Pre-Breeding of Wheat (*Triticum aestivum* L.) for Biomass Allocation and Drought Tolerance

By

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A Thesis Submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy (PhD) in Plant Breeding

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December 2018

Thesis Abstract

Bread wheat (*Triticum aestivum* L., 2n=6x=42) is the third most important cereal crop globally after maize and rice. However, its production and productivity is affected by recurrent drought and declining soil fertility. Wheat cultivars with a well-balanced biomass allocation and improved root systems have better water- and nutrient-use efficiency and, hence, increased productivity under dry-land farming systems. The overall objective of this study was to develop breeding populations of wheat with enhanced drought tolerance and biomass allocation under water-limited conditions. The specific objectives of the study were: (i) to evaluate agronomic performance and quantify biomass production and allocation between roots and shoots in selected wheat genotypes in response to different soil water levels to select promising genotypes for breeding for drought tolerance and carbon (C) sequestration, (ii) to determine variance components and heritability of biomass allocation and grain yield related traits among 99 genotypes of bread wheat and triticale (*Triticosecale* Wittmack) to optimize biomass partitioning for drought tolerance, (iii) to deduce the population structure and genome-wide marker-trait association of yield and biomass allocation traits in wheat to facilitate marker-assisted selection for drought tolerance and C sequestration, and (iv) to estimate the combining ability of selected wheat genotypes and their progenies for agronomic traits, biomass allocation and yield under drought-stressed and non-stressed conditions for future breeding and genetic advancement for drought tolerance and C sequestration. To achieve these objectives, different experiments were conducted.

In the first study, 99 wheat genotypes and one triticale accession were evaluated under drought-stressed and non-stressed conditions in the field and greenhouse using a 10×10 alpha lattice design with two replications. Data on the following phenotypic traits were collected: days to heading (DTH), number of productive tillers per plant (NPT), plant height (PH), days to maturity (DTM), spike length (SL), thousand kernel weight (TKW), root and shoot biomass (RB and SB), root to shoot ratio (RS) and grain yield (GY). There was significant ($p<0.05$) genotypic variation for grain yield and biomass production. The highest grain yield of 247.3 g m^{-2} was recorded in the genotype LM52 and the least was in genotype Sossognon with 30 g m^{-2}. Shoot biomass ranged from 830g m^{-2} (genotype Arenza) to 437 g m^{-2} (LM57), whilst root biomass ranged between 140 g m^{-2} for LM15 and 603 g m^{-2} for triticale. Triticale also recorded the highest RS of 1.2, while the least was 0.30 for LM18. Water stress reduced total biomass production by 35% and RS by 14%. Genotypic variation existed for all measured traits useful for improving drought tolerance, while the calculated RS values can improve accuracy in estimating C sequestration potential of wheat. The following genotypes: BW140, BW141, BW152, BW162, LM26, LM47, LM48, LM71, LM70 and LM75 were selected for further development based on their high grain and biomass production, low drought sensitivity and marked genetic diversity.

In the second study, data obtained from the above experiment were subjected to analyses of variance to calculate variance components, heritability and genetic correlations. Significant ($p \leq 0.05$) genetic and environmental variation were observed for all the traits except for spike length. Drought stress decreased the heritability of RS from 47 to 28% and GY from 55 to 17%. The genetic correlations between RS with PH, NPT, SL, SB and GY were weaker under drought-stress ($r \leq - 0.50$; $p<0.05$) compared to non-stressed condition, suggesting that lower root biomass under drought stress compromises wheat productivity. The negative genetic correlation between GY and RS ($r = -0.41$ under drought-stressed and $r = -0.33$ under non-stressed conditions; $p<0.05$), low heritability (<42%) and high environmental variance (>70%) for RS observed in this population constitute several bottlenecks for improving GY and RS simultaneously. However, indirect selection for DTH, PH, RB, and TKW, could help optimize RS and simultaneously improve drought tolerance and yield under drought-stressed condition.

In the third study, the 99 wheat genotypes and one triticale accession were genotyped using 28,356 DArTseq derived single nucleotide polymorphism (SNPs) markers. Phenotypic and genomic data were subjected to genome wide association study (GWAS). Population structure analysis revealed seven clusters with a mean polymorphic information content of 0.42, showing a high degree of diversity. A total of 54 significant marker-trait associations (MTAs) were identified. Twenty-one of the MTAs were detected under drought-stressed condition and 11% were on the genomic loci where quantitative trait loci (QTLs) for GY and RB were previously identified, while the remainder are new events providing information on biomass allocation. There were four genetic markers, two under each water treatment, with pleiotropic genetic effect on RB and SB that may serve as a means for simultaneous selection. Significant MTAs observed in this study will be useful in devising strategies for marker-assisted breeding to improve drought tolerance and to enhance C sequestration capacity of wheat.

Lastly, 10 better performing and genetically diverse wheat genotypes selected during the first experiment were crossed using a half diallel mating design to generate F_1 families. The parents and crosses were evaluated using a completely randomized block design with 2 replications under a controlled environment condition. Significant ($p<0.05$) genotype by water regime interaction effects were recorded for RB, SB, RS and GY. Root and shoot biomass were reduced by 48 and 37%, respectively, due to drought stress hindering biomass allocation patterns and hence C sequestration potential of the tested genotypes. Further, drought stress reduced RS and GY by 18 and 28%, respectively compared with the non-stressed treatment. Analysis of variance showed that both general combining ability (GCA) and specific combining ability (SCA) effects were significant ($p<0.05$) in conditioning the inheritance of grain yield and related traits and biomass allocation. Non-additive gene effects were more important in controlling the inheritance of the measured traits under drought-stressed and non-stressed conditions. Parental genotypes LM47 and BW140 had significant and positive GCA effects for root and shoot biomass and GY under drought-stressed conditions. These are recommended for recurrent selection programs to improve the respective traits. The crosses BW141×LM48 and LM47×LM75 were good specific combiners for biomass allocation and GY under drought stress, while BW141×LM48 and LM48×LM47 were good combiners under non-stressed condition. These families are selected for advanced breeding to develop pure line cultivars. The preliminary results suggest that simultaneous improvement of grain yield and root biomass can be realized to improve drought tolerance and C sequestration ability in wheat.

Overall, the study detected marked phenotypic and genetic variation among diverse set of wheat genetic resources and candidate crosses for drought tolerance and biomass allocation through field and greenhouse based experiments and genomic analyses. The selected parents and novel crosses are useful for wheat breeding to enhance drought tolerance, yield and yield components and biomass allocation for C sequestration. This is the first study that evaluated biomass allocation in wheat as a strategy to improve drought tolerance and carbon sequestration.

Declaration

I, **Isack Mathew**, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other University.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
    a. Their words have been re-written but the general information attributed to them has been referenced.
    b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.
5. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in the references sections.

Signed

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Isack Mathew

As the candidate's supervisors, we agree to the submission of this thesis:

……….

Prof. Hussein Shimelis (Supervisor)

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Dr. Vincent Chaplot (Co-Supervisor)

Acknowledgements

I would like to express my gratitude to my supervisors Professor Hussein Shimelis and Doctor Vincent Chaplot for their encouragement, guidance and support throughout the course of this study. I have gained a lot of knowledge from their constructive comments, suggestions and review of my work. I am forever indebted to you, the only way to repay your sterling investment in me is for me to continue until we achieve what we set out to achieve: breeding wheat for drought tolerance and carbon sequestration. I would also like to acknowledge Dr. Macdex Mutema, who was there for me in the infancy of my PhD study. As I set out into the unknown, he guided and offered a steady hand. I appreciate, dearly.

I would like to acknowledge the Water Research Commission (project number WRC K5-2721-4) for funding my bursary. In the same vein, I would like to acknowledge Dr. Rebecca Zengeni for support. Highly appreciated.

The staff of African Centre for Crop Improvement (ACCI), past and present, is acknowledged for excellence. I would like to mention, Rowelda Donnelly and Lyndre Anderson, who went beyond the call of administrators' duty to make my study a pleasure. Forever, engraved in my heart. Then there was Andile Mshengu, a friend and a sister, thank you.

The ACCI staff at Ukulinga Farm and the Controlled Environment Facility were excellent for the successful trials, forever indebted.

I would like to acknowledge my fellow postgraduate students whom I have shared endless arguments, research experience, light moments and above all academic engagements. These are friends that I will never forget and will always meet in the circles of life. Such is life. Special thanks to Admire Shayanowako, Learnmore Mwadzingeni, Kwame Shamuyarira, Cousin Musvosvi, Terence Tapera, Marilyn Christian, Sibusiswangaye Mdluli and Nokuthula Hlanga.

