wheat genotypes under non-stress and water stress conditions.

Parameters	Non-stress			Water stress		
	PC1	PC2	PC3	PC1	PC2	PC3
A	**0.97**	0.14	0.06	**0.98**	-0.01	0.13
gs	0.26	**0.93**	0.09	-0.16	**0.94**	-0.14
C_i	0.34	0.64	-0.09	0.09	0.21	**0.79**
T	0.001	**0.86**	0.30	-0.29	**0.92**	-0.10
A/C_i	**0.83**	0.06	-0.33	0.18	**0.92**	0.23
C_i/C_a	0.38	**-0.67**	0.34	**-0.86**	.039	-0.01
WUE$_{inst}$	**0.97**	0.12	0.10	**0.98**	-0.11	0.09
WUE$_i$	**0.97**	0.06	0.12	**0.96**	-0.21	0.08
WUE	-0.33	0.13	**0.87**	-0.04	0.26	**-0.83**
GY	0.31	-0.02	**0.82**	-0.84	-0.25	0.04
Total variance explained (eigenvalues)	4.26	2.40	1.77	4.85	2.71	1.36
% of total variance	42.66	24.05	17.70	48.53	27.10	13.65
Cumulative variance (%)	42.66	66.71	84.42	48.53	75.64	89.30

A- Photosynthetic rate, gs- stomatal conductance, T-Transpiration, C_i-internal CO_2 concentration, A/C_i- ratio of photosynthetic rate and internal CO_2 concentration, C_i/C_a- ratio of internal CO_2 concentration and atmospheric CO_2, WUE$_i$- intrinsic water-use efficiency, WUE$_{inst}$- instantaneous water-use efficiency, WUE-water-use efficiency at whole-plant level, GY- grain yield. Vector loadings > 0.6 are boldfaced.
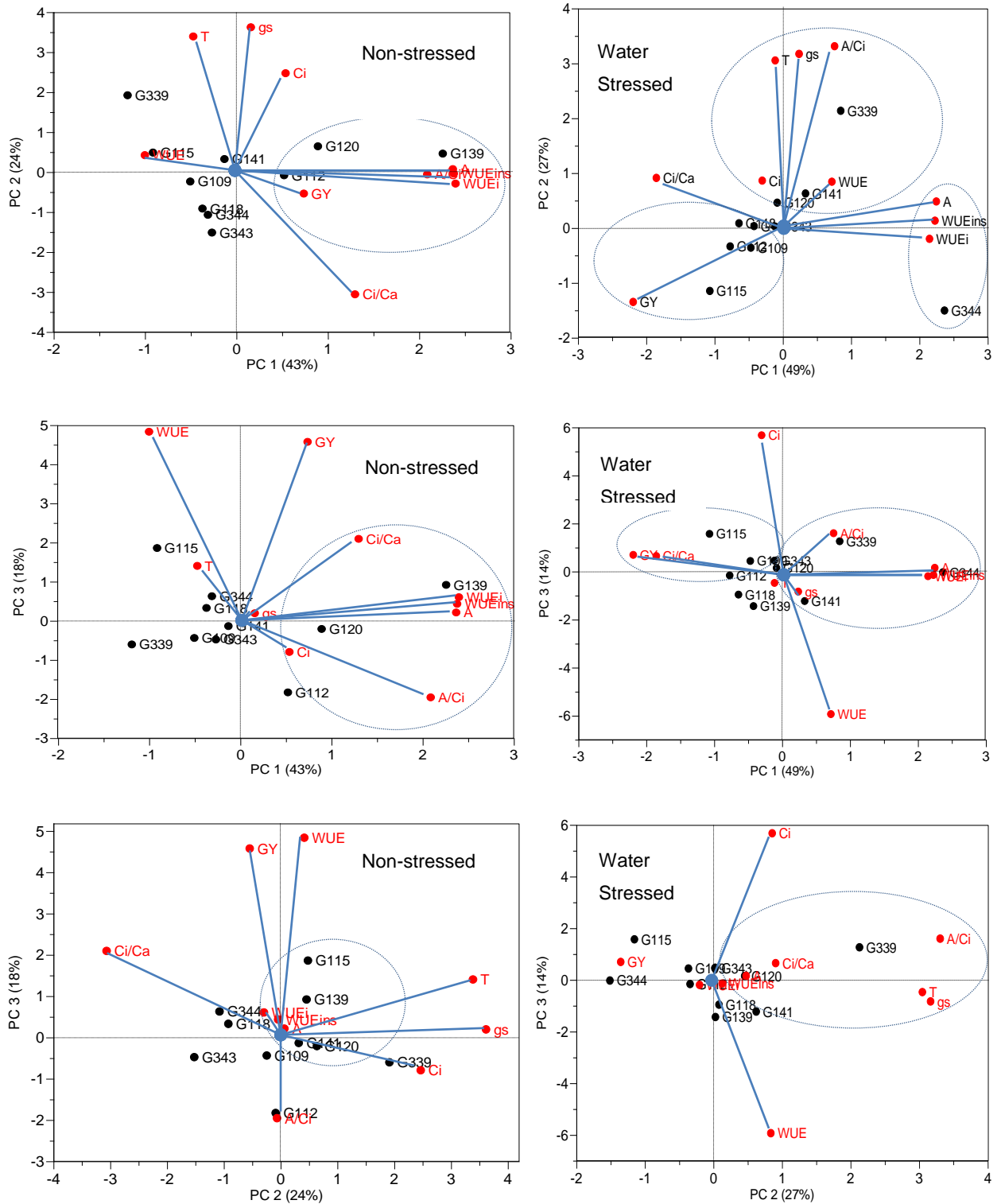
Figure 2. 2: Rotated principal component scores and percent explained variance of PC1 vs PC2, PC1 vs PC3 and PC2 vs PC3 showing the grouping of wheat genotypes based on leaf gaseous exchange parameters, water-use efficiency and grain yield under non-stress and water stress conditions. A- Photosynthetic rate, *gs-* stomatal conductance, *T-*

Transpiration, C_i-internal CO_2 concentration, A/Ci- ratio of photosynthetic rate and internal CO_2 concentration, C_i/Ca- ratio of internal CO_2 concentration and atmospheric CO_2, WUE_i- intrinsic water-use efficiency, WUE_{inst}- instantaneous water-use efficiency, WUE-water-use efficiency at whole-plant level, GY- grain yield.

Table 2. 7: Principal component scores of PC1 vs PC2, PC1 vs PC3 and PC2 vs PC3 showing the grouping of wheat genotypes based on leaf gaseous exchange parameters under water stress conditions.

	PC1 vs PC2									
Leaf gas exchange parameters	**G339**	**G115**	**G118**	**G109**	**G343**	**G141**	**G344**	**G112**	**G120**	**G139**
A	High	Low	Low	Low	Low	Low	High	Low	Low	Low
T	High	Low	Low	Low	Low	High	High	Low	High	Low
gs	High	Low	Low	Low	Low	High	High	Low	High	Low
Ci	Low	Moderate	Low	Low	Moderate	Low	Low	Low	Low	Low
A/Ci	High	Low	Moderate	Moderate	Low	High	High	Moderate	High	Low
Ci/Ca	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
WUEi	High	Low	Low	Low	Low	Low	High	Low	Low	Low
WUEinst	High	Low	Low	Low	Low	Low	High	Low	Low	Low
	PC1 vs PC3									
	G339	**G115**	**G118**	**G109**	**G343**	**G141**	**G344**	**G112**	**G120**	**G139**
A	High	Low	Low	Low	Low	High	High	Low	Low	Low
T	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
gs	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Ci	High	High	High	High	High	High	High	High	High	High
A/Ci	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Ci/Ca	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
WUEi	High	Low	Low	Low	Low	High	High	Low	Low	Low
WUEinst	High	Low	Low	Low	Low	High	High	Low	Low	Low
	PC2 vs PC3									
	G339	**G115**	**G118**	**G109**	**G343**	**G141**	**G344**	**G112**	**G120**	**G139**
A	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
T	High	Low	Low	Low	Low	High	Low	Low	Low	High
gs	High	Low	Low	Low	Low	High	Low	Low	Low	High

Ci	High	High	Low	Low	Low	High	Low	Low	Low	High
A/Ci	High	Low	Low	Low	Low	High	Low	Low	Low	Low
Ci/Ca	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
WUEi	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
WUEinst	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low

Table 2. 8: Principal component scores of PC1 vs PC2, PC1 vs PC3 and PC2 vs PC3 showing the grouping of wheat genotypes based on leaf gaseous exchange parameters under non-stress conditions.

PC1 vs PC2										
Leaf gas exchange parameters	**G339**	**G115**	**G118**	**G109**	**G343**	**G141**	**G344**	**G112**	**G120**	**G139**
A	Low	Low	Low	Low	Low	Low	Low	High	High	High
T	High	High	Low	Low	Low	Low	Low	High	High	High
gs	High	Low	Low	Low	Low	Low	Low	High	High	High
Ci	High	Low	Low	Low	Low	Low	Low	Moderate	Moderate	Moderate
A/Ci	Low	Low	Low	Low	Low	Low	Low	High	High	High
Ci/Ca	Low	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
WUEi	Low	Low	Low	Low	Low	Low	Low	High	High	High
WUEinst	Low	Low	Low	Low	Low	Low	Low	High	High	High
PC1 vs PC3										
	G339	**G115**	**G118**	**G109**	**G343**	**G141**	**G344**	**G112**	**G120**	**G139**
A	Low	Low	Low	Low	Low	Low	Low	High	High	High
T	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
gs	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Ci	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
A/Ci	Low	Low	Low	Low	Low	Low	Low	High	High	High
Ci/Ca	Low	Low	Low	Low	Low	Low	Moderate	Moderate	Moderate	Moderate

WUEi	Low	Low	Low	Low	Low	Low	Low	High	High	High
WUEinst	Low	Low	Low	Low	Low	Low	Low	High	High	High

PC2 vs PC3										
	G339	**G115**	**G118**	**G109**	**G343**	**G141**	**G344**	**G112**	**G120**	**G139**
A	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
T	High	Low	Low	High	Low	High	Low	High	High	Low
gs	High	Low	Low	High	Low	High	Low	High	High	Low
Ci	High	Low	Low	High	Low	High	Low	High	High	Low
A/Ci	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Ci/Ca	Moderate	Moderate	Moderate	Moderate	Low	Moderate	Moderate	Low	Moderate	Moderate
WUEi	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
WUEinst	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low

2.6 Discussion

Breeding high-yielding wheat genotypes for dry environments requires identification of drought tolerant and water-use efficient germplasm for use in improvement programmes. The current study determined drought tolerance of dryland wheat genotypes based on leaf gas exchange and water-use efficiency in order to identify promising genotypes for drought tolerance breeding. Results of the current study showed that water stress reduced photosynthetic rate (A), stomatal conductance (gs) and transpiration rate (T). Stomatal closure due to water stress has been reported to cause a decline in A and internal CO_2 concentration (C_i) (Moud and Yamagish 2006). However, in the current study, C_i was high under water stressed condition despite a reduction in A, gs and T (Table 2.4). The increase in C_i is an indication that A, gs and T were predominantly reduced by non-stomatal limitation such as reduced adenosine triphosphate (ATP) and activity ribulose-1,5- bisphosphate (RubP) carboxylase/ oxygenase under water stressed condition (Tezara et al., 1999; Parry et al. 2002; Santos et al., 2009; Jackson et al., 2016). The mean reduction in A due to water stress was 50.00% which was more than the mean decline in gs (20.45%) suggesting the role of non-stomatal limitations to reduction in A. Furthermore, results of the present study showed that A was poorly correlated with gs further confirming the role of non-stomatal limitations to A (Cornic, 2000; Dong et al., 2016). On the contrary, Flexas et al., (2009) reported that stomatal limitation is characterised by a daily maximum value of gs of greater than 0.05–0.10 mmol $H_2Om^{-2} s^{-1}$, whereas non-stomatal limitation is characterised by a value of gs of less than this threshold.

The mean stomatal conductance under water stressed condition in the current study was 0.35 mol $H_2Om^{-2} s^{-1}$ suggesting stomatal limitations to A. Singh and Raja Reddy (2011), suggested that gs value above 0.04 mol H_2O $m^{-2} s^{-1}$ is the main cause of reduced A under water stressed condition. A gs below (0.04 mol $H_2Om^{-2} s^{-1}$) suggest that the reduction in A is due to non-stomatal limitations. Photosynthesis requires diffusion of CO_2 from the atmosphere into the leaf through the stomata to the intercellular air spaces then across the mesophyll and to the site of carboxylation in the chloroplast stroma (Flexas et al., 2007). Stomatal limitations to photosynthesis results from the low CO_2 availability caused by limited diffusion through the stomata

and mesophyll cells (Hu et al., 2010). A number of studies have reported similar results where a reduction in A was a result of low gs (Chaves et al., 2003; Ripley et al. 2007; Xu and Zhou 2008). It is possible that the reduction in A observed in the current study could have been due to both non-stomatal and stomatal limitations (e.g. mesophyll conductance) (Jackson et al., 2016).

Stomatal closure reduces the rate of transpiration under water stress condition leading to improved water-use efficiency; however, this can lead to a reduced CO^2 influx into the leaves (Lawson and Blatt 2014). Water-use efficiency is an important physiological adaptation mechanism that may improve yield and drought tolerance of crops under limited water conditions (Medrano et al., 2015). In the present study, water stress significantly reduced intrinsic water-use efficiency (WUE_i) by 28.72% across all wheat genotypes. However, genotype G344 and 339 showed higher WUE_i values (8.81 and 3.93 µmol (CO_2) m^{-2} (H_2O respectively)). Similarly, instantaneous water-use efficiency (WUE_{inst}) was reduced by 42.86% across all wheat genotypes; however, genotypes G344 and G339 showed higher values (0.26 and 0.14 µmol (CO_2) m^{-2} (H_2O), respectively) under water stressed condition. In addition, these genotypes maintained higher A values (2.54 and 1.64 µmol CO_2 m^{-2} s^{-1}) under water stressed condition suggesting maintenance of WUE improved photosynthetic rates of these genotypes under water stressed condition. Maintenance of high WUE_i and A rates by G344 and G339 suggests these genotypes are drought tolerant and can be successfully grown in drought prone environments. The high WUE_i and WUE_{inst} of genotypes G344 and G339 under water stressed condition could be due to efficient control of gs (0.28 and 0.43 mol $H_2O m^{-2}$ s^{-1}) resulting in high A (2.54 and 1.65 µmol CO_2 m^{-2} s^{-1}). Important physiological traits for improving WUE at the leaf level is A and gs. Therefore, the selection of wheat genotypes with higher A and a low gs under water stress will lead to improved WUE (Singh and Raja Reddy 2011). Increased WUE enables the absorption of carbon due to reduced water loss (Farquhar et al., 1989). Additionally, genotype G344 showed a higher WUE at the whole plant level (0.6 kg m3) compared to other genotypes under water stressed condition. Low WUE indicates a high water-use or consumption by the plant and a high WUE indicates less water consumption (Farooq et al., 2009). Furthermore, low water-use under water stressed condition is indicative of drought tolerance. Results of the present study suggest that genotype G344 is water-use efficient and drought tolerant suitable for improving yields in dry

environments. Michihiro et al., (1994) also reported that wheat genotypes with high WUE under water stress condition are drought tolerant, whereas drought sensitive genotypes have low WUE. The wheat genotypes such as G344 and G339 which showed high WUE_i and WUE_{inst} under water stressed condition showed no significant differences in terms of grain yield compared to other genotypes. This is possibly because after water stress relief, plants were able to recover from the effect of water stress quickly and resume normal plant physiological functioning.

Associations among important indicators of drought tolerance is important for strategic breeding for drought tolerance improvement. In the current study, highly significant and positive correlations were observed between gs and T ($r = 0.91$; $P < 0.001$) under water stressed condition. This indicates that transpiration rate was greatly influenced by stomatal conductance. Similar findings have been reported on various C3 plants under water stress condition (Monneveux et al., 2006; Silva and Costa 2009; Silva et al., 2013). *Ci/Ca* negatively correlated with WUE_i ($r = -0.90$; $P < 0.001$) and WUE_{inst} ($r = -0.94$; $P < 0.001$) under water stressed condition (Table 2.5). Studies have reported that Ci/Ca is the inverse of WUEi (Brodribb 1996), which suggest that as the rate of WUE_i increases, the rate of C_i/Ca decreases. Furthermore, WUE_i and WUE_{inst} were highly correlated with A under water stressed condition (Table 2.5), suggesting improved WUE increased A in the current study. Similar studies where A was correlated with WUE under water stressed condition have been reported (Singh and Raja Reddy 2011; Medrano et al., 2015). Water-use efficiency at the plant level was poorly and negatively correlated with WUE at the leaf level (WUE_i and WUE_{inst}). This indicates that the relationship that existed between WUE_i and WUE_{inst} ($r = 0.99$; $P < 0.001$) did not have an influence on WUE at the whole-plant level (Table 2.5). Medrano et al. (2015) reported negative and non-significant association between WUE at the leaf level with WUE at the whole-plant level. This further suggested that improved water-use efficiency at the leaf level does not improve whole-plant water-use efficiency.

Principal component analysis under water stressed condition indicated that *A, gs, T, A/C_i, C_i/Ca,* WUE_{inst} and WUE_i explained most of the variation (PC1 and PC2) in the current study (Table 2.6). Principal component analysis biplot allowed the identification of drought tolerant genotypes such as G344 and G339 based on their high values of *A, gs, T, A/C_i,* WUE_i and WUE_{inst} under water stressed condition (Figure 2.2) (Table

2.7). Several other studies associated leaf gas exchange parameters and water-use efficiency as indicators of drought tolerance in different crop species (Lawlor and Tezara 2009; Silva and Costa 2009; Silva et al., 2013; Mashilo et al., 2017). In conclusion, the current study detected significant genetic variation for drought tolerance among the tested wheat genotypes using physiological parameters. Genotypes G339 and G344 were identified to be drought tolerant with efficient photo-assimilation rate, transpiration rate, stomatal conductance, carboxylation efficiency and water-use under water stressed condition.

References

Abdullah F., Hareri M., Naaesan M., Ammar M.A., Kanbar O.Z. (2011) Effect of drought on different physiological characters and yield components in different varieties of Syrian durum wheat. Journal of Agricultural Science 3:127–133.

Akhkha A., Boutraa T., Alhejely A. (2011) The rate of photosynthesis, chlorophyll content, dark respiration, proline and Abscicic acid (ABA) in wheat (Triticum durum) under water deficit conditions. International Journal of Agricultural and Biology 13:215–221.

Athar H.R., Ashraf M. (2009) Strategies for crop improvement against salinity and water stress: an overview. In: Ashraf M., Ozturk M., Athar H.R., editors. Salinity and water stress: improving crop efficiency. Germany: Spinger-Verlag; p. 1–16. Bartels DA, Furini JI, Salamini F. 1996. Responses of plants to dehydration stress: a molecular analysis. Plant Growth Regulation 20:111–118.

Blum A. (1996) Crop responses to drought and the interpretation of adaptation. Plant Growth Regulation 20:135–148.

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.

Blum A. (2009) Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. Field Crops Research 112:119–123.

Brodribb T. (1996) Dynamics of changing intercellular CO_2 concentration (Ci) during drought and determination of minimum functional Ci. Plant Physiology 111:179–185.

Chaves M.M., Maroco J., Pereira J.S. (2003) Understanding plant response to drought-from genes to the whole plant. Functional Plant Biology 30:239–264.

Chen C., Wang E., Yu Q. (2010) Modelling the effects of climate variability and water management on crop water productivity and water balance in the North China Plain. Agriculture Water Management 97:1174–1183.

Cornic G. (2000) Drought stress inhibits photosynthesis by decreasing stomatal aperture, not by affecting ATP synthesis. Trends Plant Science 5:187–188.

Deng X.P., Shan L., Zhang H.P., Turner N.C. (2006) Improving agricultural water use efficiency in arid and semiarid areas of China. Agriculture Water Management 80:23–40.

Dong B., Liu M., Shao H., Li Q., Shi L., Du F., Zhang Z (2008) Investigation on the relation between leaf water use efficiency and physio-biochemical traits of winter wheat under rained condition. Colloids and Surfaces B 62:280–287.

Dong C., Shao L., Wang M., Liu G., Liu H., Xie B., Li B., Fu Y., Liu H (2016) Wheat carbon dioxide responses in space simulations conducted at the Chinese Lunar Palace-1. Agronomy Journal 108:32–38.

Dube E., Mare-Patose R., Kilian W., Barnard A., Tsilo T.J. (2015) Identifying high-yielding dryland wheat cultivars for the summer rainfall area of South Africa. South African Journal of Plant Soil 33:77–81.

Ehleringer J.R., Hall A.E., Farguhar G.D. (1993) Introduction: water use in relation to productivity. In: Ehleringer J.R., Hall A.E., Farguhar G.D, editors. Stable isoptopes and plant carbon water relations. San Diego: Academic Press:3–7.

FAO. 2002. Plant production and protection series. No. 30. [accessed 10 November 2015]. www.fao.org/docrep/006/ y4011e/y4011eoo.htm#.

Farooq M., Wahid A., Kobayashi N., Fujita D., Basra S.M.A. (2009) Plant drought stress: effect, mechanism and management. Agronomy for Sustainable Development 29:185–212.

Farquhar G.D., Ehleringer J.R., Hubick K.T. (1989) Carbon isotope discrimination and photosynthesis. Annual Review of Plant Physiology 40:503–537.

Flexas J., Barón M., Bota J., Ducruet J.M., Gallé A., Galmés J., Jiménez M., Pou A., Ribas-Carbó M., Sajnani C. (2009) Photosynthesis limitations during water stress acclimation and recovery in the drought-adapted Vitis hybrid Richter-110 (V. *berlandieri* ×V. *rupestris*). Journal of Experimental Botany. 60:2361–2377.

Flexas J., Ortuno M.F., Ribas-Carbó M., Diaz-Espejo A., Florez-Sarasa I.D., Medrano H. (2007) Mesophyll conductance to CO_2 in Arabidopsis thaliana. New Phytologist. 175:501–511.

Hu L.X., Wang Z.L., Huang B.R. (2010) Diffusion limitations and metabolic factors associated with inhibition and recovery of photosynthesis from drought stress in a C3 perennial grass species. Acta Physiologeae Plantarum 139:93–106.

Jackson P., Basnayake J., Inman-Bamber G., Lakshmanan P., Natarajan S., Stokes C. (2016) Genetic variation in transpiration efficiency and relationships between whole plant and leaf gas exchange measurements in Saccharum spp. And related germplasm. Journal of Experimental Botany 67:861–871.

Lantican M., Pingali P., Rajaram S. (2001) Growth in wheat yield potential marginal environments. In: Reeves J., McNab A., Rajaram S., editors. Proceedings of the Warren E. Kronstad Symposium, Ciudad Obregón, Sonora, Mexico: 73–79.

Lawlor D.W., Tezara W. (2009) Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes. Annals of Botany 103:561–579.

Lawson T., Blatt M.R. (2014) Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. Plant Physiology 164:1556–1570.

Mashilo J., Odindo A.O., Shimelis H.A., Musenge P., Tesfay S.Z., Magwaza L.S. (2017) Drought tolerance of selected bottle gourd [*Lagenaria siceraria* (Molina) Standl.] landraces assessed by leaf gas exchange and photosynthetic efficiency. Plant Physiology and Biochemistry 120:75–87.

Medrano H., Tomasa M., Martorell S., Flexas J., Hernandez E., Rossello J., Poub A., Escalona J.S., Bota J. (2015) From leaf to whole-plant water use efficiency

(WUE) in complex canopies: limitations of leaf WUE as a selection target. Crop Journal 3:220–228.

Michihiro W., Lui J.C.B., Garvalho G.C. (1994) Cultivar difference in leaf photosynthesis and grain yield of wheat under soil water deficit conditions. Jap Journal of Crop Science 63:339 344.

Monneveux P., Rekika D., Acevedo E., Merah O. (2006) Effect of drought on leaf gas exchange, carbon isotope discrimination, transpiration efficiency and productivity in the field grown durum wheat genotypes. Plant Science 170:867–872.

Moud A.A.M., Yamagishi T. (2006) Gas exchange responses of different wheat (*Triticum aestivum* L.) cultivars to water stress condition. International Journal of Agriculture and Biology 1:102–105.

Mwadzingeni L., Shimelis H., Tesfay S., Tsilo T.J. (2016) Screening of bread wheat genotypes for drought tolerance using phenotypic and proline analyses. Frontier Plant Science 7:1–12.

Parry M.A.J., Andralojc P.J., Khan S., Lea P.J., Keys A.J. (2002) Rubisco activity: Effects of drought stress. Annals of Botany 89:833–838.

Ripley B.S., Gilbert M.E., Ibrahim D.G., Osborne C.P. (2007) Drought constraints on C4 photosynthesis: stomatal and metabolic limitations in C3 and C4 subspecies of *Alloteropsis semialata*. Journal of Experimental Botany 58:1351–1363.

Santos M.G., Ribeiro R.V., Machado E.C., Pimentel C. (2009) Photosynthesis parameters and leaf water potential of five common bean genotypes under mild water deficit. Biologia Plantarum 53:229–236.

Silva A.L.C, Costa W.A.J.M. (2009) Varietal variation in stomatal conductance, transpiration and photosynthesis of commercial sugarcane varieties under two contrasting water regimes. Tropical Agriculture Research and Extension 12:97–102.

Silva M.A., Jifon J.L., Santos C.M., Jadoskki C.J., Silva J.A.G. (2013) Photosynthetic capacity and water use efficiency in sugarcane genotypes subjected to water deficit during early growth phase. Brazilian Archives of Biology and Technology 56:735–748.

Singh S.K., Raja Reddy K. (2011) Regulation of photosynthesis, fluorescence, stomatal conductance and water-use efficiency of cowpea (*Vigna unguiculata* [L.] Walp.) under drought. Journal of Photochemistry and Photobiology B: Biology 105:40–50.

Sourour A., Afel O., Mounir R., Mongi B.Y. (2017) A review: morphological, physiological, biochemical and molecular plant responses to water deficit stress. International Journal of Engineering Science 6:01–04.

Tambussi E.A., Bort J., Araus J.L. (2007) Water use efficiency in C4 cereal under Mediterranean conditions: a review of some physiological aspects. CIHEAM (Options Mediterranean's: Series B 57:189–203.

Tezara W., Mitchell V.J., Driscoll S.D., Lawlor D.W. (1999) Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. Nature 401:914–917.

Varga B., Vida G., Varga-László E., Bencze S., Veisz O. (2015) Effect of simulating drought in various phenophases on the water use efficiency of winter wheat. Journal of Agronomy and Crop Science 201:1–9.

Xu Z., Zhou G. (2008) Response of leaf stomatal density to water status and its relationship with photosynthesis in a grass. Journal of Experimental Botany 59:3317–3325.

Yildirim A., Sönmezoğlu Ö.A., Sayaslan A., Koyuncu A.M., Güleç T., Kandemir N. (2013) Marker-assisted breeding of a durum wheat cultivar for γ-gliadin and LMW-glutenin proteins affecting pasta quality. Turkish Journal of Agriculture and Forestry 37:527–533

CHAPTER 3

Morpho-physiological traits associated with water-use efficiency in selected dry land wheat (*Triticum aestivum* L.) genotypes

Abstract

Morphological and physiological traits associated with enhanced water-use efficiency (WUE) are key components for drought tolerance. The objective of the present study was to examine associations between morphological and physiological traits of common wheat under drought stress in order to identify unique traits that may be used as direct or indirect selection criteria for improving WUE and drought tolerance in wheat. Ten selected and genetically diverse wheat genotypes were assessed under non-stressed (NS) and water-stressed (WS) conditions using a randomised complete block design with three replications. A significant ($P<0.05$) genotype x water condition interaction was observed for some studied traits suggesting varying genotypic responses under NS and WS conditions. Instantaneous water use-efficiency was positively and significantly correlated with number of leaves ($r = 0.76$; $P < 0.001$), number of tillers (NT) ($r = 0.67$; $P = 0.03$), plant height (PH) ($r = 0.72$; $P = 0.01$), dry biomass (DM) ($r = 0. 81$; $P < 0.001$) and grain yield (GY) ($r = 0.70$; $P = 0.02$) under WS condition. Further, these traits were also positively and significantly correlated with photosynthetic rate. Among assessed traits, number of tillers and dry biomass respectively recorded high heritability values of 81.40 and 83.12% and genetic advance of 48.22 and 63.80 g/plant, respectively under WS condition. This indicates that genetic gain can be realised for enhanced WUE in wheat incorporating these traits. Further, GY was significantly and positively correlated with NT ($r = 0.95$; $P < 0.001$) and DM ($r = 0.92$; $P < 0.001$) under WS condition. The following genotypes: G339, G343 and G344 which exhibited high NT and DM under WS condition were selected with enhanced water-use efficiency for breeding and sustainable wheat production under dryland environments.