Here, I mention my family, the people I love the most. Thank you for every sacrifice.

I would like to remember many people I have met because of this work. I will never recall your names but you left a lasting impression, I have learnt a lot about people, about breeding and other countries because of you. Continue to be good.

Dedication

To every father who toiled in foreign lands to raise a child, teaching them the value of hardwork and sacrifice.
To every mother who woke up early every morning to care of a child, teaching them gentleness and good-heartedness.
To every family that stood side by side, showing one another that unity prevails over adversity.

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Publications Pertaining to This Thesis

Chapter 2

Mathew I, Shimelis H, Mutema M, Chaplot V (2017) What crop type for atmospheric carbon sequestration: Results from a global data analysis. Agriculture, Ecosystems and Environment 243:34-46 https://doi.org/10.1016/j.agee.2017.04.008

Mathew I, Shimelis H, Mutema M, Minasny B, Chaplot V. Crops for increasing soil carbon and soil fertility. Under review in Plant and Soil.

Chapter 3

Mathew I, Shimelis H, Mutema M, Clulow A, Zengeni R, Mbava N, Chaplot V (2019) Selection of wheat genotypes for biomass allocation to improve drought tolerance and carbon sequestration into soils. Journal of Agronomy and Crop Science 00:1–16 https://doi.org/10.1111/jac.12332

Chapter 4

Mathew I, Shimelis H, Mwadzingeni L, Zengeni R, Mutema M, Chaplot V (2018) Variance components and heritability of traits related to root: shoot biomass allocation and drought tolerance in wheat. Euphytica 214:225 https://doi.org/10.1007/s10681-018-2302-4

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Introduction to thesis

Background and constraints to crop production

Global crop production is challenged by numerous biotic and abiotic stresses contributing to low yields and quality. Drought stress and poor soil fertility are among the major challenges affecting food production (Fahad et al. 2017). The rise in atmospheric carbon dioxide (CO_2) over the years has led to global warming, which has caused dramatic increase in the frequency and intensity of drought in many parts of the world (Zougmoré et al. 2018). Agricultural production is also curtailed by highly degraded soils that cannot support sustainable crop production due to long term soil carbon (C) depletion by land mismanagement (Lal 2004). The Southern Africa region is experiencing declining rainfall and most of its agricultural lands are characterised by degraded soils (Mapfumo et al. 2017). In addition, biotic stress pressure is projected to increase with climate change (Zavala et al. 2017), further threatening crop production and food security.

Drought stress and low soil C as key constraints to wheat production

Wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD) is the third most important cereal crop next to maize and rice globally. It is cultivated on a total of 240 million hectares (Portmann et al. 2010). Wheat is the main staple food crop and supports about 30% of the global population (Lobell and Gourdji et al. 2012; Pfeifer et al. 2014). Presently about 730 million tons of wheat is produced annually which is far below global demand due to high population pressure and rapid urbanization (FAO 2016). Wheat production has stretched across diverse environments characterized by a wide range of constraints including drought and poor soils. Several climate prediction models forecast erratic rainfall distribution due to climate change, which will increase the frequency and intensity of drought in sub-Saharan Africa (SSA) (Hegerl et al. 2018). This will further reduce wheat production and productivity. As a result, there is need to develop and deploy drought resilient and high performing wheat varieties adapted to SSA agro-ecologies. Prolonged periods of drought will have higher impact in sub-Saharan Africa agriculture since 90% of crop production is dependent on rain-fed agriculture (Rockström et al. 2010). In SSA, terminal drought stress frequently lead to crop failures due to depletion of residual soil moisture (Haque et al. 2016). In addition, the impact of drought is exacerbated by low soil fertility, a consequence of historical loss of soil C and inherent nutrient deficiency (Tully et al. 2015). Soil C is a vital constituent of soil organic matter formation which is responsible in maintenance of nutrient and hydrological cycles that are important for crop production. As a result there is need to restore soil C and improve its ability to support plant growth for enhanced crop yields.

Mitigating drought stress and low soil C impact on wheat production

Several strategies including intensive irrigation and fertilizer inputs have been adopted widely to reduce the impacts of drought and low soil fertility, respectively. However, there are concerns on their environmental sustainability. For instance, by 2050 only 5% of agriculture will be irrigated in SSA due to declining water resource (Calzadilla et al. 2014) and this will intensify competition for water between agriculture and human consumption. Similarly, excessive and misuse of fertilizers causes widespread acidification and environmental pollution in intensive farming systems.

Breeding for drought tolerance and enhanced C sequestration in crops is believed to be a viable option to address current drought and poor soil fertility challenges in wheat production. This option is regarded to be economic and environmentally sustainable. Improving drought tolerance and C sequestration in crops will increase productivity with positive impact on the environment. Wheat has higher potential for soil C inputs amounting up to 70% of its total below ground carbon (Martens et al. 2009). In comparison, maize only deposited 30% of its total below ground carbon (Balesdent and Balabane 1992). It is envisaged that improved cultivars will also have higher adoption rates among farmers because they will fit in their cropping systems seamlessly. The genetic variation in the C sequestration potential of different genotypes has not been adequately investigated compared with the various studies made available on drought tolerance. There is need to compare the potential of wheat to sequester C compared with other crops and evaluate the possibility of simultaneous improvement of its C sequestration capacity and drought tolerance without compromising yield potential.

Breeding for drought tolerance and C sequestration ability in wheat

Studies indicated that drought tolerance and C sequestration can be improved simultaneously through selection of relevant traits such as high rooting capacity and balanced biomass partitioning between roots and shoots (Rebetzke et al. 2013). However, drought tolerance is a polygenic trait that is controlled by many minor genes. It is also subject to genotype x environment interaction that require selection of genotypes across representative test environments and using robust genomic tools. This will enhance breeding for drought tolerance and yield gains. C sequestration has never been pursued as a breeding objective in annual crops and may be affected by unfavourable source-sink competition between above and below ground parts.

The critical aspects in breeding for drought tolerance and C sequestration in wheat include altering biomass allocation between roots and shoots. After assimilation, C is allocated to different parts of the plant based on the source-sink balance and optimal partitioning theory (Poorter and Nagel 2000). Intuitively, larger and denser roots have higher C sink strength and are important for C sequestration and drought tolerance. Denser root systems would be particularly important in SSA where there is over reliance of wheat production on sub-soil moisture which causes post-anthesis terminal drought (Haque et al. 2016). Larger and deeper root systems will enable wheat to access soil moisture and reduce the impact of edaphic drought stress. For C sequestration, Paustian et al. (2016) estimated that enhancing root biomass in annual crops can potentially store an equivalent of approximately 1 pentagram of C per annum to the soil or 20% of agricultural C emissions, which would greatly contribute to restoring soil C. Up to 80% of soil C originate from plant root turnover in the soil (Yang et al. 2012) and this opens opportunities for incorporating plant residue for soil C restitution. Thus, enhancing the root system of wheat will improve drought tolerance and C sequestration capacity since an efficient and large root system will increase moisture extraction and C deposition in the soil.

Phenotypic and genetic analyses of drought tolerance and C sequestration

Conventionally, crop genotypes are evaluated under multiple environments to quantify the genetic and environmental components and to establish yield stability. The partitioning of genetic and environmental components is important because quantitative traits such as biomass, yield and yield related traits are known to be highly affected by genotype and environmental conditions. The inclusion of root traits in breeding programs is key in drought tolerance and C sequestration (Osmont et al. 2007) but have been neglected due to difficulties associated with root phenotyping. In the past, selection for drought tolerance has mainly been based on above ground traits (White et al. 2015). Various molecular technologies have been developed to circumvent the impact of environmental variance on selection. However, to utilize molecular based techniques effectively, the genotypes must be well-phenotyped initially under *ex situ* conditions to establish true associations between markers and traits, especially for traits such as C sequestration that have not been widely investigated.

Strategies for breeding for drought tolerance and C sequestration

Due to the complexity of drought tolerance and, possibly, C sequestration, complementary genomic and conventional techniques must be used to improve selection efficiency. The germplasm must be effectively and efficiently screened under natural or controlled environments to identify suitable genotypes. The phenotypic and genetic data are then used in combination to establish marker trait associations for marker-assisted selection. Genome wide association studies (GWAS) based on micro-array diversity array technologies (DArTs) derived single nucleotide polymorphism (SNP) markers have gained popularity in understanding population structures, dissecting complex traits and discovery of associated markers in many crops, including wheat (Gupta et al. 2008). The identified markers can then be used for marker-assisted selection to eliminate environmental variance and improve selection gains provided there is adequate genetic variation in the population (Xu et al. 2012).