Keywords: Breeding, drought stress, drought tolerance, morphology, water-use efficiency, wheat

3.1 Introduction

Water-use efficiency (WUE) is an important physiological parameter that can improve wheat yields under water-limited conditions (Condon et al., 2004; Mbave, 2013; Varga et al., 2015). Water-use efficiency at the whole-plant level is the ability of genotypes to produce biomass or yield per unit water used (Blum, 2005). Two components of WUE are recognized namely: instantaneous water-use (WUE_{inst}) and intrinsic water-use (WUE_i). WUE_{inst} is defined as the ratio of photosynthetic rate and transpiration rate, whereas, WUE_i is defined as the ratio of photosynthetic rate and stomatal conductance (Ehleringer et al., 1993; Tambussi et al., 2007). Both WUE_{inst} and WUE_i are considered to be important short-term physiological responses to measure water-use efficiency (Ehleringer et al., 1993).

Dryland wheat (*Triticum aestivum* L.) production and productivity is affected by recurrent droughts associated with global climate change. This presents a need to develop drought resilient and adapted wheat cultivars. Water use efficiency of crop genotypes can be improved at the physiological level by improving carboxylation efficiency and/or increasing CO_2 diffusion in the mesophyll cells (Flexas et al., 2013; 2016). Therefore, wheat improvement programs may select genotypes adapted to water-deficits, using an integrated approach involving physiological and morphological drought adaptive responses. Further, understanding morphological and physiological response mechanisms under water-deficits is useful in identifying yield related traits that could contribute to enhanced yields under water-stressed conditions (Liu et al., 2015). The magnitude of associations between morphological and physiological traits can serve as selection guide for water-use efficient, high yielding and drought tolerant genotypes for cultivation in water-limiting environments (Farshadfar et al., 2013).

Morphological traits associated with WUE and drought tolerance are key for cultivar development under drought conditions (Chen et al., 2012). Wheat ideotypes with reduced leaf area, smaller plant size and few productive tillers are amongst the ideal attributes that have been reported to improve WUE under limited water conditions (Fleury et al., 2010). Reduced leaf area improves WUE because the amount of water loss per unit leaf area is reduced as a result of fewer stomata number per leaf (Anyia and Herzog, 2004; Blum, 2005). Reduced plant size and reduced number of tillers under limited water conditions has been reported to contribute to efficient water-use in

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wheat (Akram, 2011). In addition, yield components such as the number of tillers, grain number per spike, number of fertile tillers per plant, thousand seed weight, peduncle length, awn length, plant height, spike length, number of kernels per spike and grain weight per spike contribute to wheat yield response (Blum, 2005; Nouri-Ganbalani et al., 2009; Aminzadeh, 2010). This suggests that morphological traits are critical in determining the production and productivity of wheat in water-limited environments.

Due to their high heritability and correlation with grain yield, most morphological traits can be used as indirect selection criteria for drought tolerance breeding and cultivar development in wheat (Chen et al., 2014; Abdolshahi et al., 2015). Genetic variation and higher heritability are necessary preconditions to enhance selection response. The expression and heritability of quantitative traits is influenced by genotypic differences, environmental influences and genotype by environment (G x E) interaction effects (Crossa et al., 1990). Genetic parameters such as genotypic and phenotypic coefficient of variation, heritability and genetic advance are important determinants of selection gains (Maniee et al., 2009). Therefore, understanding the morphological basis of drought tolerance in wheat is an overriding consideration to identify and select unique traits which can be used as indirect selection criteria for drought tolerance improvement.

The International Maize and Wheat Improvement Centre (CIMMYT) and various national wheat improvement programs are engaged in development and deployment of wheat germplasm with abiotic and biotic stress tolerance for breeding or direct production in target production environments. In an attempt to select wheat genotypes that are adapted to water scarce conditions, diverse wheat genotypes were acquired from CIMMYT's pre-breeding drought and heat tolerance nurseries. From this genetic pool, 10 genetically superior wheat genotypes developed for cultivation in dry environments were selected. The selected wheat genotypes possessed varied morphological and physiological responses that may be associated with water-use efficiency for drought tolerance breeding. Therefore, these genetic resources should be evaluated using various morpho-physiogical traits associated with drought tolerance. The objective of the present study was to examine associations between morphological and physiological traits of selected wheat genotypes under drought stress in order to identify unique traits that may be used as direct or indirect selection criterion for improving water-use efficiency and drought tolerance in wheat.

3.2 Materials and methods

3.2.1 Plant materials

Ten genetically diverse wheat genotypes developed for cultivation in dry environments were sourced from CYMMIT and used for the study (Table 3.1). Initially, a set of 100 diverse wheat genotypes were acquired from CYMMIT's drought tolerance nursery. Preliminary field experiments (data not presented) identified the 10 selected genotypes as agronomically suitable with outstanding yield and yield-related traits under South African conditions. The genotypes were further selected based on their parentage.

Table 3. 1: Names and pedigree information of 10 wheat genotypes used in the present study.

Genotype CODE	Name/Pedigree
G109	BABAX/3/PRL/SARA//TSI/VEE#5/4/CROC_1/AE.SQUARROSA (224)//2*OPATA
G112	CHIBIA/WEAVER
G115	CROC_1/AE.SQUARROSA (213)//PGO/3/NG8319//SHA4/LIRA
G118	CROC_1/AE.SQUARROSA (224)//OPATA/3/RAC655
G120	CROC_1/AE.SQUARROSA (224)//2*OPATA/3/2*RAC655
G139	SW89.5277/BORL95//SKAUZ
G141	SW94.60002/4/KAUZ*2//DOVE/BUC/3/KAUZ/5/SW91-12331
G339	KABY//2*ALUBUC/BAYA
G343	ALTAR 84/AEGILOPS SQUARROSA (TAUS)//OCI/3/VEE/MJI//2*TUI
G344	MILAN/KAUZ//PRINIA/3/BABAX

3.2.2 Study site

The experiment was conducted at the University of KwaZulu-Natal's Ukulinga Research Farm, Pietermaritzburg, KwaZulu-Natal Province (29°37′00″S30°23′34″E). The soil at the site is characterized by clay loam soils with the following physical properties: Clay % =28%; Silt % = 48.94% and Coarse sand = 10.35%. The trial was planted under a rain-out condition using custom-made plastic mulches which allowed the crops to grow under natural growing conditions excluding rainfall.

3.2.3 Experimental design and crop establishment

The experiment was laid out as a 2 x 10 factorial treatment structure using a complete randomized block design replicated 3 times. The two factors were water condition (non-stressed [NS] and water-stressed [WS] and genotypes (10 levels; see Table 3.1). Seeds were planted in single rows of 1.4 m long for each genotype. Two seeds were planted per hole and later thinned to one after two weeks of emergence. Intra-row and inter-row spacing were 15 and 40 cm, respectively. Based on soil fertility analysis N, P and K were applied at a rate of 200, 20 and 0 kg/ha, respectively. Water stress was imposed at heading stage by withholding irrigation for 10 days. Plants in the NS treatment were watered daily to maintain soil moisture content close to 30% volumetric water content until physiological maturity. Volumetric soil moisture content was measured using a handheld soil moisture probe (Type ML2X attached to HH2 moisture meter, Delta devices, England). Measurements were made every second day after imposing water stress. Plants in the WS treatment were re-irrigated after 10 days following water stress and both NS and WS treatments were fully irrigated until physiological maturity stage. Weed control was done manually. The experiment was planted on the 11th January 2016 and harvested on the 20th May 2016.

3.3 Data collection

3.3.1 Morphological traits

Data was collected on the following morphological traits: number of leaves per plant (NL) where counted manually. Number of tillers per plant (NT) was measured by counting the number of productive tillers at physiological maturity from three randomly selected plants. Plant height (PH) was measured at physiological maturity, using a plant height ruler from the soil surface until the tip of the spike (Mwadzingeni et al., 2016). The leaf area (LA) (cm^2) was calculated following the method of (Pommel et al., 2006):

$$LA = L \times W \times A.$$

Where:

L= Leaf length

W= Leaf width

A is a constant value which is 0.75 (Pommel et al, 2006).

Leaf area ratio (LAR) (m^2/m^2) was calculated as the ratio of LA and dry mass (DM) according to Amanullah et al., (2007):

$$LAR = LA/ DM$$

At maturity, the whole plant was harvested except the roots and placed in the oven for 48 h at 70°C in order to determine dry biomass (DM) according to Sharma et al., (2012).

3.3.2 Yield and yield components

The following yield and yield components were measured: number of spikes per plant (NSS) was counted. The spike length (SL) per plant was measured in centimetres (CM) using a rule. Grain yield (GY) was determined as weight (grams) per plant (Mwadzingeni et al., 2016).

3.3.3 Physiological traits

The LI-COR 6400 XT portable photosynthesis system equipped with an LED red/blue light source (6400-02B) (Licor Biosciences, Inc. Lincoln, Nebraska, USA) was used to measure the following leaf gas exchange parameters: stomatal conductance (gs) (mol H_2O m^{-2} s^{-1}), photosynthetic rate (A) (μmol CO_2 m^{-2} s^{-1}) and transpiration rate (T) (mmol H_2O m^{-2} s^{-1}). Intrinsic water use efficiency (WUE_i) (μmol (CO_2) m^{-2} (H_2O) was calculated as the ratio of A and gs and instantaneous water-use efficiency (WUE_{inst}) (μmol (CO_2) m^{-2} (H_2O), was calculated as the ratio of A and T (Tambussi *et al.*, 2007). Water-use efficiency at the whole-plant level (kg m^{-3}) was calculated as the ratio of grain yield to water used by the plant according to Mbave (2013) as follows:

$$WUE = GY/ET$$

Where:

WUE – water use efficiency

GY – grain yield

ET- evapotranspiration

ET was obtained from the weather station at Ukulinga Research Farm and was calculated based on the water balance equation following the method of (Chen et al., 2010) as follows:

$$ET=CR+P+DW-D-R$$

Where:

ET- evapotranspiration

CR- Capillary rise

P- Precipitation

D- Drainage

R- Runoff

DW (mm) - Change in soil moisture content

3.4   Data analysis

3.4.1   Analysis of variance

Data was subjected to analysis of variance (ANOVA) using GenStat (Version 17, VSN International, Hempstead, UK). The replications and blocks were treated as fixed factors, whereas genotypes, water treatments and their interaction were considered as random factors. The Least Significant Difference (LSD) was used to separate treatment means at 5% level of significance.

3.4.2   Correlation analysis

Pearson correlation analysis was performed using SPSS 16.0 (SPSS, 2007) to determine the level of association among the studied parameters under NS and WS conditions. Significance tests of the correlation were determined using the Student t-test.

3.4.3   Estimation of genetic parameters

Phenotypic and genotypic variances for the studied morphological traits were estimated from the analysis of variance (ANOVA). Genotypic and phenotypic

coefficients of variation were calculated according to (Singh and Chaudhary, 1977). Broad-sense heritability (H^2) was estimated according to (Falconer, 1989). Heritability values were categorized as low (0-30%), moderate (30-60%) and high (≥60%) (Robinson et al., 1949). Genetic advance (GA) was estimated according to (Johnson et al., 1955) using a 5% selection intensity (k = 2.06, where k is the selection intensity factor).

3.4.4 Principal component analysis

Principal component analysis (PCA) based on the correlation matrix was performed using SPSS 16.0 (SPSS, 2007).

3.5 Results

3.5.1 Soil water content

Soil moisture content differed between non-stressed and water-stressed treatments (Figure 3.1). Soil water content under water-stressed condition decreased to approximately 5% after 10 days of irrigation water was withheld to impose water stress. Signs of leaf rolling and wilting of leaves were observed in water drought-stressed plants at maximum stress (10 days). In contrast, under non-stressed condition, soil water content was maintained at ~ 25% throughout the study.
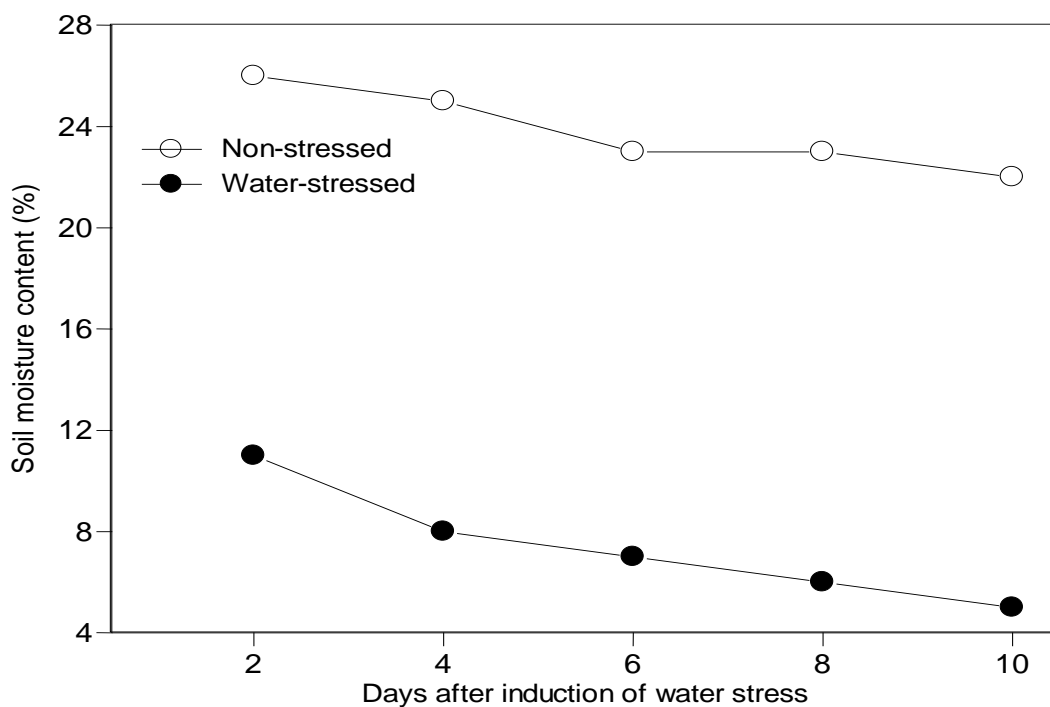
Figure 3. 1: Mean volumetric soil moisture content (%) of wheat genotypes grown under non-stressed and water-stressed conditions.

3.5.2 Effect of genotype, water-stress and genotype x water condition interaction effect on morphological and physiological traits

Analysis of variance showing mean squares and the significance test of the studied morphological and physiological traits among 10 selected wheat genotypes tested under non-stressed and water-stressed conditions is presented in Table 3.2. Significant differences ($P < 0.05$) were observed among wheat genotypes with respect to number of tillers, number of leaves, dry mass, leaf area and leaf area ratio (Table 3.2). Similarly, photosynthetic rate, stomatal conductance and transpiration rate, instantaneous water-use efficiency, water use efficiency and intrinsic water-use efficiency also differed significantly ($P < 0.05$) (Table 3.2) suggesting possible variability regarding genotypic responses. A significant genotype x water condition interaction ($P < 0.05$) with respect to number of tillers, plant height, dry matter and leaf area ratio, photosynthetic rate, instantaneous water-use efficiency and intrinsic water-use efficiency were recorded suggesting varying responses among the tested wheat genotypes under both non-stressed and water-stressed conditions (Table 3.2).

Table 3. 2: Analysis of variance showing mean square values and significance tests of morpho-physiological parameters of the 10 wheat genotypes tested under non-stressed and water-stressed conditions.

		Morphological traits								
Source of variance	df	LA	NL	NT	SL	NSS	PH	DM	LAR	GY
Genotypes (G)	9	145.32*	0.39*	24.21**	2.90ns	5.56ns	165.89ns	107.79**	5.71**	0.038ns
Stress conditions (W)	1	10.96ns	0.66ns	344.00**	0.20ns	0.26ns	865.11ns	86.40*	123.35*	0.020ns
G x W	9	59.73ns	0.28ns	32.10**	1.97ns	6.48ns	77.95*	98.03**	5.74**	0.032ns
Residual	36	43.80	0.34	5.14	2.09	2.94	76.06	15.85	1.37	0.031

		Physiological traits					
Source of variance	df	A	gs	T	WUE	WUE_{inst}	WUE_i
Genotypes (G)	9	2.13**	0.01**	3.69**	0.04**	0.01**	14.22**
Stress conditions (W)	1	9.45**	0.14**	26.04*	23.44**	0.05**	17.58ns
G x W	9	2.86**	0.007ns	1.90ns	0.04ns	0.02**	17.29**
Residual	36	0.35	0.003	0.62	2.15	0.03	3.11

LA =leaf area, NL = number of leaves, NT =number of tillers, SL = Spike length, NSS = Number of spikes per spikelet, PH =plant height, DM = dry matter, LAR = leaf area ratio, GY = grain yield, A = Photosynthetic rate, gs = stomatal conductance, T = transpiration rate, WUE = water-use efficiency, WUE_{inst} = instantaneous water-use efficiency, WUE_i = intrinsic water-use efficiency, * Significant at 0.05 probability level, ** Significant at 0.01 probability level, ns = non-significant.

3.5.3 Mean response of wheat genotypes for selected morphological traits under non-stressed and water-stressed conditions

Mean response for morphological and physiological traits of wheat genotypes tested under non-stressed and water-stressed conditions are presented in Table 3.3. Significant genotypic differences ($P < 0.05$) were observed with respect to leaf area (LA), under non-stressed condition. G343 recorded a mean LA of 51 cm^2, whereas the lowest leaf area was recorded for G120 with a value of 28 cm^2. Under water stress condition, non-significant differences were detected with regards to LA. Reduction in LA due to water stress was 3.79 cm^2. Non-significant ($P > 0.05$) genotypic response were observed with regards to number of leaves (NL) under NS condition. However, significant differences ($P < 0.05$) were observed under water-stressed condition with regards to NL. Genotypes G343 and G344 recorded the highest NL ($\approx$ 4) under water-stressed condition. Water stress increased NL by 14% across all genotypes. Overall, the genotypes G344, G141, G118 and G139 showed increased NL under water-stressed condition. The highest reduction in NL of 22.44% was observed for G120 due to water stress. Highly significant ($P < 0.001$) differences were observed among wheat genotypes under non-stressed condition with regards to number of tillers (NT). Genotypes G141, G339, G343 and G344 recorded the highest NT (> 15) under non-stressed condition. Under water-stressed condition, genotypes G339, G343 and G344 also recorded the highest NT. Conversely, the lowest NT were observed for G109 under both test conditions. The highest reduction of 51% for NT was observed for G112 due to water stress. Overall, water stress reduced number of tillers by 35%.

Non-significant ($P > 0.05$) differences were observed with regards to spike length under both non-stressed and water-stressed conditions. Likewise, non-significant differences were detected with regards to number of spikes per spikelet among test genotypes under both test conditions. Plant height (PH) differed significantly ($P < 0.05$) among the test genotypes under non-stressed condition. Genotypes G339 and G112 produced taller plants with values of 82 and 85 cm, respectively under non-stressed condition. G120 recorded the lowest PH of 60 cm under non-stressed condition. Under water-stressed condition, non-significant differences were observed among genotypes with regards to plant height. Highly significant ($P < 0.001$) genotypic response with

respect to dry biomass (DM) was observed under both non-stressed and water-stressed conditions (Table 3.3). Genotypes G344, G112 and G118 recorded higher DM of 19.33, 23.00 and 23.67 g, respectively under non-stressed condition compared with other genotypes such as G343, G115, G120 and G139 which recorded lower DM values ranging from 7.67 to 10.67g. Under water-stressed condition, genotypes G120, G115 and G344 recorded higher DM values of 15, 18 and 22 g, respectively which was the highest compared with G139, G343 and G339 which recorded low DM of 5 and 7 g, respectively. Moreover, water stress increased DM of genotypes G120, G344 and G109by 8, 14 and 25%, respectively. Further, the reduction in DM due to water stress was 34% across all genotypes. Significant differences ($P < 0.05$) were observed in terms of leaf area ratio (LAR) among wheat genotypes under non-stressed condition. Genotype G343 recorded significantly ($P < 0.05$) higher LAR value (8.46), which was higher than the rest of the tested genotypes which recorded LAR of < 5. Under water-stressed condition, non-significant differences ($P > 0.05$) were observed amongst the genotypes with regards to LAR. Water stress reduced LAR by 83% among the test genotypes. There were non-significant differences observed for grain yield (GY) under both test conditions.

Table 3. 3: Mean values for selected morphological traits of 10 wheat genotypes tested under non-stressed and water-stressed conditions.

Genotypes	LA (cm^2)			NL			NT			SL		
	NS	WS	R %	NS	WS	R %	NS	WS	R %	NS	WS	R %
G109	37.40b	37.00	1.07	3.00	2.66a	11. 33	8.78abc	4.44cd	49.43	10.00	11.00	-10.00
G112	43.10bc	41.00	4.87	3.00	2.77ab	7.67	10.33cd	5.00bc	51.60	11.00	12.00	-9.09
G115	40.40bc	38.00	5.94	3.00	2.77abc	7.67	11.11abc	8.22d	26.01	10.00	12.00	-20.00
G118	36.90b	36.00	2.44	3.00	3.11ab	-3.67	11.78e	9.00cd	23.60	10.00	10.00	0.00
G120	28.60a	31.00	-8.39	4.00	3.11abcd	22.25	12.44a	9.22cd	25.88	10.00	10.00	0.00
G139	44.20bc	44.00	0.45	3.00	3.22d	-7.33	14.33de	9.66a	32.59	11.00	10.00	9.09
G141	36.90b	41.00	-11.11	3.00	3.22abc	-7.33	15.78bc	9.88cd	37.39	12.00	11.00	8.33
G339	37.70b	40.00	-6.10	4.00	3.33bcd	16.75	17.22e	11.11c	35.48	12.00	11.00	8.33
G343	51.00c	35.00	31.37	4.00	3.55cd	11.25	17.67ab	11.11ab	37.13	10.00	9.00	10.00
G344	39.80bc	39.00	2.01	3.00	3.77abcd	-25.67	18.89de	12.77c	32.40	11.00	11.00	0.00
Mean	39.70	38.20	3.79	3.30	3.15	-14.0	13.83	9.04	34.63	10.70	10.70	0.00
P-value	0.03	0.43		0.83	0.03		<0.001	0.002		0.3	0.4	
LSD $_{(0.05)}$	12.07	10.80		1.30	0.6		3.3	3.5		2.2	2.7	
SE	5.75	5.10		0.60	0.3		1.6	1.7		1.1	1.3	

Table 3.3: (Continued)

Genotypes	NSS			PH (cm)			DM (g)			LAR			GY (g/plant)		
	NS	WS	R %	NS	WS	R %	NS	WS	R %	NS	WS	R %	NS	WS	R %
G109	17.00	19.00	-11.76	73.97bcd	59.63	19.39	13.33abc	16.67cd	-25.06	2.96a	0.64	78.38	0.35	0.21	40
G112	19.00	21.00	-10.53	84.97d	67.73	20.29	23.00e	9.67ab	57.96	1.89a	0.60	68.25	0.34	0.26	23.53
G115	17.00	21.00	-23.53	67.97ab	65.63	3.44	8.00ab	18.00cd	-55.56	3.85a	0.57	85.19	0.37	0.30	18.92
G118	19.00	20.00	-5.26	71.74abcd	66.63	7.12	23.67e	10.33ab	56.36	1.74a	0.53	69.54	0.37	0.35	5.41
G120	18.00	18.00	0.00	59.30a	58.97	0.56	8.33ab	15.33bc	-8.43	3.54a	0.53	85.03	0.3	0.30	0
G139	18.00	16.00	11.11	70.27abc	67.30	4.23	10.17ab	5.00a	50.84	4.26a	0.68	84.04	0.35	0.03	91.43
G141	20.00	20.00	0.00	72.17abcd	64.63	10.45	16.67cd	10.33ab	38.03	2.57a	0.65	74.71	0.29	0.26	10.34
G339	19.00	18.00	5.26	81.97cd	66.73	18.59	13.67bcd	6.67a	51.21	3.19a	0.60	81.19	0.3	0.27	10
G343	19.00	16.00	15.79	77.4bcd	63.20	18.35	7.67a	6.33a	58.20	8.46b	0.54	93.62	0.34	0.23	32.35
G344	20.00	20.00	0.00	75.2bcd	78.53	-4.43	19.33de	22.00d	-13.81	2.06a	0.49	76.21	0.28	0.38	-35.71
Mean	18.6	18.9	0.00	73.50	65.90	10.34	14.38	12.03	16.34	3.45	0.58	79.62	0.32	0.35	19.63
P-value	0.12	0.08		0.047	0.48		<0.001	<0.001		0.003	0.76		0.14	0.6	
LSD (0.05)	2.2	3.2		13.59	16.15		5.9	6.1		2.67	0.23		0.07	0.01	
SE	1.1	1.7		6.46	7.68		2.8	2.9		1.27	0.1		0.03	0.005	

LA=leaf area, NL=number of leaves, NT=number of tillers, SL=spike length, NSS=number of spikelets/spike, PH=plant height, DM=dry mass, LAR=leaf area ratio, GY=grain yield. LSD = Least significance difference SE = Standard error. Means followed by the same letter (s) are not significantly different at 5% level of significance. NS - Non-stressed condition, WS – Water-stressed condition.

3.5.4 Mean response of wheat genotypes for some physiological traits under non-stressed and water-stressed conditions

Mean response of the studied physiological traits among wheat genotypes tested under non-stressed and water-stressed conditions are presented in Figure. 3.2. Highly significant genotypic differences ($P < 0.05$) were observed with respect to photosynthetic rate (A) under both water conditions (Figure 3.2 A). Genotypes G120 and G139 recorded significantly higher A of 2.47 and 4.47 µmol CO_2 m^{-2} s^{-1} under non-stressed condition, respectively. The rest of the genotypes recorded A values of < 2 µmol CO_2 m^{-2} s^{-1} under non-stressed condition. Under water-stressed condition, G344 and G339 showed significantly ($P < 0.05$) higher A of 2.54 and 1.65 µmol CO_2 m^{-2} s^{-1} compared to other genotypes (Figure 3.2 A). Genotypes G112, G118, G120 and G139 recorded low A values yielding a reduction of 81, 66, 87 and 85% due to water stress, respectively. Genotypes G339, G118, G139 and G120 showed significantly ($P < 0.05$) higher gs of 0.56, 0.46, 0.47 and 0.48 mol H_2O m^{-2} s^{-1} under non-stressed condition (Fig. 3.2B). Non-significant ($P > 0.05$) differences were observed among wheat genotypes with regards to gs under water-stressed condition. Also, non-significant ($P > 0.05$) differences were detected among genotypes with respect to transpiration rate (T) under non-stressed condition (Figure 3.2 C). However, highly significant genotypic differences ($P < 0.001$) were observed under water-stressed condition with respect to T. Genotype G343 had a significantly lower T under water-stressed condition (8.05 mmol H_2O m^{-2} s^{-1}) and a 31% reduction due to water stress. On the contrary, genotypes G339, G141 and G139 showed significantly higher T values of 11.84, 11.40 and 11.01 mmol H_2O m^{-2} s^{-1} under water-stressed condition. The highest reduction in T of 22.06 and 30.9% was observed for G112 and G343 due to water stress (Figure 3.2 C).