Successful breeding for drought tolerance and C sequestration will depend on availability of adequate genetic variation for selection. The presence of genetic variation is highly reported for root traits that improve tolerance to drought, hypoxia and ion toxicity stresses in cereals (Kell 2011; Lynch and Wojciechowski 2015). This will provide opportunities for marker-assisted selection (MAS) to improve C sequestration and drought tolerance. The The International Maize and Wheat Improvement Center (CIMMYT) maintains a repository gene bank for wheat. This is an important reservoir for genes to improve locally adapted genotypes. In South Africa, the Agricultural Research Council-Small Grain Institute (ARC-SGI) spearheads breeding programs to improve wheat for water-limited environments and maintain the national gene bank. These genetic resource are vital to select desirable genotypes exhibiting the promising traits. Such genotypes can be used in developing new breeding populations through targeted crosses of complementary parents with subsequent genetic analysis and selection.

Importance of combining ability in cultivar development

The success of future breeding or genetic advancement depends on the combining ability of the selected individuals and their progenies. Combining ability measures the relative ability of an individual to pass hereditary traits to its offspring (Aly 2013) and can be used to select best parents for future breeding or families for genetic advancement (Goldringer et al. 1997). Combining ability analysis enables the breeder to determine the mode of gene action for a particular trait, which is a requisite for devising the most appropriate selection strategy (Gowda et al. 2012). Combining ability of parents and progenies can be determined using several genetic designs including line x tester, factorial or North Carolina Designs and diallel, among others. Diallel is the choice of mating design since it involves crosses of a set of parents in desirable pair of combinations (Hayman 1954). Diallel genetic design will aid in generating breeding populations and to identify best parents and families for future breeding and genetic advancement for drought tolerance and C sequestration ability.

Problem statement

A set of diverse wheat germplasm derived from CIMMYT was previously characterized for drought tolerance using phenotypic and proline analyses (Mwadzingeni et al. 2017). The germplasm exhibited wide variation for agronomic traits and grain yield under drought-stressed and non-stressed conditions for effective breeding. However, their biomass allocation pattern is yet to be optimized for attaining high yields especially under low input production systems with limited water availability and variable soils properties. The germplasm needs to be well-characterized particularly for biomass allocation to roots, shoots and grain to develop breeding populations with enhanced drought resilience and C sequestration potential. The genetic and environmental factors governing biomass allocation and grain yield in wheat has not been adequately assessed to initiate appropriate breeding methods to develop cultivars with enhanced biomass allocation for high yield and C sequestration, especially in water limited environments.

General aim

In general, this study aimed to improve drought tolerance and enhance C sequestration capacity of wheat to increase food production under water-limited conditions.

Specific objectives

- To evaluate agronomic performance and quantify biomass production and allocation between roots and shoots in selected wheat (*Triticum aestivum* L.) genotypes in response to different soil water levels to select promising genotypes for breeding for drought tolerance and C sequestration.
- To determine variance components and heritability of biomass allocation and grain yield related traits among 99 genotypes of bread wheat and triticale (*Triticosecale* Wittmack) to optimize biomass partitioning for drought tolerance.
- To deduce the population structure and genome-wide marker-trait association of yield and biomass allocation traits in wheat to facilitate marker-assisted selection for drought tolerance and C sequestration.
- To estimate the combining ability of selected wheat genotypes and their progenies for agronomic traits, biomass allocation and yield under drought-stressed and non-stressed conditions for future breeding and genetic advancement for drought tolerance and C sequestration.

Hypotheses

i. There is significant variation in agronomic traits, biomass allocation and grain yield among wheat genotypes evaluated under drought-stressed and non-stressed conditions.

ii. Genetic and environmental factors affect biomass allocation, yield and yield-related traits.

iii. The DArTseq markers and biomass allocation traits are significantly associated under drought-stressed and non-stressed conditions.

iv. The selected parental genotypes and their progenies have desirable combining ability for agronomic traits, biomass allocation and grain yield under drought-stressed and non-stressed conditions.

Outline of thesis

This thesis consists of a total of five chapters as outlined below (Table 0.1). Chapters 1-5 were written following the University of KwaZulu-Natal's dominant thesis format with the abstract, introduction, materials and methods, results, discussion and conclusion sections. Due to their interdependence, the chapters contain some unavoidable overlaps and repetitions of references and introduction sections. The general discussion and recommendations from the study are presented at the end. The reference style used in the thesis is based on the referencing format used in Euphytica International Journal of Plant Breeding. Chapter 1 is a quantitative review seeking to establish the link between plant biomass and soil organic carbon and was published in the Journal of Agriculture, Ecosystems and Environment (doi.org/10.1016/j.agee.2017.04.008). Chapter 2 was accepted for publication in Journal of Agronomy and Crop Science. Chapter 3 was published in Euphytica (doi: 10.1007/s10681-018-2302-4). Chapters 4 and 5 are being prepared for submission for publication.

Table 0.1 Structure of the thesis showing chapter number and title

Chapter	Title
---	Introduction to thesis
1	What crop type for atmospheric carbon sequestration: Results from a global data analysis
2	Selection of wheat genotypes for biomass allocation to improve drought tolerance and biomass allocation
3	Variance components and heritability of traits related to root: shoot biomass allocation and drought tolerance in wheat
4	Genome wide association study of drought tolerance and biomass allocation in wheat
5	Combining ability of selected wheat genotypes for drought tolerance and biomass allocation
---	An overview of research findings

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Chapter 1 What crop type for atmospheric carbon sequestration: Results from a global data analysis

Abstract

Sequestration of atmospheric carbon (C) into soils is a strategy to compensate for anthropogenic emissions of carbon dioxide. The response of soil organic carbon stocks (SOCs) to crop types is yet to be determined under different environments. The objectives of this study were to elucidate the impact of crop type on the allocation of atmospheric C to shoots and roots, and ultimately to the soils and to determine its association with soil carbon stocks. Three hundred and eighty-nine field trials were compared to determine allocation of biomass and C in plants and SOCs under fields of different crop types. Grasses had the highest plant biomass production (19.80 ±1.16 Mg ha^{-1} yr^{-1}), followed by cereals (9.44±0.45 Mg ha^{-1} yr^{-1}), fibre (7.90±1.00 Mg ha^{-1} yr^{-1}), legumes (3.29±0.63 Mg ha^{-1} yr^{-1}), and oil crops (3.05±1.16 Mg ha^{-1} yr^{-1}) showing significant differences ($p<0.05$). Maize (6.3±0.34 Mg C ha^{-1} yr^{-1}) had the highest plant C amongst summer crops, while wheat (2.2±0.35 Mg C ha^{-1} yr^{-1}) had the highest plant C amongst winter crops. In all the studies, crops allocated more C to their shoots than roots yielding root C: shoot C (Rc/Sc) ratios below magnitude. The greatest C allocation to roots was in grasses (Rc/Sc=1.19±0.08), followed by cereals (0.95±0.03), legumes (0.86±0.04), oil crops (0.85±0.08), and fibre crops (0.50±0.07). There was evidence that high plant C stocks were found in crops grown under carbon rich clayey soils of tropical humid areas. Natural grasses and cereals should be promoted as they appeared to yield greater potential for atmospheric carbon sequestration in plants and soils. Overall, the study evaluated the relative potential of the main crop types to sequester atmospheric C useful in screening of crop types for carbon efficiency and for development of plant soil C input models.

Key words: Organic carbon; Climate change; Biomass; Land rehabilitation

1.1 Introduction

Plant C is important in the global C cycle because annually more than 10% of all atmospheric CO_2 passes through the plant-soil-atmosphere interface (Raich and Porter 1995). Therefore, fostering the ability of plants to fix atmospheric CO_2 presents a huge potential to reduce atmospheric C concentration. To our knowledge, there are many studies focusing on tillage impacts on SOCs but there are a few which investigated the quantitative relationship between plant C and SOCs. Balesdent and Balabane (1996) reported that root derived C accounts for between 60 and 75% of SOCs showing that root biomass and C are important determinants of

SOCs. Most studies that investigated C allocation patterns in plants reported that grasses accumulate higher amounts of C than crops. The variations in plant C allocations suggest that there could be significant differences in plant C allocation across crop types, climatic zones, and soil types. The differences are certainly critical in the eventual deposition of plant C into soil C pools and can be used to select crop varieties with superior C sequestration potential. Therefore, it is important to understand the C input of different parts (root vs shoot) in order to strategize options that aim at increasing SOCs (Rasse et al. 2005). The disparities in land management practices, crop types and environmental conditions make it difficult to compare carbon sequestration potential of the different main crops. However, data from various studies across the world can provide an opportunity for comprehensive analysis seeking to draw general understanding on the allocation of carbon to shoots and roots and the correlations between plant C and soil C. The data need to be integrated over time, space and climate through focused data analysis and interpretation for wider application. Therefore, the objectives of this paper were to integrate results from different studies worldwide in order to evaluate differences in root and shoot biomass, and C stocks of the main crops and then to deduce the extent to which the stocks correlated to soil C under different environmental conditions. Information on the differences in biomass and carbon allocation and their relationship with SOCs is useful to estimate the relative potential of the main crops to sequester atmospheric C and enable preliminary screening of crop genotypes for carbon sequestration potential. The determination of C allocation between shoots and roots reflects the differential investment of C between the two parts and it was hypothesized that production of crops with high root biomass investment may improve SOCs and drought tolerance.