Highly significant differences ($P < 0.001$) were observed among the tested wheat genotypes under non-stressed condition with regards to intrinsic water-use efficiency (WUE$_i$) (Figure 3.2 D). Genotypes G120 and G139 recorded significantly higher WUE$_i$ values of 5.10 and 9.42 µmol (CO_2) m^{-2} (H_2O) under non-stressed condition. Under water-stressed condition, most of the tested wheat genotypes showed significantly ($P < 0.05$) lower WUE$_i$ values of 0.99-1.82 µmol (CO_2) m^{-2} (H_2O) except G344 which

recorded WUE_i of 8.81 μmol (CO_2) m^{-2} (H_2O). Further genotypes G112, G139 and G120 showed severe reduction in WUE_i of 77, 83 and 60%, respectively (Figure 3.2 D). Significant genotypic response with respect to instantaneous water-use efficiency (WUE_{inst}) was observed under both test conditions (Figure 3.2 E). Genotypes G120 and G139 showed significantly ($P < 0.001$) higher WUE_{inst} (>0.16 μmol (CO_2) m^{-2} (H_2O)) under non-stressed condition. Under water-stressed condition, genotype G344 showed significantly ($P < 0.05$) higher WUE_{inst} value of 0.26 μmol (CO_2) m^{-2} (H_2O). Water-stressed reduced WUE_{inst} of the genotypes G112 and G139 by 75 and 82% respectively, whereas the lowest reduction of 40 and 38% were recorded for G109 and G141, respectively (Figure. 3.2 E). Highly significant ($P < 0.001$) differences were observed under both non-stressed and water-stressed conditions with respect to whole-plant water-use efficiency (WUE) (Figure 3.2 F). Genotype G115 showed significantly ($P < 0.001$) higher WUE value (0.07 kg m^3) under non-stressed condition. Under water-stressed condition, G112 showed a low WUE value of 0.04 kg m^3 compared to G115 which had significantly higher value (0.06 kg m^3) than G112 under water-stressed condition (Figure 3.2 F).
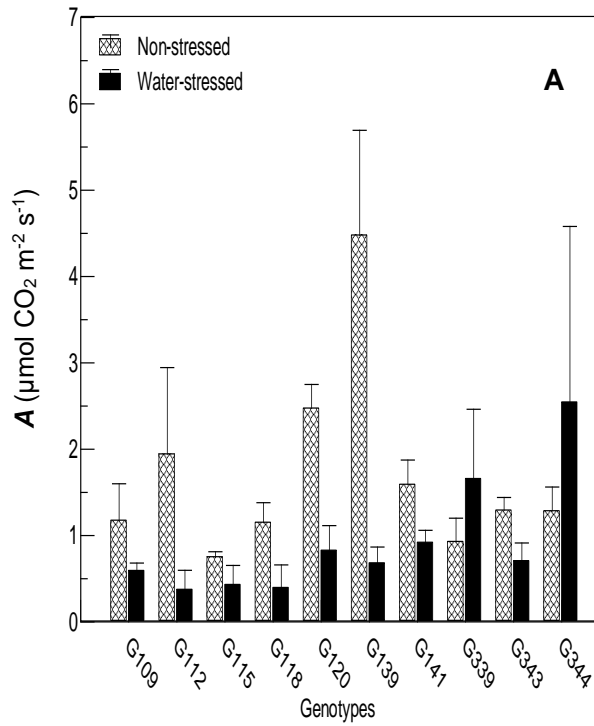
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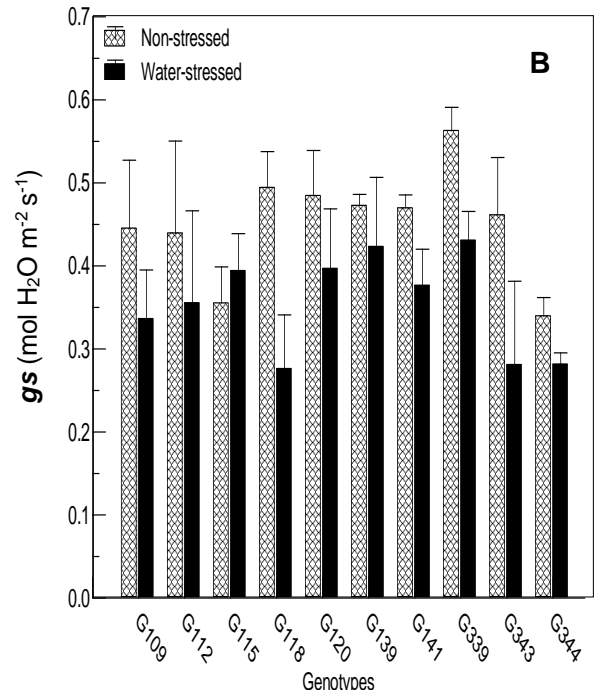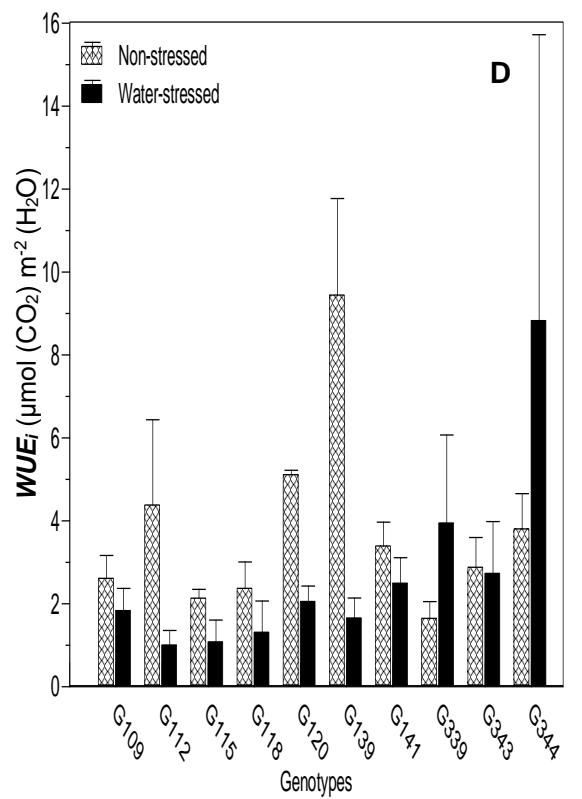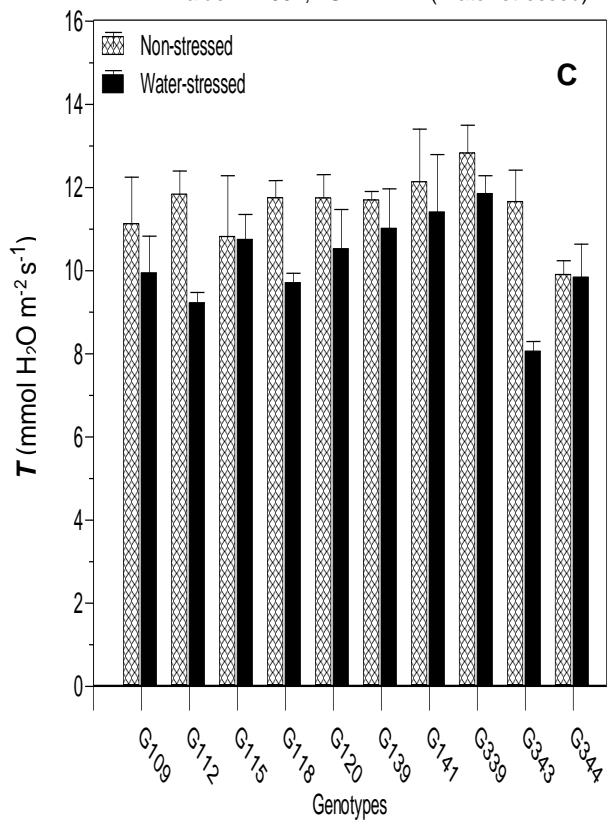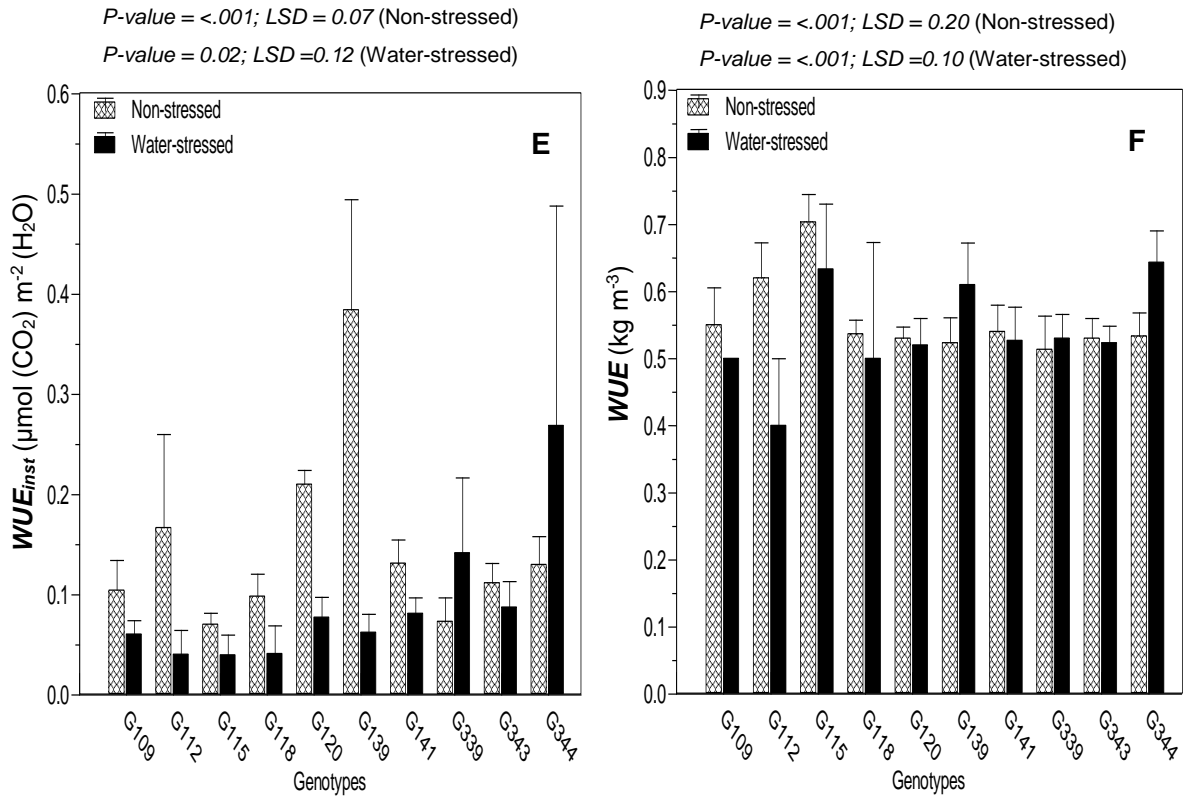
Figure 3. 2: Mean values for some physiological traits among 10 wheat genotypes tested under non-stressed and water-stressed conditions. *A =* Photosynthetic rate, *gs* =stomatal conductance, *T*=Transpiration, WUE$_i$=intrinsic water =use efficiency, WUE$_{inst}$= instantaneous water-use efficiency, WUE-water-use efficiency at whole-plant level.

3.5.5 Associations among morphological and physiological traits

The level of associations among the studied traits under non-stressed and water-stressed conditions are presented in Table 3.4. Under non-stressed condition, number of tillers was positively and significantly correlated with number of spikelets per spike ($r = 0.70$; $P = 0.02$), plant height ($r = 0.94$; $P < 0.001$), dry mass ($r = 0.97$, $P < 0.001$), grain yield ($r = 0.93$; $P < 0.001$) and leaf area ratio ($r = 0.82$; $P < 0.001$). Number of spikelets per spike positively and significantly correlated with plant height ($r = 0.70$; $P = 0.02$), dry mass ($r = 0.66$; $P = 0.03$), grain yield ($r = 0.67$; $P = 0.03$), leaf area ratio ($r = 0.63$; $P = 0.04$) and transpiration rate ($r = 0.75$; $P = 0.019$) under non-stressed condition. Plant height was significantly and positively correlated with dry mass ($r =$

0.91; $P < 0.001$), grain yield ($r = 0.99$; $P < 0.001$) and leaf area ratio ($r = 0.83$; $P < 0.001$) under non-stressed condition. Dry mass was positively correlated with grain yield ($r = 0.89$; $P < 0.001$) and leaf area ratio ($r = 0.88$; $P = 0.002$) under non-stressed condition. Grain yield was positively and significantly correlated with leaf area ratio ($r = 0.84$; $P = 0.002$) under non-stressed condition. Leaf area ratio was not correlated to any physiological trait under non-stressed condition. Further, photosynthetic rate was positively and significantly correlated with WUE_{inst} ($r = 0.99$; $P < 0.001$) and WUE_i ($r = 0.97$; $P < 0.001$) under non-stressed condition. Stomatal conductance negatively and significantly correlated with whole-plant water-use efficiency (WUE) ($r = -0.68$; $P = 0.029$) whereas, water use efficiency at the whole plant level also negatively and significantly correlated with transpiration rate ($r = -0.74$; $P = 0.02$) under non-stressed condition.