1.2 Materials and Methods

1.2.1 Study setup

The study is based on data collected mostly from field experiments conducted under various standard farming practices. Literature search was conducted on electronic academic databases using search engines such as Google Scholar, Refseek, Science Direct, SciFinder, Scopus, Springer Link and Web of Science. Key words such as carbon allocation, carbon partition, root: shoot biomass carbon, plant carbon sequestration, rhizodeposition and plant/soil organic C stocks were used to search for journal articles published from 1990 to time of the literature search in early 2016.

In order to be included in the analysis, studies had to report on root and shoot biomass, root and shoot C concentration or stocks, and soil organic C stocks (SOCs) measured during the experimental periods. In some papers, these variables were derived from root: shoot ratios, harvest indices, soil organic C concentration or referred publications on the same experiment. A total of 42 journal articles (Appendix 1.1) detailing different studies across the world were obtained using the above criteria. Names of authors, year of paper publication, geographical location of experimental site, nature of experiment, crop type(s) used in the experiments, quantitative information on plant biomass, C variables and environmental conditions were captured onto a database. Long-term climate variables (such as MAP: mean annual precipitation and MAT: mean annual temperature), soil properties (including pH, texture and bulk density) and tillage operations were used to stratify the observations in the database. These environmental factors influence SOCs and plant C through their effects on crop productivity, microbial activity and soil properties.

1.2.2 Definitions of Environmental factors

This analysis considered the following environmental factors long-term mean annual precipitation (MAP) and mean annual temperature (MAT), geographical location as defined by coordinates (LAT: latitude and LON: longitude) and soil properties (clay content, bulk density and pH) (Table 1.1). In the cases where MAP and MAT were not provided in the papers, the data were obtained from Climate data (2016) (www.climatedata.eu) using the location coordinates (LAT and LON). Climate is further categorized into tropical (hot and wet), subtropical (warm and arid to humid) and temperate (cold and arid to moist) according to MAP and MAT. Soil texture was derived from the journal articles and categorized following Mutema et al. (2015). Soil bulk density (BD) was cited from the articles and where BD was given for different horizons, the average for the whole profile was calculated. Soil pH (acidity or alkalinity) used in the current paper is based on calcium chloride ($CaCl_2$) scale averaged across the soil profile.

Table 1.1 Environmental factors and their classes used in the study

Factor	Remarks	Categories	Symbol	Factor class
Climatic region	Based on the 30 year average annual temperature and precipitation for the study site	Precipitation >1000mm Temperature $>20^{o}$C	Hot and wet	Tropical
		Precipitation 300-1000mm Temperature 10-20^{o}C	Warm and arid-humid	Sub-tropical
		Precipitation <800mm Temperature $<10^{o}$C	Cool and arid to moist	Temperate
Clay content (%)[b]	Soil texture based on the clay content or dominant fraction	>32% clay	Tex	Clay
		20-32% clay		Loam
		<20% clay		Sand
Soil pH*	Soil pH as cited in the paper	<5	pH	H. acidic
		5.1-6.5		Acidic
		6.6-8.0		Basic
		8<		H. basic
Soil bulk density (gcm^{-3})	Average bulk density of soil profile	1.3<	BD	Low
		<1.3		High
Tillage	Agronomic practices involving soil disturbance as cited by the authors	Deep ploughing	Tillage	Conventional
		Targeted ploughing		Minimum
		No ploughing at all		No-till

[a] Categories adapted from Mutema et al. 2015

*H. acidic/basic=highly acidic/basic

1.2.3 Definitions of biomass variables

All definitions adopted in the paper are strictly for purposes of the current analysis and are not for universal application. Natural grass refers to native and/or pasture grasses which are distinctly different from cereal grain crops; and for simplicity are referred as grass. Shoot biomass was defined as total above ground biomass (leaves and stems) excluding grain, lint or pods. Root biomass referred to all biomass found below the soil surface (crown roots, rhizomes and nodules) excluding yield (such as pods in groundnuts). Total biomass was the summation of root and shoot. If root and shoot biomass were not explicitly cited in the articles, they were derived from ratios and harvest indices provided by or referred to by the authors) to enhance the current analysis.

1.2.4 Definitions of carbon allocation variables

This paper reviews data on soil organic C stocks (SOCs), root and shoot C stocks and their ratios as defined in Table 1.2. The root (Rcs) and shoot (Scs) C stocks were defined as the total amount of C measured in the respective plant parts. These C stocks in the two parts were summed up to derive total plant C stocks (Pcs). Where plant C was stated in terms of C content per mass basis it was converted to absolute quantities on an annual basis (Mg C $ha^{-1}yr^{-1}$) for purposes of the current paper. It was calculated as a product of biomass of the respective plant part (roots or shoots) and the C concentration, where appropriate. Root to shoot (RS) ratios were calculated for biomass and C stocks to elucidate allocation patterns between the two plant parts. All the ratios were computed except in the cases where they were stated by the paper authors.

Soil C stocks (SOCs), as used in the current paper strictly refer to total amount of organic C in a soil profile of definite depth over one-hectare piece of land. Where it was not stated, SOCs were calculated as follows (Don et al. 2011):

$$SOCs = \sum_{i=1}^{n} SOC_{conc} * BD * soil\ volume$$

Where SOCs is the soil organic C stock (Mg C ha^{-1}), SOC_{conc} the soil C concentration per unit mass of soil (Mg C Mg^{-1}), and BD the soil bulk density (Mg m^{-3}), n=soil depth (m).

Table 1.2 Definitions of carbon and biomass variables used in this study

Variable	**Symbol**	**Units**	**Definition**
Soil organic carbon	SOCs	$MgCha^{-1}$	The total amount of soil organic carbon in the whole soil profile sampled in the experiment
Root biomass	RB	$Mgha^{-1}yr^{-1}$	The annual total amount of below ground biomass of the crop excluding harvestable parts such as pods
Shoot biomass	SB	$Mgha^{-1}yr^{-1}$	The annual total amount of above ground biomass (leaves and stems) of the crop excluding harvestable parts such as grains or pods
Plant biomass	PB	$Mgha^{-1}yr^{-1}$	The annual total of root and shoot biomass of the crop excluding harvestable parts as defined in shoots and roots variables
Plant carbon stock	Pcs	$MgCha^{-1}yr^{-1}$	Annual total amount of carbon in the whole plant reported by the authors or derived as sum of root and shoot carbon stocks
Shoot carbon stock	Scs	$MgCha^{-1}yr^{-1}$	The annual total amount of carbon in the shoot biomass reported by authors or derived as product of shoot biomass and shoot carbon concentration
Root carbon stock	Rcs	$MgCha^{-1}yr^{-1}$	The annual total amount of carbon in the root biomass reported by authors or derived as product of root biomass and root carbon concentration
Root: shoot ratio	RS		An expression of root as a fraction of shoot on a mass basis
Root: shoot ratio of carbon stock	Rcs:Scs		An expression of root carbon stocks as a fraction of shoot carbon stocks

1.2.5 Data analyses

Summary statistics were generated for plant biomass and C, and SOCs variables. The variability and distribution of datasets for the different factor strata were elucidated using box-plots. Each box-plot captured the minimum, maximum, median, mean, Q1 and Q3 values after the outliers were checked and removed from the boxplots (VSN 2015). T-tests were conducted to separate the means. A multivariate analysis, using uncentred principal component analysis (PCA), was also conducted in Statistica 7 software (StatSoft Incorporation (2004). Finally, bivariate analyses, based on Pearson's correlations, were carried out in Statistica 7 software to determine the associations between variables.