Under water-stressed condition, leaf area was positively and significantly correlated with leaf area ratio ($r = 0.65$; $P = 0.04$). Number of leaves per plant positively and significantly correlated with number of tillers ($r = 0.93$; $P < 0.001$), dry mass ($r = 0.96$; $P < 0.001$), grain yield (r = 0.95; $P < 0.001$), photosynthetic rate ($r = 0.73$; $P = 0.02$) and instantaneous water-use efficiency (WUE_{inst}) ($r = 0.77$; $P < 0.001$) under water-stressed condition. Number of tillers was positively and significantly correlated with dry mass ($r = 0.88$; $P < 0.001$), grain yield ($r = 0.95$; $P < 0.001$), photosynthetic rate ($r = 0.66$; $P = 0.04$) and WUE_{inst} ($r = 0.68$; $P = 0.03$) under water-stressed condition. Plant height, dry matter and grain yield were positively and significantly correlated with photosynthetic rate ($r = 0.76$; $P = 0.01$; $r = 0.80$; $P = 0.005$ and $r = 0.69$: $P = 0.03$) and WUE_{inst} ($r = 0.72$; $P = 0.02$; $r = 0.81$; $P = 0.02$ and $r = 0.70$; $P = 0.02$), under water-stressed condition in that order. Dry matter positively and significantly correlated with grain yield ($r = 0.92$; $P < 0.001$) whereas, photosynthetic rate was significantly and positively correlated with instantaneous water use efficiency ($r = 0.97$; $P < 0.001$), whereas, transpiration rate was positively and significantly correlated with intrinsic water use efficiency ($r = 0.81$; $P < 0.001$) under water-stressed condition.

Table 3. 4: Pearson's correlation co-efficient (*r*) describing the association between morphological and physiological traits of 10 selected wheat genotypes under non-stressed (lower diagonal) and water-stressed (upper diagonal) conditions.

Traits	LA	NL	NT	SL	NSS	PH	DM	GY	LAR	*A*	*gs*	*T*	WUEinst	WUE	WUEi
LA	1	0.01ns	-0.04ns	0.40ns	0.06ns	0.48ns	0.04ns	0.07ns	**0.65***	0.26ns	0.34ns	0.10ns	0.09ns	0.17ns	-0.05ns
NL	0.18ns	1	**0.92****	-0.51ns	-0.41ns	0.56ns	**0.96****	**0.94****	-0.43ns	**0.72***	-0.29ns	0.35ns	**0.76****	0.34ns	0.29ns
NT	0.43ns	0.38ns	1	-0.45ns	-0.38ns	0.51ns	**0.88****	**0.95****	-0.47ns	**0.65***	-0.09ns	0.49ns	**0.67***	0.52ns	0.31ns
SL	-0.00ns	-0.02ns	0.49ns	1	**0.80****	0.25ns	-0.35ns	-0.41ns	0.18ns	0.09ns	0.30ns	-0.32ns	-0.00ns	-0.07ns	-0.33ns
NSS	0.30ns	0.04ns	**0.70***	0.60ns	1	0.28ns	-0.28ns	-0.37ns	-0.19ns	0.01ns	-0.17ns	-0.56ns	-0.02ns	-0.17ns	-0.52ns
PH	0.41ns	0.35ns	**0.94****	0.33ns	**0.70***	1	0.55ns	0.52ns	-0.33ns	**0.76***	-0.27ns	-0.23ns	**0.72***	0.38ns	-0.33ns
DM	0.48ns	0.47ns	**0.97****	0.36ns	**0.66***	**0.91****	1	**0.92****	-0.40ns	**0.80****	-0.24ns	0.42ns	**0.81****	0.26ns	0.39ns
GY	0.39ns	0.33ns	**0.93****	0.34ns	**0.67***	**0.99****	**0.89****	1	-0.33ns	**0.69***	-0.05ns	0.51ns	**0.70***	0.49ns	0.31ns
LAR	0.22ns	0.08ns	**0.82****	0.28ns	**0.63***	**0.83****	**0.84****	**0.84****	1	-0.29ns	0.59ns	0.29ns	-0.42ns	-0.07ns	0.21ns
A	0.06ns	-0.08ns	-0.02ns	0.09ns	-0.10ns	-0.01ns	-0.07ns	-0.04ns	-0.07ns	1	-0.13ns	0.14ns	**0.97****	0.38ns	0.17ns
gs	0.05ns	0.54ns	0.04ns	0.28ns	0.07ns	-0.08ns	0.04ns	-0.12ns	-0.35ns	0.17ns	1	0.49ns	-0.22ns	0.11ns	0.36ns
T	0.09ns	0.45ns	0.65ns	**0.77***	**0.75***	0.52ns	0.59ns	0.54ns	0.62ns	0.04ns	**0.86****	1	0.05ns	0.25ns	**0.81****
WUEinst	0.06ns	-0.10ns	-0.00ns	0.07ns	-0.11ns	0.00ns	-0.06ns	-0.03ns	-0.04ns	**0.99****	0.11ns	0.00ns	1	0.35ns	0.11ns
WUE	-0.16ns	-0.46ns	-0.50ns	-0.41ns	-0.41ns	-0.31ns	-0.54ns	-0.27ns	-0.34ns	-0.11ns	**-0.68***	**-0.74***	-0.08ns	1	-0.20ns
WUEi	0.08ns	-0.14ns	0.06ns	0.10ns	-0.02ns	0.11ns	0.00ns	0.08ns	0.05ns	**0.97****	0.01ns	0.01ns	**0.98****	-0.02ns	1

LA=leaf area, NL=number of leaves, NT=number of tillers, SL=spike length, NSS=number of spikelets/spike, PH=plant height, DM=dry matter, LAR=leaf area ratio, GY=grain yield, *A*=Photosynthetic rate, *gs*-=stomatal conductance, *T*=Transpiration, WUE$_i$=intrinsic water-use efficiency, WUE$_{inst}$=instantaneous water-use efficiency, WUE=water-use efficiency at whole-plant level. * Significant at 0.05 probability level, ** Significant at 0.01 probability level, ns-non-significant. Significant correlation coefficients (*r* ≥ 0.7) are boldfaced.

3.5.6 Estimates of genetic parameters amongst studied morphological traits under non-stressed and water-stressed conditions

Estimates of genetic parameters among the studied morphological traits are summarized in Table 3.5. The analysis between two water conditions revealed that for all the traits studied, phenotypic variance was relatively higher than genotypic variance. A lower genotypic variance was observed for grain yield (0.00 g/plant), spike length (0.17 cm) and number of spikes per spikelet (0.46) under non-stressed condition. Furthermore, a higher phenotypic variance was observed for leaf area (78.6 cm^2) and plant height (94.0 cm), whereas, a lower phenotypic variance was observed on grain yield (0.00 g/plant), spike length (1.87 cm), number of spikelets per spike (2.12) and leaf area ratio (5.43). Environmental variance was higher for leaf area (45.5 cm^2) and plant height (62.8 cm) under non-stressed condition.

Under water-stressed condition, all the tested traits showed lower genotypic variance except for dry matter and number of tillers with values of 28.0 and 8.27%, respectively. Leaf area and dry matter were the only traits that recorded higher phenotypic variance values of 40.2 and 40.5%, respectively, whereas the rest of the traits showed low phenotypic variance (Table 3.5). Furthermore, environmental variance was generally lower for all the traits except for plant height (88.7 cm) and leaf area ratio (39.4) under water-stressed condition. Leaf area ratio and dry matter recorded high values of GCV (50.06 and 40.8%) and PCV (67.4 and 47.50%), under non-stressed condition. Under water stress condition, number of tillers and dry matter recorded high values of GCV (32.0 and 44.10%) and PCV (39.3 and 53.03%), respectively. Leaf area, number of tillers, plant height, dry matter and leaf area ratio recorded high heritability and genetic advance (GA) values under non-stressed condition (Table 3.5). Under water-stressed condition, leaf area showed low heritability but high genetic advance. Number of tillers and dry matter recorded high heritability and genetic advance values under water-stressed condition (Table 3.5). Leaf area recorded the higher GA under water-stressed condition.

Table 3. 5: Estimates of variance components, genotypic and phenotypic coefficients of variation, broad-sense heritability and genetic advance of morphological traits assessed among 10 wheat genotypes under non-stressed and water-stressed conditions.

	Non-stressed condition								Water-stressed condition							
Traits	σ^2_g	σ^2_p	σ^2_e	X	GVC (%)	PCV (%)	H^2 (%)	GA	σ^2_g	σ^2_p	σ^2_e	X	GVC (%)	PCV (%)	H^2 (%)	GA
LA	31.1	78.60	45.5	39.6	14.10	14.10	100.00	35.60	0.75	40.20	39.40	38.00	2.28	16.70	13.66	122.1
NL	-	-	0.58	3.0	-	18.60	-	-	0.08	0.21	0.13	3.00	9.43	15.30	61.72	24.75
NT	10.7	14.30	3.61	14.0	23.40	27.0	86.50	31.30	8.27	12.50	4.21	9.00	32.00	39.30	81.40	48.22
SL	0.17	1.87	1.70	11.0	3.75	12.40	30.20	41.20	0.08	2.46	2.38	11.00	2.57	14.30	18.03	79.07
NSS	0.46	2.12	1.66	19.0	3.57	7.66	46.60	16.50	1.59	5.79	4.20	19.00	6.64	12.70	52.40	24.17
PH	31.2	94.0	62.8	73.5	7.59	13.20	57.54	73.60	-	-	88.70	66.00	-	14.30	-	-
DM	32.6	44.20	11.70	14.0	40.8	47.50	85.80	55.40	28.00	40.50	12.50	12.00	44.10	53.00	83.12	63.80
GY	0.00	0.00	0.00	0.34	8.32	8.82	94.28	9.360	0.00	0.00	0.00	0.30	18.90	18.90	100	18.89
LAR	3.00	5.43	2.43	3.46	50.10	67.40	74.33	90.60	-	-	0.02	0.60	-	21.10	-	-

δp^2=Phenotypic variance, δg^2=genotypic variance, δe^2=environmental variance, X=grand mean, GCV=genotypic coefficient of variation, PCV=phenotypic coefficient of variation, H^2=broad-sense heritability, GA=genetic advance, LA=leaf area, NL=number of leaves, NT=number of tillers, SL=spike length, NSS=number of spikelets/spike, PH=plant height, DM=dry mass, GY=grain yield, LAR=leaf area ratio.

3.5.7 Principal component analysis

Principal component analysis (PCA) showing eigenvectors, eigenvalues, and percent variance of morphological and physiological traits of selected 10 wheat genotypes under non-stressed and water-stressed conditions is presented in Table 3.6. Under non-stressed condition, PCA revealed 3 principal components (PC) which accounted for 79.27% of the total variation. PC1 accounted for 39.52% of total variation and was associated with number of tillers, plant height, dry mass, grain yield and leaf area ratio. Photosynthetic rate, instantaneous water-use efficiency and intrinsic water-use efficiency were positively correlated with PC2 which accounted for 21.10% of the total variation. PC3 accounted for 18.64% total variation and positively correlated stomatal conductance and transpiration rate.

Under water-stressed condition, PCA revealed 3 principal components which accounted for 80.84% of the total variation. Number of leaves, number of tillers, plant height, dry mass, grain yield and photosynthetic rate PC1 which accounted for 42.84% of the total variation. PC2 accounted for 21.87% and was positively correlated with transpiration rate and negatively associated with spike length and number of spikelets per spike. Leaf area and leaf area ratio were positively correlated with PC3 and the PC accounted for 16.13% of the total variation.

Table 3. 6: Principal component analysis showing eigenvectors, eigenvalues, and percent variance of morphological and physiological traits of selected 10 wheat genotypes under non-stressed and water-stressed conditions.

Traits	Non-stressed			Water-stressed		
	PC1	PC2	PC3	PC1	PC2	PC3
LA	0.50	0.10	0.13	0.20	-0.31	**0.75**
NL	0.35	-0.14	0.67	**0.87**	0.30	-0.19
NT	**0.96**	0.00	0.15	**0.82**	0.45	-0.10
SL	0.31	0.07	0.32	-0.06	**-0.86**	0.37
NSS	**0.69**	-0.09	0.12	-0.06	**-0.89**	-0.12
PH	**0.96**	0.02	0.00	**0.75**	-0.44	-0.02
DM	**0.97**	-0.05	0.16	**0.93**	0.27	-0.10
GY	**0.95**	0.00	-0.03	**0.85**	0.42	0.00
LAR	**0.89**	-0.02	-0.28	-0.39	0.05	**0.83**
A	-0.04	**0.99**	0.09	**0.94**	-0.16	0.06
gs	-0.10	0.07	**0.97**	-0.16	0.13	**0.82**
T	-0.18	0.04	**0.91**	0.27	**0.69**	0.52
WUE$_{inst}$	-0.02	**0.99**	0.04	**0.92**	-0.11	-0.10
WUE	-0.40	-0.05	-0.69	0.35	0.20	0.17
WUEi	0.07	**0.99**	-0.05	0.25	0.56	0.35
Total variance explained (eigenvalues)	5.92	3.16	2.79	6.42	3.28	2.41
% of total variance	39.52	21.1	18.64	42.84	21.87	16.13
Cumulative variance (%)	39.52	60.63	79.27	42.84	64.71	80.84

LA=leaf area, NL=number of leaves, NT=number of tillers, SL=spike length, NSS=number of spikelets/spike, PH=plant height, DM=dry mass, LAR=leaf area ratio, GY=grain yield, *A*=Photosynthetic rate, *gs*=stomatal conductance, *T*=Transpiration rate, WUE$_i$=intrinsic water-use efficiency, WUE$_{inst}$=instantaneous water-use efficiency, WUE=water-use efficiency at whole-plant level. Factor loadings > 0.70 are boldfaced.

3.6 Discussion

Breeding for wheat genotypes with high water-use efficiency requires identification of drought-adaptive morphological traits. Morphological traits correlated with yield expression under drought stress condition are useful parameters as indirect selection criterion for breeding (Chen et al., 2014; Mwadzingeni et al., 2017). The current study investigated morphological responses of wheat to drought stress in order to identify

traits that may be used for improving water-use efficiency of wheat under water-limited conditions.