1.3 Results

1.3.1 Global variation of the environmental factors, plant biomass and C stocks

Data was collated from studies spread from dry to wet, and cool to hot environments (Table 1.3). Similarly, soil properties were variable as shown by SOCs which ranged from a minimum 1.41 in a soyabean field in Uruguay (Mazzilli et al. 2015) to 197 Mg C ha^{-1} under a grassland in USA (Lee et al. 2007) with a mean of 55.82±2.54 Mg C ha^{-1} (n=281). Plant biomass and C were minimum in wheat under sub-tropical India (Manna et al. 2005) and highest in maize grown under wet and warm conditions in China (Zhang et al. 2015). Shoot and root biomass and C stocks also showed wide variability across the environments and crop types.

Table 1.3 Summary statistics of soil carbon stocks (SOCs), plant variables and environmental factors

Statistic	**Plant[a] and environmental[b] variables**													
	MAP	**MAT**	**Clay**	**BD**	**pH**	**PB**	**SB**	**RB**	**Pcs**	**Scs**	**Rcs**	**RS**	**Rcs/ Scs**	**SOCs**
	mm	°C	%	Mg m^{-3}		------Mg ha^{-1} yr^{-1} ------			-----Mg C ha^{-1} yr^{-1} ------					Mg C ha^{-1}
N	344	313	247	171	327	360	343	329	288	281	301	317	267	281
Mean	1001.02	18.89	29.05	1.33	6.51	8.05	6.61	1.83	3.75	2.93	0.88	0.34	0.32	55.82
Median	844	18.2	18	1.3	6.5	5.3	4.8	1.12	3.04	2.41	0.42	0.26	0.22	37.84
Min.	119.1	1.1	5	0.97	4.6	0.12	0.09	0.02	0.06	0.04	0.01	0.02	0.01	1.41
Max.	1727	41	74.7	1.75	8.36	42.79	36.74	15.13	19.29	16.57	10.52	2.26	1.67	197.13
Quartile 1	704	9.4	15	1.16	5.53	2.98	2.2	0.7	1.25	0.94	0.17	0.19	0.15	23.3
Quartile 3	1437.5	24.25	51.2	1.42	7.7	11.28	9	2.16	5.41	4.41	0.77	0.41	0.42	85.43
SD	416	11.08	18.98	0.19	1.07	7.44	6.26	2.22	3.25	2.51	1.48	0.24	0.3	42.59
SEM	22.43	0.63	1.21	0.01	0.06	0.39	0.34	0.12	0.19	0.15	0.09	0.01	0.02	2.54
%CV	41.56	58.66	65.33	13.96	16.37	92.32	94.81	121.39	86.8	85.83	169.03	71.53	94.65	76.3
Skewness	0.09	0.55	0.65	0.63	0.04	1.85	2.15	3.12	1.78	1.8	3.58	3.05	1.83	1.05
Kurtosis	-1.07	-0.24	-1.03	-0.42	-1.29	3.95	5.87	11.4	4.54	5.47	14.11	15.76	3.74	0.28

N=number of observations, Min and Max= minimum and maximum, respectively, Q1 and Q3=first and third quartile, SD=standard deviation, SEM=standard error of mean and CV=coefficient of variation. MAP=mean annual precipitation, MAT=mean annual temperature, BD=bulk density, pH=soil acidity/alkalinity, PB=total plant biomass, SB=shoot biomass, RB=root biomass, Pcs=total plant carbon stock, Scs=shoot carbon stock, Rcs=root carbon stock, RS=root biomass:shoot biomass, Rcs/Scs=root:shoot carbon stock ratio, SOCs=soil organic carbon stock. [a] See Table 1.2 for description and units, [b] See Table 1.1 for description and units

1.3.2 Plant biomass and C allocations to shoots and roots

Grasses had the highest mean biomass (PB) (19.80±1.16 Mg ha^{-1} yr^{-1}, n=29) (Fig 1.1a), with reported values ranging from 10.96 to 29.50 Mg ha^{-1} yr^{-1}. Amongst the cereals, maize had the highest mean PB (11.24±0.77 Mg ha^{-1} yr^{-1}, n=60), while rice, sorghum, triticale and wheat had mean PB of about 9.5 Mg ha^{-1} yr^{-1}. Legumes and brassicas tended to have lower Pb than sunflower and fibre crops. Shoot (SB) (Fig 1.1b) and root (RB) biomass (Fig 1.1c) followed a similar trend to PB. Grasses, with an average Rb of 7.23±0.30 Mg ha^{-1} yr^{-1} (n=25), accumulated about three times more root biomass than maize, which had the greatest RB amongst the cereals (Fig 1.1c). All the plants generally accumulated more shoot than root biomass because RS values were below 1, except some few grass studies whose RS ratios even reached 1.6 (Fig 1.1d).

1.3.3 Plant C stocks and their allocation in shoot and roots

Plant C concentration was not significantly different within species so plant C stocks were more determined by biomass accumulation. Grasses also amassed the highest total plant C stocks (Pcs) (6.80±0.57 Mg C ha^{-1} yr^{-1}), and were followed by maize (6.30±0.34 Mg C ha^{-1} yr^{-1}), cotton (4.3±0.47 Mg C ha^{-1} yr^{-1}) and soyabean (3.00±0.48 Mg C ha^{-1} yr^{-1}) (Fig. 1.2a). The mean shoot C stocks (Scs) in grass (3.20±0.43 Mg C ha^{-1} yr^{-1}) were 120% lower than in maize, sorghum and cotton (Fig 1.2b). Soyabean had higher Pcs and Scs than the other legume crops, while fibre crops had, generally, greater Pcs and Scs compared to the legumes. Grasses exhibited the highest mean Rcs being nearly three times that of maize (Fig. 1.2c). Rcs were consistently less than 1 Mg C ha^{-1} yr^{-1} with very little variation among the annual crops, except maize. The root to shoot C stocks (Rcs/Scs) ratios were also greatest under grasses where the mean was 0.82 showing that grasses can store up to 45% of their C stocks in the roots. The Rcs/Scs ratios were much were lower in the other crops. For instance, maize (0.20) and wheat (0.30) store only 16% and 23%, respectively, of their total C stocks in the roots (Fig. 1.2d).