3.6.1 Morphological response of wheat under water-stressed and non-stressed conditions

Water stress has been shown to reduce plant growth, which in the current study was evident from the reduced leaf area, number of leaves, and number of tillers, dry mass and leaf area ratio (Table 3.3). Plant height, number of leaves, dry matter and leaf area are among important morphological adaptation mechanisms under water-stressed conditions (Farooq et al., 2009). Plants reduce plant height in response to water stress in order to invest more biomass to the root to increase water uptake. This is associated with increased root to shoot ratio. Plants exposed to drought stress decrease the carbon and nitrogen supply to shoot development and reallocate more resources to root growth and development, thus maintaining a higher root: shoot ratio as an adaptation trait to drought resistance (Yin et al., 2005; Villagra and Cavagnaro, 2006; Erice et al., 2007). It has been reported that a reduction in plant height to threshold of 70 to 100 cm is required for efficient partitioning of resources to grain yield (Araus et al., 2008; Mbave, 2013). Most of the tested genotypes in the current study maintained plant height below this threshold suggesting poor partitioning of resources (Table 3.3). Increased plant height for G344 suggest efficient biomass partitioning for high yield development in this genotype. However, increased plant height did not translate into increased grain yield under water stress condition, possibly because taller plants tends to lodge resulting in lower grain yield (Tomm et al., 2000) (Table 3.3). Liu et al. (2015) also reported non-significant correlation between plant height and grain yield in wheat. However, selection based on increased plant height could result in lower grain yield as more assimilates are translocated to biomass production rather than grain yield. However, the current study showed that wheat genotypes such as G344, G120, G109 and G115 showed increased biomass production under water stress condition (Table 3.3). In wild emmer wheat, high biomass production was associated with drought tolerance (Merchuk-Ovnat et al., 2018). However, non-significant correlation between plant height and dry matter ($r = 0.55$; $P > 0.05$) in the current study suggested a higher plant height did not have any

relationship with production of biomass production among the studied wheat genotypes (Table 3.3).

Results of the current study indicated that water stress significantly reduced dry biomass of genotype G112, G118, G139 and G339 by more than 50%. It has been reported that wheat tends to reduce stem dry mass in order to provide more assimilates to the grain filling (Abayomi et al., 1999; Gupta et al., 2001; Anjum et al., 2011; Vurayai et al., 2011; Mbave, 2013). Reduction in growth as indicated by reduced dry mass production in the current study can be considered as a possibility to preserve carbohydrates in the form of simple sugars to sustain metabolism, prolonged energy supply and better recovery after stress relief (Bartels and Sunkar, 2005). This is further supported by non-significant differences among genotypes with regards to grain yield after re-watering in the water-stressed treatment (Table 3.3). Guttieri et al., (2001), showed that reduced accumulation of dry mass under water-stressed conditions was due to decreased leaf number, leaf area and an increase in leaf senescence. Genotypes G118 and G339 showed reduced number of leaves due to water stress, whereas the rest of the tested genotypes showed increased number of leaves and leaf area (Table 3.3). The reduction in number of leaves and leaf area in response to drought stress are drought-avoidance mechanisms that limit transpirational water losses (Schuppler et al., 1998). This may further result in low yield gains due to reduced assimilation rates and low photosynthetic capacity of the plants (Allahverdiyev and Huseynova, 2017). Blum (1996) also reported that the number of leaves per plant determines the potential radiation interception for photosynthesis per unit leaf area. In the present study leaf area ratio was reduced by water stress, however, no significant difference were observed among the genotypes. The reduction in leaf area ratio is mainly due to lower leaf area and dry mass (Nagai and Makino, 2009; Solomon and Labuschagne, 2009). Furthermore, leaf area ratio was positively correlated with leaf area ($r = 0.65$; $P = 0.04$) under water-stressed condition in the current study, suggesting that as the leaf area increased it led to an increased leaf area ratio.

3.6.2 Broad-sense heritability and genetic advances of morphological traits

Genetic variation of morphological traits is key for trait based breeding in crop improvement programmes. In the current study, considerable genetic variation was

observed for some studied traits (Table 3.5). Higher proportion of phenotypic variance than genotypic variance were noted among the studied traits (Table 3.5). Higher PCV and GCV values indicate greater contribution of genetic and environmental components, in that order, influencing phenotypic variation among the studied wheat genotypes. Morphological traits such as number of tillers and dry mass exhibited high PCV values under both non-stressed and water-stressed conditions (Table 3.5). These suggested selections based on the traits would be an effective approach for increasing considerable genetic gain in wheat improvement programs. Further, leaf area, number of tillers, plant height, dry matter and leaf area ratio showed high heritability and genetic advanced values under non-stressed condition (Table 3.5). Plant height, dry matter and leaf area ratio exhibited high heritability and genetic advance values under non-stressed condition. Similar to the current study, high levels of genotypic variance and heritability have been reported for morphological traits such as spike length, number of tillers, number of spikelets per spike and plant height in wheat which may improve their selection gains (Chen et al., 2012; Mwadzingeni et al., 2017). On the contrary, number of tillers and grain yield showed high heritability and genetic advance under water-stressed condition. High values for heritability and genetic advance among the studied traits is indicative of additive gene action in the inheritance of these traits indicating selection for these traits can advance genetic gains in wheat breeding (Rana et al., 2015). Further, higher heritability indicates that the environmental effect on expression of traits was minimal. Traits such as leaf area, spike length and plant height exhibited low heritability under water-stressed condition (Table 3.5). This suggested selection to improve this trait may be relatively slow. Traits such as leaf area, number of leaves, spike length, number of spikes per spikelet, plant height and grain yield showed low or high values of heritability or genetic advance (Table 3.5). The low genetic advance observed for some traits may be compensated for by their high heritability. However, since high heritability does not always indicate a high genetic gain, heritability is recommended to be considered in association with high genetic advance for effect selection and trait introgression.

3.6.3 Association among morpho-physiological traits with water-use efficiency among wheat genotypes

Water-use efficiency is an important trait that can improve wheat yield levels under water-limited environments (Condon et al., 2004; Mbave, 2013; Varga et al., 2015). Breeding for stress-tolerance improvement must not only rely on direct selection for high grain yield under water-stressed conditions among diverse genotypes (Liu et al., 2015). Morphological traits that can improve WUE are useful indirect selection criterion for breeding for drought tolerance. Secondary traits have been successfully used for genetic improvement of wheat adapted to dry environments (Reynolds and Tuberosa, 2008; Chen et al., 2012). This may aid in accelerated breeding and cultivar development. In the present study, number of leaves per plant, number of tillers, and dry matter positively and significantly correlated with grain yield, photosynthetic rate and instantaneous water-use efficiency under water-stressed condition. This suggested the identified traits could be useful indirect selection indicators for measuring water-deficit-stress tolerance in wheat (Liu et al., 2015). This is because traits such as number of fertile tillers are associated with high photosynthetic rate (Table 3.4), which ultimately contributes to high shoot biomass (Munns et al., 2010). Improving crop photosynthesis and WUE can potentially be achieved by increased leaf photosynthetic and lower transpiration rates (Zhu et al., 2010). In the current study, genotypes G339, G343 and G344 exhibited high number of leaves and number of tillers under water-stressed condition. Further, these genotypes exhibited high photosynthetic rate and instantaneous water-use efficiency under water-stressed condition (Figure 3.2 A & C). Two of these genotypes, G343 and G344 showed slight lower transpiration rates under water stress condition, except for G339 (Figure 3.2 C). This suggest that these genotypes have efficient regulation of control of transpiration and photosynthetic rates under water stress. Similar to results of the present study, Théroux et al., (2015) reported that poplar clones with delayed decline in mesophyll conductance in response to water stress exhibited higher photosynthetic rate and water-use efficiency. Several other studies have shown that genotypes with reduced transpiration rates may have significantly increased yields levels (Sinclair et al., 2005; Messina et al., 2015). Reduced transpiration at high vapor pressure deficit results in plant-water conservation therefore minimizing crop failure and increasing water-use

efficiency (Ryan et al., 2016) and drought tolerance (Sinclair et al., 2017). The genotypes identified in the current study were previously identified as drought tolerant characterized by efficient photo-assimilation rate, transpiration rate and carboxylation efficiency (Tshikunde et al., 2018). In the current study, plant height also positively and significantly correlated with photosynthetic rate and instantaneous water-use efficiency under water-stressed condition (Table 3.5). This suggested that plant height could aid in breeding wheat for increased water-use efficiency. Previous studies have also reported positive correlations between plant height, grain yield per plant, plant photosynthetic rate, stomatal conductance and transpiration rate with drought tolerance in wheat (He and Rajaram, 1994; Garcial del Moral et al., 2003; Sayar et al.,2005; Mwadzingeni et al., 2016). According to Falconer and Mackay (1996), correlated response of grain yield for effective selection depends on correlation of secondary traits with grain yield. Dry mass has been reported to be the main trait for increasing grain yield, due to the association of dry mass with more assimilates essential for grain filling and formation (Abdoli et al., 2013). A similar trend was observed in the current study were grain yield correlated with dry mass ($r = 0.89$; $P < 0.001$) under non-stressed condition. A study in durum wheat (Khokhar et al., 2018) showed that grain yield under water-stressed condition was positively correlated with dry mass and number of tillers. In addition, these two traits were reportedly important morphological traits for increasing grain yield under water stress condition in wheat (Simane et al., 1998). Other traits such as number of spikelets per spike, spike length and plant height were not correlated to grain yield under water stress condition in the current study. Results of the current study contradicted those by (Pirdashti et al., 2004; Chen et al., 2012) who reported positive correlations between numbers of spikelets per spike, spike length and plant height and grain yield under water stress condition in wheat. Positive correlations of yield related traits with grain yield in the present study suggest genetic gains for grain yield can be achieved through direct or indirect selection of yield-contributing traits which may ultimately improve and enhance productivity (Mwadzingeni et al., 2017).

3.6.4 Principal component analysis involving morpho-physiological traits

Principal component analysis under non-stressed and water-stressed condition in the current study indicated that number of tillers, spike length, number of leaves, plant height, dry mass, grain yield, photosynthetic rate, instantaneous water-use efficiency and intrinsic water-use efficiency explained most of the variation (PC1 and PC2) which accounted for more than 60% total variation (Table 3.6). The identified morpho-physiological traits have been shown to be useful for breeding wheat for drought tolerance (Chen et al., 2012; Mwadzingeni et al., 2016; Merchuk-Ovnat et al. 2018). The combination of the studied morpho-physiological traits is useful to identify wheat genotypes for drought tolerance breeding (Merchuk-Ovnat et al., 2018).

3.6.5 Conclusions

The current study sought to identify morphological traits that may be used as direct or indirect selection criterion to improving water-use efficiency and drought tolerance in wheat. Several traits such as number of leaves, number of tillers, plant height and dry mass were significantly correlated with photosynthetic rate and instantaneous water use-efficiency in the current study. These suggest that such traits could be used as indirect selection criterion for breeding wheat for high water-use efficiency and photosynthetic capacity. Further, traits such as number of tillers and dry matter exhibited high values for heritability and genetic advance values indicating genetic gains incorporating this trait to improve WUE in wheat is possible. Number of tillers and dry matter were also significantly and positively correlated with grain yield under water stress conditions suggesting the possible effectiveness in increasing grain yield under water stress condition. Overall, genotypes G339, G343 and G344 which exhibited high NT and DM under WS condition were selected with enhanced water-use efficiency for breeding and to boost wheat production under dryland environments.

References

Abayomi Y.A., Wrght D. (1999) Effects of water stress on growth and yield of spring wheat (*Triticum aestivum* L.) cultivars. Tropical Agriculture 76:120-125.

Abdoli M., Saeidi M., Jalali-Honarmand S., Mansourifar S., Ghobadi M., Cheghamirza K. (2013) Effect of source and sink limitation on yield and some agronomic characteristics in modern bread wheat cultivars under post anthesis water deficiency. Acta Agriculturae Slovenica 101:173 -182

Abdolshahi R., Nazari M., Safarian A., Sadathossini T.S., Salarpour M., Amiri H. (2015) Intergrated selection criteria for drought tolerance in wheat (*Triticum aestivum* L.) breeding programs using discriminant analysis. Field Crops Research 174:20-29.

Amanullah J., Hassan M.J., Nawab K., Ali A. (2007) Response of specific leaf area (SLA), leaf area index (LAI) and leaf area ratio (LAR) of maize (i L.) to plant density, rate and timing of nitrogen application. World Applied Science Journal 2:235-43.

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

Allahverdiyev T., Huseynova I. (2017) Influence of water deficit on photosynthetic activity, dry Matter partitioning and grain yield of different durum and bread wheat genotypes. Cereal Research Communications 45:432–441.

Aminzadeh G. (2010) Evaluation of seed yield stability of wheat advanced genotypes in Ardabil, Iran. Research Journal of Environmental Sciences 4:478-482.

Anjum S.A., Xie X.-U., Wang L.C., Saleem M.F., Man C., Lei W. (2011) Morphological, physiological and biochemical responses of plants to drought stress. African Journal of Agricultural Research 6:2026-2032.

Anyia A., Herzog H. (2004) Water-use efficiency, leaf area and leaf gas exchange of cowpea under mid-season drought. European Journal of Agronomy 20:327-339.

Araus J.L., Slafer G.A., Royo C., Serret M.D. (2008) Breeding for yield potential and stress adaptation in cereals. Plant Science 27:377-412.

Bartels D., Sunkar R. (2005) Drought and salt tolerance in plants. Critical Reviews in Plant Sciences 24:23-58.

Blum A. (1996) Crop responses to drought and the interpretation of adaptation. Plant Growth and Regulation 120:135-148.

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.

Chen W., Deng X.-P., Eneji A.E., Wang L.L., Xu Y., Cheng Y. (2014) Dry-matter partitioning across parts of the wheat internode during the grain filling period as influenced by fertilizer and tillage treatments. Communications in Soil Science and Plant Analysis 45:1799-1812.

Chen X., Min D., Yasir T.A., Hu Y.G. (2012) Evaluation of 14 morphological, yield-related and physiological traits as indicators of drought tolerance in Chinese winter bread wheat revealed by analysis of the membership function value of drought tolerance (MFVD). Field Crops Research 137:195-201.

Condon A.G., Richards R.A., Rebetzke J.G., Farquhar D.G. (2004) Breeding for high water use efficiency. Journal of Experimental Botany 55:2447–2460.

Crossa J., Gauch H.G., Zobel R.W. (1990) Additive main effects and multipicative interction analysisof two international maize cutivar trials. Crop Science 30:493-500.