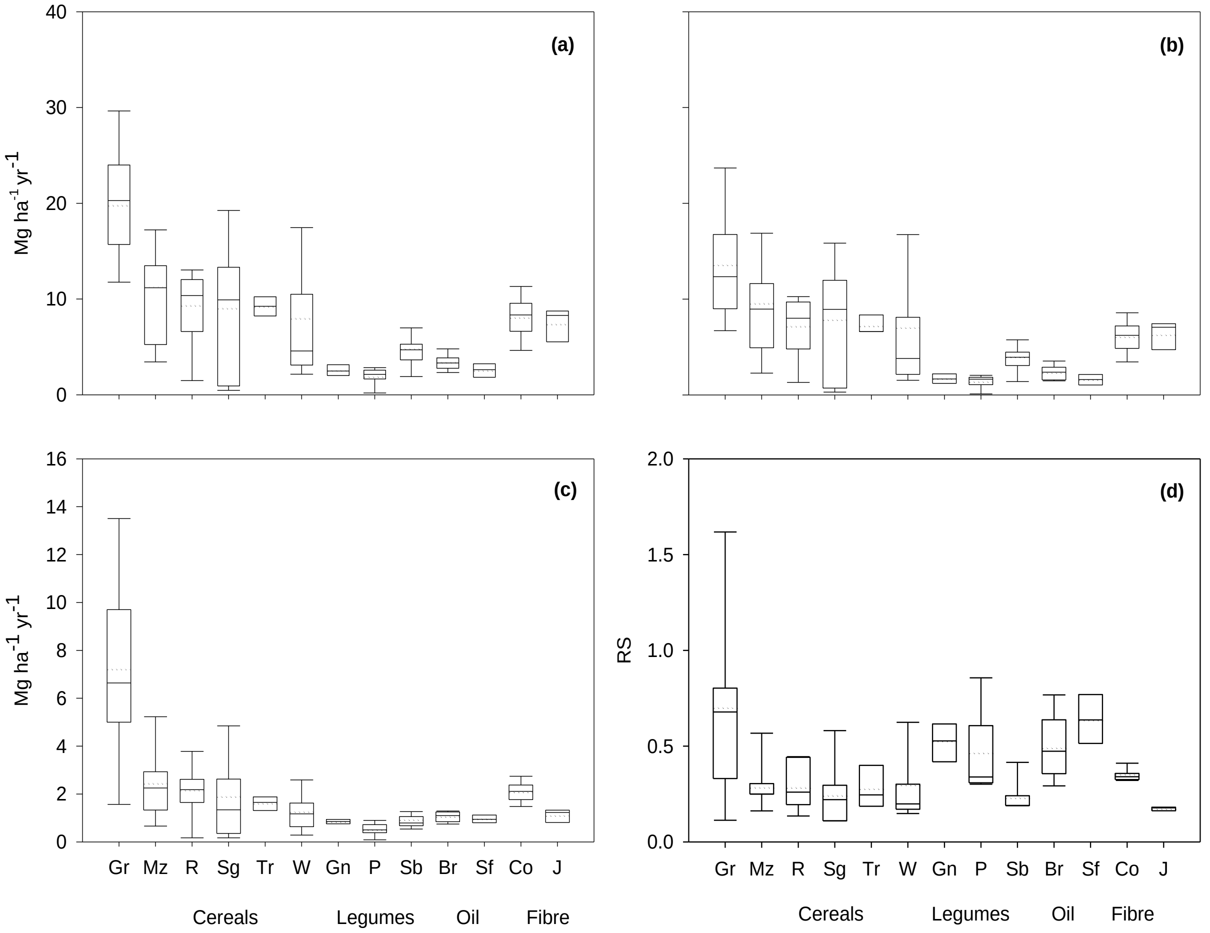

Fig 1.1 Biomass in (a) total plant, (b) shoot, (c) roots and (d) RS ratios of biomass for different crops grouped by crop type (cereals, legumes, oil and fibre crops) and grass.

Each box plot shows the minimum, maximum, median, quartile 1 (25%) and quartile 3 (75%) values. Gr=grass; Mz=maize; R=rice; Sg=sorghum; W=wheat; Tr=triticale; Sb=soyabean; P=peas; Gn=groundnuts; Br=brassicas; Sf=sunflower; Co=cotton; J=jute

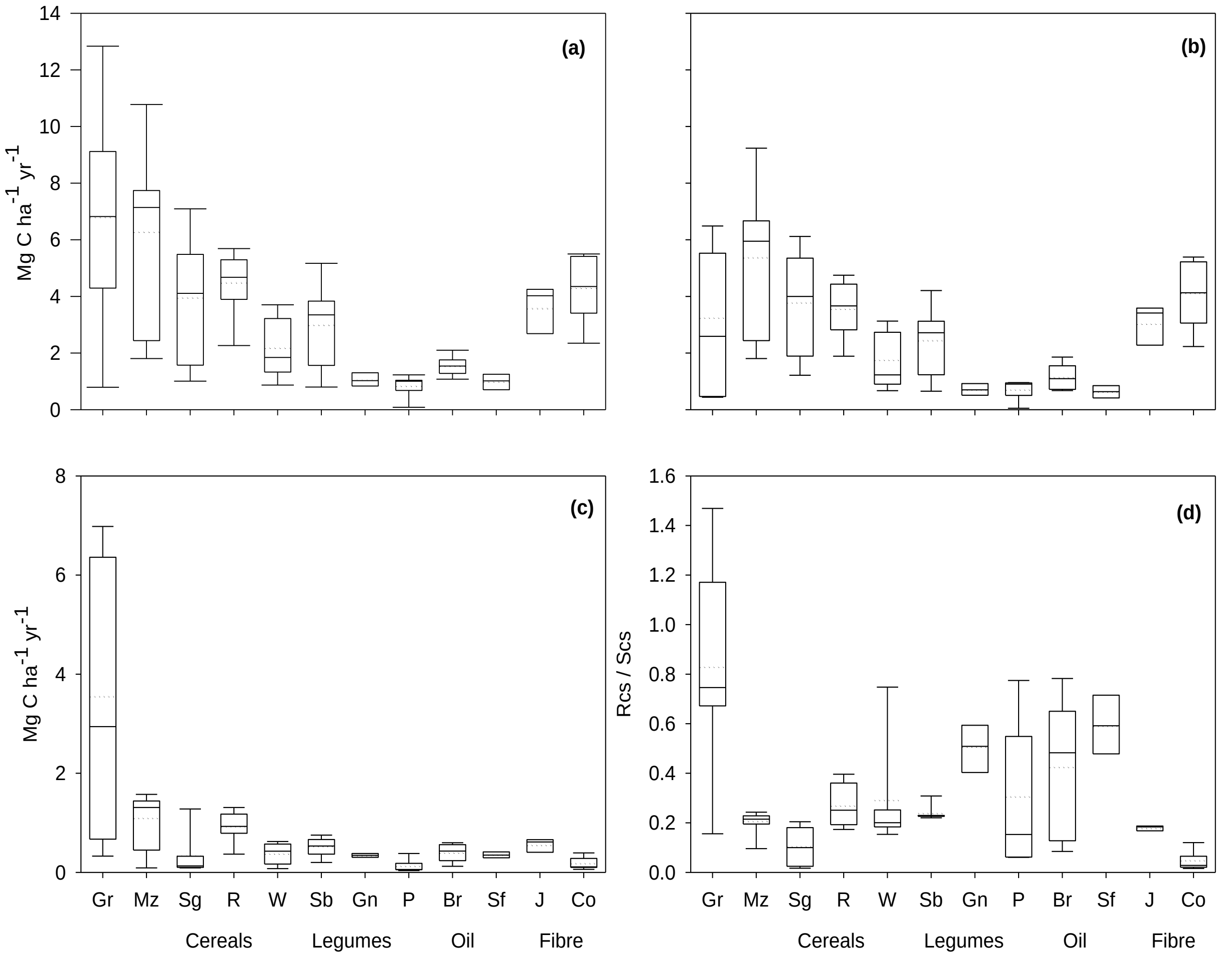

Fig 1.2 Carbon stocks in (a) total plants, (b) shoots, (c) roots, and (d) RS ratios of the C stocks for different crops grouped by crop types (cereals, legumes, oil and fibre crops) and grass.

Each box plot shows the minimum, maximum, median, quartile 1 (25%) and quartile 3 (75%), values. Br=brassicas; Co=cotton; Gn=groundnuts; Gr=grass; J=jute; Mz=maize; P=peas; R=rice; Sb=soyabean; Sf=sunflower; Sg=sorghum; W=wheat

1.3.4 A comparison of SOCs under different crops

A comparison across all the experimental sites showed that soils under maize and soybean had the highest mean SOCs (Fig 1.3). Uncharacteristically high SOCs were recorded for soils where maize is grown in rotation with soyabean. Soils under grasses also tended to have lower SOCs than soils under maize and soybean. However, the SOCs under grass varied greatly and the mean was greater than the other crop types. The average SOCs from soils under most crops were below 50 Mg C ha^{-1} in comparison to 60 Mg C ha^{-1} under grass and 80 Mg C ha^{-1} under maize/soyabean. The SOCS under the same crop varied across climatic regions with the tropical regions supporting the highest mean stocks across all crop types (Fig 1.4a). The SOCs associated with maize and soybean were similar and highest under tropical regions but soyabean had significantly higher SOCs compared to maize under sub-tropical regions (Fig 1.4b and 2.4c). Wheat had higher SOCs compared to maize under sub-tropical regions but was consistently associated with the lowest SOCs under the other regions (Fig 1.4b-d).

1.3.5 The variation of plant biomass with climatic factors

There was a general increase of PB from subtropical (mean of 7.4±0.58 Mg ha-^{1} yr^{-1}) to tropical climate zone (mean of 7.8±0.93 Mg ha-^{1} yr^{-1}) and temperate (mean of 8.0±0.73 Mg ha-^{1} yr^{-1}) (Fig 1.5a). Shoot biomass accumulation (SB) showed a different trend with tropical climate exhibiting the highest Sb (Fig 1.5b). The trend in RB also showed a general increase sub-tropical to tropical with a sharp increase under temperate climate (Fig 1.5c). In all cases, sub-tropical climates regions exhibited lower biomass compared to tropical and temperate regions. The RS ratios showed a sharp decrease under tropical compared to both sub-tropical and temperate regions (Fig 1.5d).

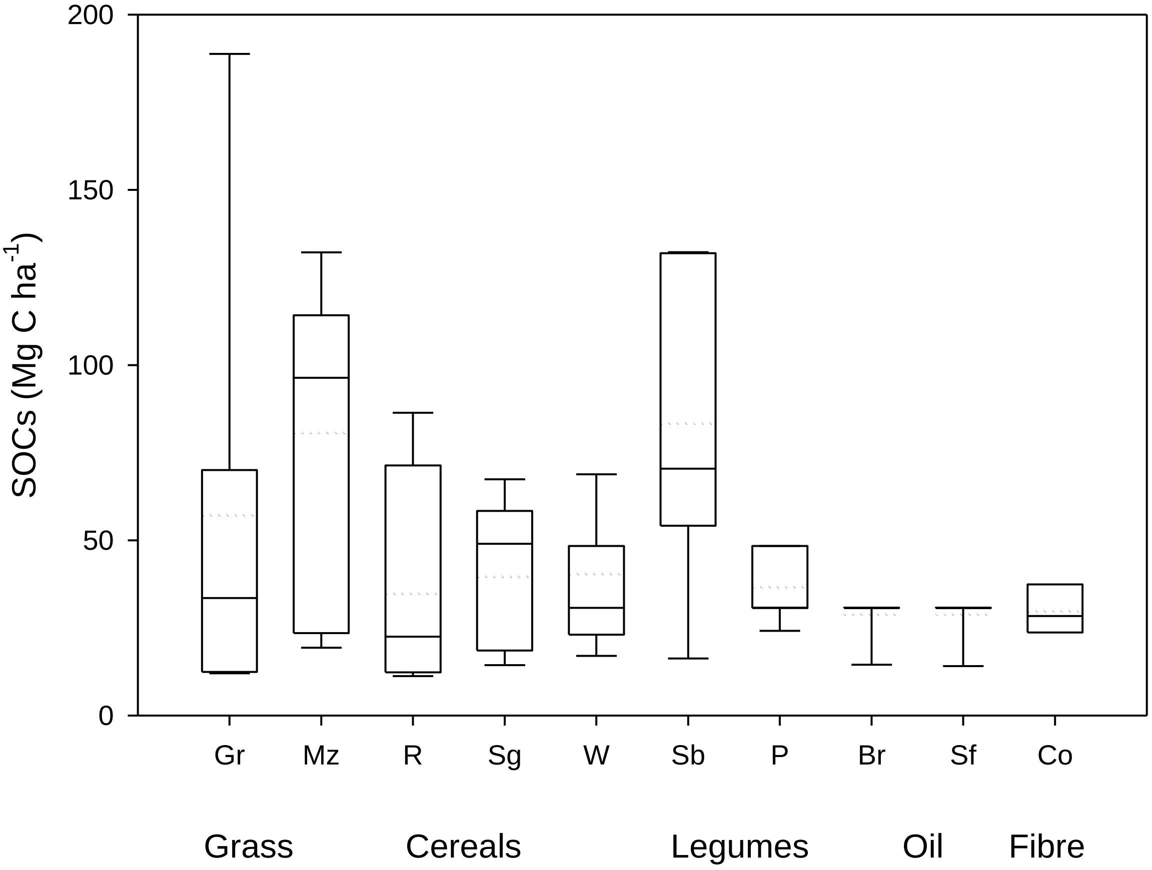

Fig 1.3 Soil organic C stocks in fields planted to different crop types.

Each box plot shows the minimum, maximum, median, quartile 1 (25%) and quartile 3 (75%) values. Br=brassicas; Co=cotton; Gn=groundnuts; Gr=grass; Mz=maize; P=peas; R=rice; Sb=soyabean; Sf=sunflower; Sg=sorghum; W=wheat

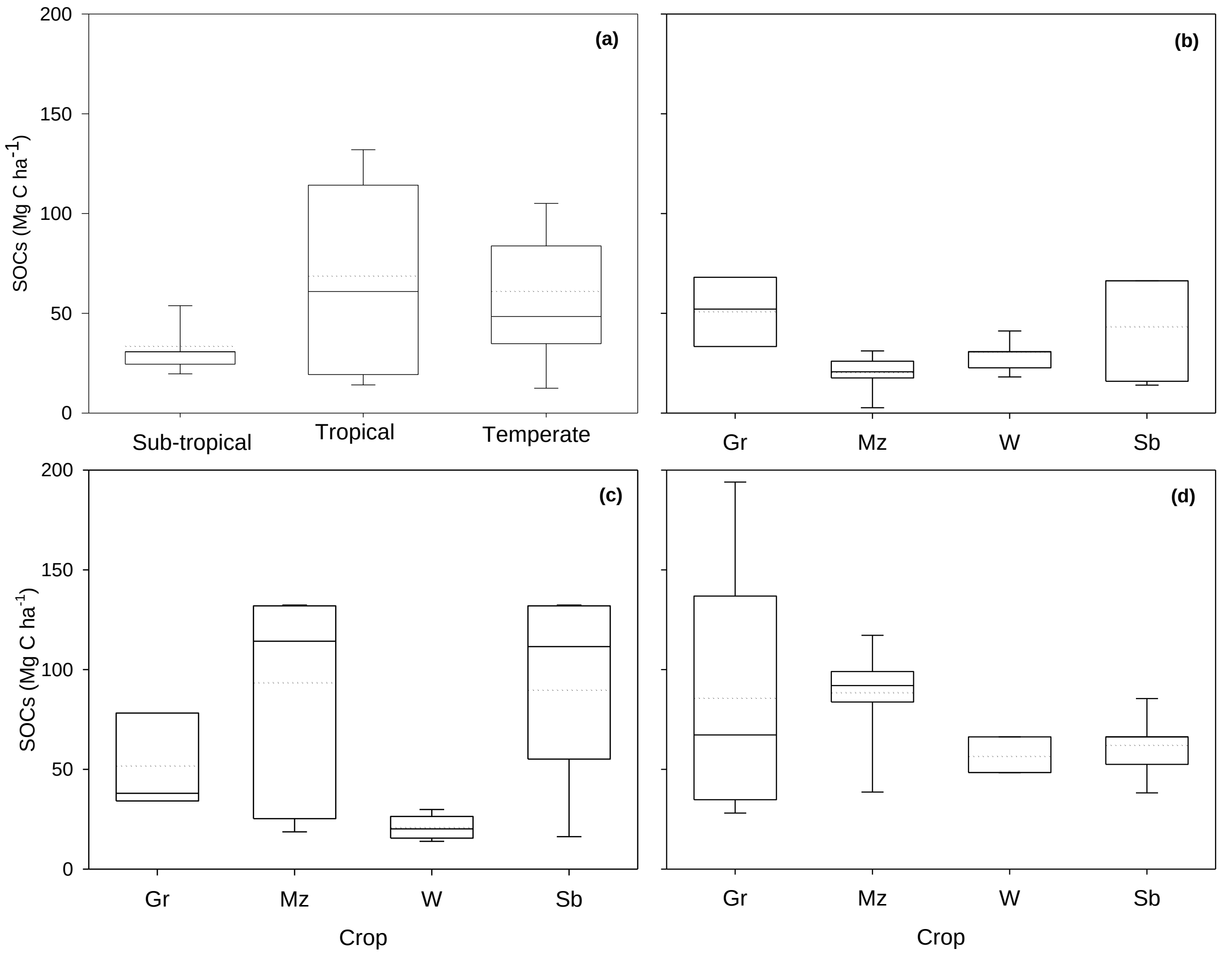

Fig 1.4 Soil organic C stocks (SOCs) measured in different climatic regions (a) and SOCs associated with selected crops under (b) subtropical, (c) tropical and (d) temperate climatic regions.

Gr=grass, Mz=maize, W=wheat and Sb=soyabean. See Table 1.1 for climate categories.