Ehleringer J.R., Hall A.E., Farguhar G.D. (1993) Introduction: water use in relation to productivity. In: Ehleringer JR, Hall AE, Farguhar G.D, Eds. Stable isotopes and plant carbon water relations. San Diego. Academic Press: 555:3–8.

Erice G., Irigoyen J.J., Sanchez-Dıaz M., Avice J.J., Ourry A. (2007) Effect of drought, elevated CO_2 and temperature on accumulation of N and vegetative storage proteins (VSP) in taproot of nodulated alfalfa before and after cutting. Plant Science. 172:903-912.

Falconer D.S. (1989) Introduction to quantitative genetics. Longman Science and Technology., London And New York.

Falconer D.S., Mackay T.F.C. (1996) Introduction to Quantitative genetics.4th edition. Longmans Green, Harlow, Essex, UK.

Farooq M., Wahid A., Kobayashi N., Fujita D., Basra S.M.A. (2009) Plant drought stress: effects, mechanisms and management. Agronomy for Sustainable Development 29:185-212. .

Farshadfar E., Poursiahbidi M.M., Safavi S.M. (2013) Assessment of drought tolerance in land race of bread wheat based on resistance/tolerance indices. International Journal of Advances in Biological and Biomedical Research. 1:143-158.

Fleury D., Jefferies S., Kuchel H., Langridge P. (2010) Genetic and genomic tools to improve drought tolerance in wheat. Journal of Experimental Botany 61:3211-3222.

Flexas J., Niinemets U., Gallé A., Barbour M.M., Centritto M., Diaz-Espejo A., Douthe C., Galmés J., Ribas-Carbo M., Rodriguez P.L. (2013) Diffusional conductances to CO_2 as a target for increasing photosynthesis and photosynthetic water-use efficiency. Photosynthesis Research 117:45-59.

Flexas J., Díaz-Espejo A., Conesa M.A., Coopman R.E., Douthe C., Gago J., Gallé A., Galmés J., Medrano H., Ribas-Carbo M. (2016) Mesophyll conductance to CO_2 and Rubisco as targets for improving intrinsic water use efficiency in C_3 plants. Plant Cell Environment 39:965-982.

Garcial del Moral L.F., Rharrabti Y., Villegas D., Royomm C. (2003) Evaluation of grain yield and its components in Durum Wheat under Mediterranean conditions: An Ontogenic Approach. Agronomy Journal. 95:388-396.

Gupta N.K., Gupta S., Kumar A. (2001) Effect of water stress on physiological attributes and their relationship with growth and yield in wheat cultivars at different growth stages. Journal of Agronomy 86:1437-1439.

Guttieri M.J., Stark J.C., Obrien K., Souza E. (2001) Relative sensitivity of spring wheat grain yield and quality parameters to moisture deficit. Crop Science 41:327-335.

He Z., Rajaram S. (1994) Differential responses of bread wheat characters to high temperature. Euphytica 72:197–203.

Johnson H.W., Robinson H.F., Comstock R.E. (1955) Estimates of genetic and environmental variability in soybeans. Agronomy Journal 47:314-318.

Khokhar M., Hussain M., Anwar J., Zulkiffal M., Muzaffar Iqbal M., Baz Khan S., Arif Khan M., Qayyum A., Sabir W., Mehmood S. (2018) Correlation and path analysis for yield and yield contributing characters in wheat (*Triticum aestivum* L.).

Liu H., Meuwissen T., Sorensen A., Berg P. (2015) Upweighing rare favourable alleles increases long-term genetic gain in genomic selection programs genetic selection evolution. Genetics Selection Evolution 47:19-32.

Maniee M., Kahrizi D., Mohammadi R. (2009) Genetic variability of some morpho-physiological traits in durum wheat (*Triticum turgidum* var. durum). Journal of Applied Science 9:1383–1387.

Mbave Z.A. (2013) Water stress effects on growth, yield and quality of wheat (*Triticum aestivum* L.). MSc dissertation. University of Pretoria.

Merchuk-Ovnat, L., Fahima, T., Krugman, T., Saranga, Y. (2018) Ancestral QTL alleles from wild emmer wheat improve grain yield, biomass and photosynthesis across environments in modern wheat. Plant Science 251:23–34

Messina C.D., Sinclair T.R., Hammer G.L., Curan D., Thompson J., Oler Z., Gho C., Cooper M. (2015) Limited-transpiration trait may increase maize drought tolerancein the us corn belt. Agronomy Journal 107:1978-1986.

Munns R., James R.A., Sirault X.R.R., Furbank R.T., Jones H.G. (2010) New phenotyping methods for screening wheat and barley for beneficial responses to water deficit. Journal of Experimental Botany 61:3499-3507.

Mwadzingeni L., Shimelis, H, Tsilo, T.J. (2017) Variance components and heritability of yield and yield components of wheat under drought stressed and non-stressed conditions. Australian Journal of Crop Science 11: 1425-1430.

Mwadzingeni, L., Shimelis, H, Tesfay, S, Tsilo, T.J. (2016) Screening of bread wheat genotypes for drought tolerance using phenotypic and proline analyses. Frontiers in Plant Science 7:1-12.

Nagai T., Makino A. (2009) Differences between rice and wheat in temperature responses of photosynthesis and plant growth. Plant and Cell physiology 50:744-755.

Nouri-Ganbalani A., Nouri-Ganbalani G., Hassanpanah D. (2009) Effects of drought stress condition on the yield and yield components of advanced wheat genotypes in Ardabil, Iran. Journal of Food, Agriculture and Environment 7:228–234.

Pirdashti H., Sarvestani Z.T., Nematzadch G., Ismail A. (2004.) Study of water stress effects in different growth stages on yield and yield components of different rice (*Oryza sativa* L.) cultivars: 4th International Crop Science Congress 1-7.

Pommel B., Gallais A., Coque M., Quillere I., Hirel B., Prioul J.L., Andrieu B., Floriet M. (2006) carbon and nitrogen allocation and grain filling in three maize hybrids differing in leaf senescence. European Journal of Agronomy 24:203-211.

Rana J.C., Sharma T.R., Tyagi R.K., Chahota R.K., Gautam N.K., Sharma Mohar Singh P.N., Ojha S.N. (2015) Characterisation of 4274 accessions of common bean (*Phaseolus vulgaris* L.) germplasm conserved in the Indian gene bank for phenological, morphological and agricultural traits. Euphytica 205: 441-457.

Reynolds M., Tuberosa R. (2008) Translational research impacting on crop productivity in drought-prone environments. Current Opinion in Plant Biology 11:171-179.

Robinson H.F., Comstock R.E., Harvey P.H. (1949) Estimates of heretibility and the degree of dominance in corn. Agronomy Journal 41:353-359.

Ryan A.C., Dodd I.C., Rothwell S.A., Jones R., Tardieu F., Draye X., Davies W.J. (2016) Gravimetric phenotyping of whole plant transpiration responses to

atmospheric vapour pressure deficit identifies genotypic variation in water use efficiency. Plant Science 251:101-109.

Sayar R., Khemira H., Bensalem M., Kameli A. (2005) Drought tolearance eveluation test for durum wheat (*Triticum durum* Desf.), poster in International Conference on Intergrate Approches to sustain and improve plant production under drought stress. International Drought-II:3-61.

Schuppler U., He P.-H., John P.C.L., Munns R. (1998) Effect of water stress on cell division and Cdc2-Like cell cycle kinase activity in wheat leaves. Plant Physiology 117:667-678.

Simane B., Struik P.C., Rabbinge R. (1998) Growth and yield component analysis of durum wheat as an index of selection to terminal moisture stress. Tropical Agriculture 75:363-368.

Sinclair T.R., Hammer G.L., Van Oosterom E.J. (2005) Potential yield and water-use efficiency benefits in sorghum from limited maximum transpiration rate. Functional Plant Biology 32:945-952.

Sinclair T.R., Devi J., Shekoofa A., Choudhary S., Sadok W., Vadez V., Riar M., Rufty T. (2017) Limited-transpiration response to high vapor pressure deficit in crop species. Plant Science 260:109-118.

Singh R.K., Chaudhary B.D. (1977) Biometrical methods in quantitative genetic analysis. Kalnani publishers, New Delhi, India: 39-68

Solomon K.F., Labuschagne M.T. (2009) Morpho-physiological response of durum wheat genotypes to drought stress. South African Journal of Plant and Soil 26:141-146.

Sharma P., Jha, A. B., Dubey, R. S., and Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany.2012:1-26.

Tambussi E.A., Bort J., Araus J.L. (2007) Water use efficiency in C4 cereal under Mediterranean conditions: a review of some physiological aspects. Annals of Applied Botany 150:307-321.

Théroux R.G., Éthier G., Pepin S. (2015) Greater efficiency of water use in poplar clones having a delayed response of mesophyll conductance to drought. Tree Physiology 35:172-184.

Tomm G.O., Didonet A.D., Sandri J.L., Frizon M.I. (2000) Lodging in Wheat: Relationships with Soil Fertility and Plant Characteristics in Southern Brazil. Wheat in a Global Environment Developments in Plant Breeding. Springer, Dordrecht 9:647-653.

Tshikunde N.M., Odindo A., Shimelis H., Mashilo J. (2018) leaf gas exchange and water-use efficiency of dry-land wheat genotypes under water stressed and non-stressed conditions. ACTA Agriculturae Scandinavica, Section B- Soil and Plant Science 68:738-748.

Varga B., Vida G., Varga-László E., Bencze S., Veisz O. (2015) Effect of simulating drought in various phenophases on the water use efficiency of winter wheat. Journal of Agronomy and Crop Science 201:1-9.

Villagra P.E., Cavagnaro J.B. (2006) Water stress effects on the seedling growth of *Prosopis argentina* and *Prosopis alpataco*. Journal of Arid Environment 64:390-400.

Vurayai R., Emongor V., Moseki B. (2011) Effect of water stress imposed at different growth and development stages on morphological traits and yield of Bambara groundnuts (*Vigna subterranea* L. Verdc). American Journal of Plant Physiology 6:17-27.

Yin C.Y., Wang X., Duan B.L., Luo J.X., Li C.Y. (2005) Early growth,dry matter allocation and water use efficiency of two sympatric Populus species as affected by water stress. Environmental & Experimental Botany 53:315-322.

Zhu X.G., Long S.P., Ort D.R. (2010) Improving photosynthetic efficiency for greateryield. Annual Review of Plant Biology 61:235–261.

An overview of the research findings

Introduction and objectives of the study

Global wheat production and productivity is hindered by drought stress, especially under rain-fed production conditions (Li et al., 2009). There is need to improve drought tolerance and water-use efficiency of wheat to boost production (Medrano et al., 2015). The aim of this study was to evaluate and select drought tolerant wheat genotypes possessing key yield-influencing and drought-adaptive agronomic and physiological traits for high-yield potential, enhanced drought tolerance and water-use efficiency for breeding or direct production under water-limited regions in South Africa

This overview compares the original study objectives with the research findings in relation with each objective. In addition, the implications of the study are provided for drought tolerance breeding and water use efficiency in wheat.

Objectives of the study

1. To determine drought tolerance of dryland wheat genotypes based on leaf gas exchange and water-use efficiency in order to identify promising genotypes for drought tolerance breeding.
2. To examine associations between morphological and physiological traits of selected wheat genotypes under drought stress in order to identify unique traits that may be used as direct or indirect selection criterion for improving water-use efficiency and drought tolerance in wheat.

Research findings in brief:

> **Leaf gas exchange and water-use efficiency of dry-land wheat genotypes under water stress and non-stressed conditions**

In this study the physiological responses of ten genetically diverse wheat genotypes were studied under non-stressed (NS) and water stressed (WS) conditions using a 2 × 10 factorial experiment replicated 3 times. The core findings of the study were:

- Significant genetic variation was observed amongst the tested wheat genotypes using various physiological parameters.

- Genotypes G339 and G344 were identified as drought tolerant with high values of photosynthetic rate, transpiration rate, stomatal conductance, the ratio of photosynthetic rate and internal CO_2 concentration (A/Ci), intrinsic water use efficiency (WUE_i) and instantaneous water-use efficiency (WUE_{inst}). These are useful for breeding for enhanced drought tolerance and water-use efficiency to improve grain yield potential under drought stress environments.

Morpho-physiological traits associated with water-use efficiency in selected dry land wheat (*Triticum aestivum* L.) genotypes

Ten selected and genetically diverse wheat genotypes were assessed under non-stressed (NS) and water-stressed (WS) conditions using a randomised complete block design with three replications. The main findings of the study were:

- Genotypes G339, G343 and G344 produced higher number of tillers and dry biomass and recorded high heritability and genetic advances for number of tillers and dry biomass under water stress condition

- There were positive and significant correlations between number of tillers, dry biomass and grain yield under water stress condition suggesting selection for these traits will likely increase yield gains in wheat.

- Genotypes G339, G343 and G344 showed high number of tillers and biomass production under WS were identified and selected for breeding for enhanced water-use efficiency to boost wheat production under dryland environments.

Implications of the research findings

The following major implications for breeding were noted:

- The identified drought tolerance wheat genotypes can be used as parental lines to develop breeding populations to improve grain yield and drought tolerance under water limited conditions.

- Some genotypes can be used for direct cultivation following genotype-by-environment analysis to identify stable genotypes

- The identified morpho-physiological traits associated with water-use efficiency can be used as (in)direct selection criteria in improvement programmes to develop drought tolerant and water-use efficient wheat genotypes.

References

Li Y., Ye W., Wang M., Yan X. (2009) Climate change and drought: A risk assessment of crop-yield impacts. Climate Research 39:31–46.

Li Y., Li H., Li Y., Zhang S. (2017) Improving water-use efficiency by decreasing stomatal conductance and transpiration rate to maintain higher ear photosynthetic rate in droughtresistant wheat. The Crop Journal 231 – 239.

Medrano H., Tomás M., Martorell S., Flexas J., Hernández E., Rosselló J., Pou A., Escalona J.M., Bota J. (2015) From leaf to whole-plant water use efficiency (WUE) in complex canopies: Limitations of leaf WUE as a selection target. The Crop Journal 3:220-228.

Zhang S.Q., Shan L. (2002) The research advance of water use efficiency in plants. Agriculture Research In Arid Areas 20:1-5.