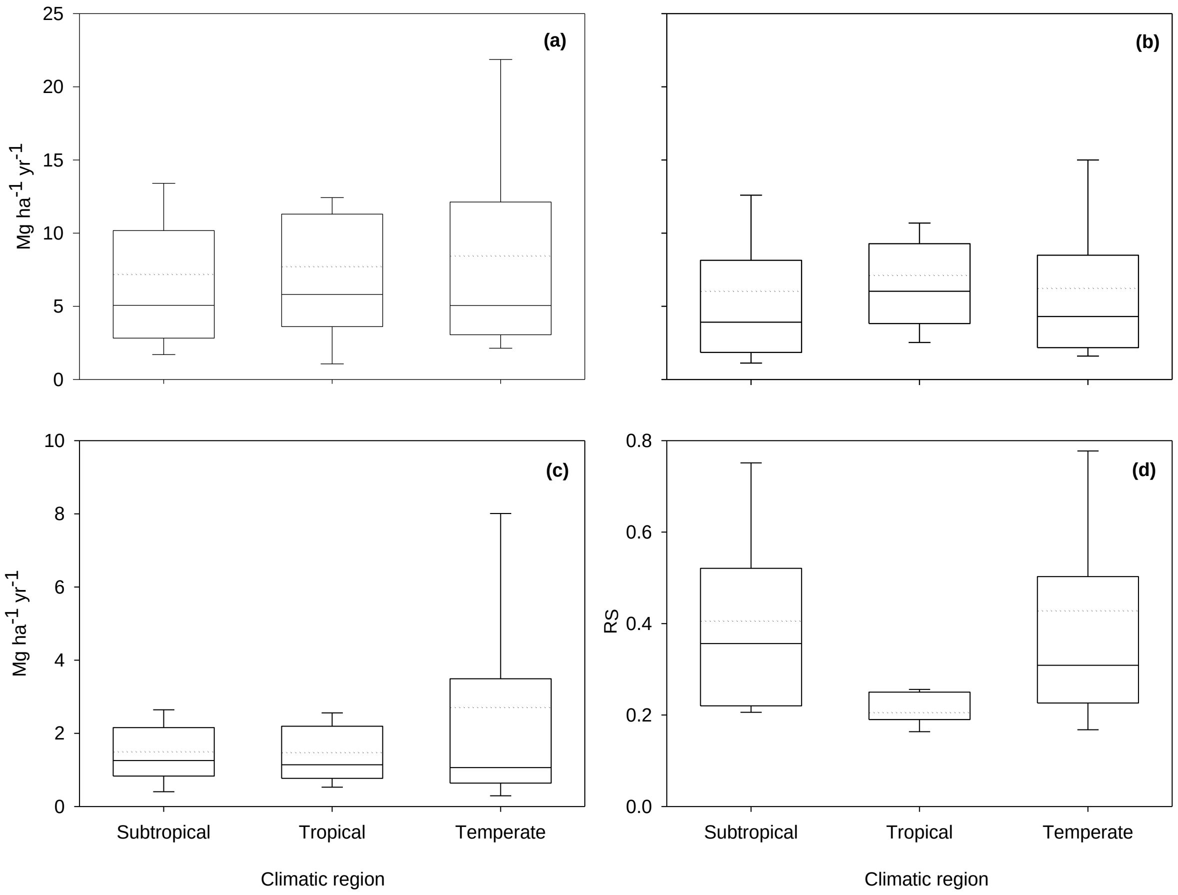

Fig 1.5 Biomass accumulation in a) total plant b) shoot and c) root fractions and d) RS ratio of biomass in different climatic zones.

Each box plot shows the minimum, maximum, median, quartile 1 (25%) and quartile 3 (75%), values. See Table 1.1 for climate categories.

1.3.6 The variation of plant C stocks with climatic factors

Plant (Pcs) and shoot (Scs) C stocks showed a general to tendency to increase from sub-tropical to tropical followed by a decline under temperate climates (Fig 1.6a and 1.6b). However, Pcs was higher under temperate compared to the subtropical although they had similar Scs. The mean Rcs increased by more than two fold from sub-tropical (0.5±0.34 Mg C $ha^{-1}yr^{-1}$) to tropical (1.0±0.64 Mg C $ha^{-1}yr^{-1}$) and threefold to (1.5±0.78 Mg C $ha^{-1}yr^{-1}$) under temperate climate (Fig 1.6c). Root C stocks (Rcs) were unexpectedly higher under temperate regions compared to the other regions and in all cases the temperate region exhibited the greatest variability for C stocks. The corresponding Rcs/Scs ratios were generally highest under temperate and least under tropical climate (Fig 1.6d). However, subtropical climates showed comparably high variation for Rcs/Scs similar to temperate climates.

1.3.7 Associations between environmental factors and plant biomass and C stocks

The first two principal components explained 55.97% of the total variation in the data (Fig 1.7). The first principal component (PC1), accounting for 32.29% of the variation, was highly correlated to total plant (PB), shoot (SB) and root (RB) biomass, and total plant (Pcs), shoot (Scs) and root (Rcs) C stocks. It was also strongly associated with soil properties of SOCs and soil clay content. The PC1 could, therefore, be regarded as an axis relating to high sequestration of C under increasing plant productivity in clayey soils. The other component (PC2), which explained 23.68% of the data variation, was strongly associated with RS and Rcs/Scs. The PCA also showed that soil pH was associated more with root variables (RB and Rcs) and the associated root: shoot ratios than the shoot variables. Root biomass and C stocks tended to be high in hot and wet climates and in soils with low bulk density as depicted by PC2.

SOCs and plant biomass exhibited a significantly positive correlation ($r=0.21$, $p<0.05$) pointing to a direct link between the two (Table 1.4). All plant variables (biomass and C stocks) showed positive associations with MAP, while they had negative correlations with MAT. Shoot biomass (SB) exhibited significant associations with both MAP and MAT in contrast to RB, which exhibited non-significant correlations to these factors. Plant C stocks (Pcs, Scs and Rcs) and SOCs were more strongly associated with MAP compared to MAT. Shoot C stocks (Scs) and Rcs had significant associations with MAP. High PB, Pcs and SOCs had a tendency to occur under regions with high precipitation.

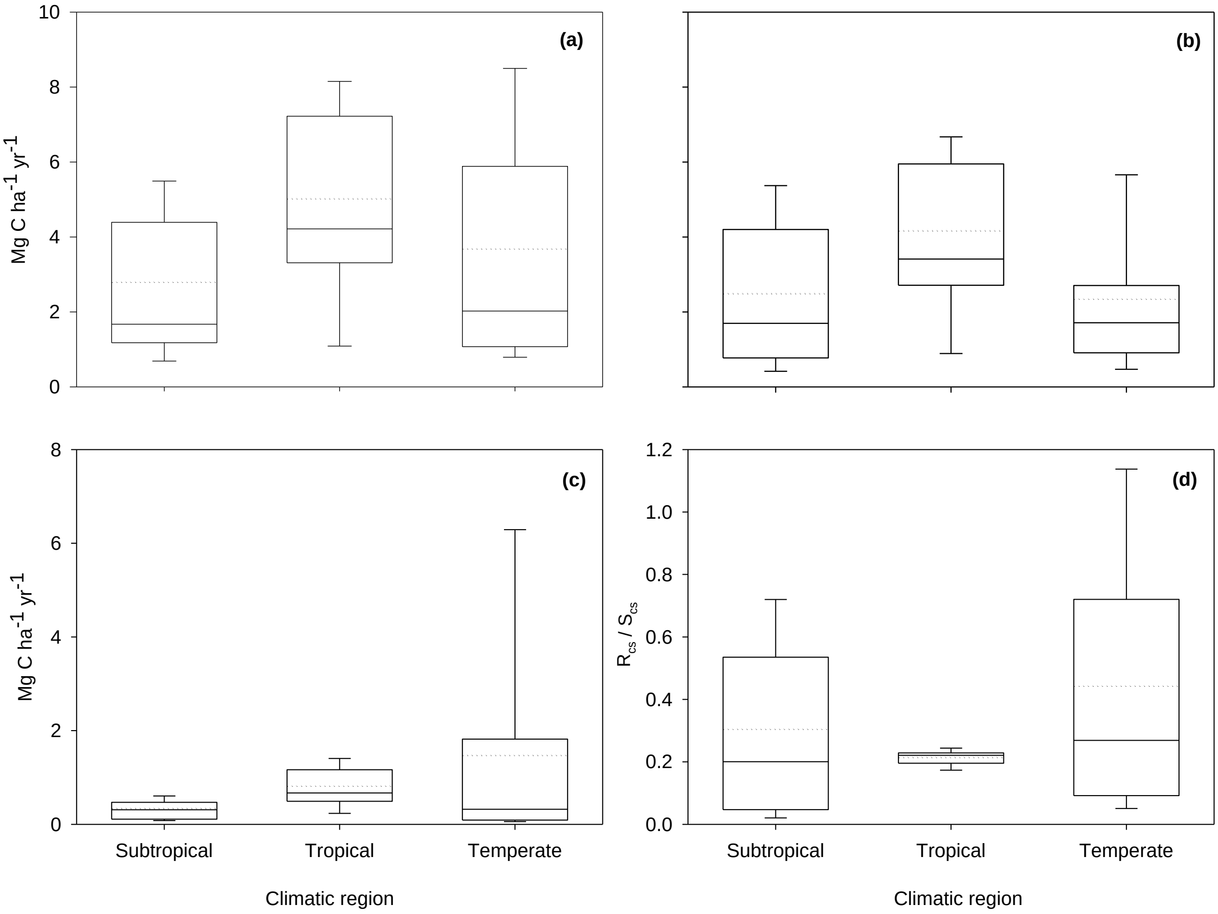

Fig 1.6 Carbon stocks in a) total plant b) shoot, and c) root fractions and d) Rcs/Scs in different climatic zones.

Each box plot shows the minimum, maximum, median, quartile 1 (25%) and quartile 3 (75%), values. See Table 1.1 for climate categories.

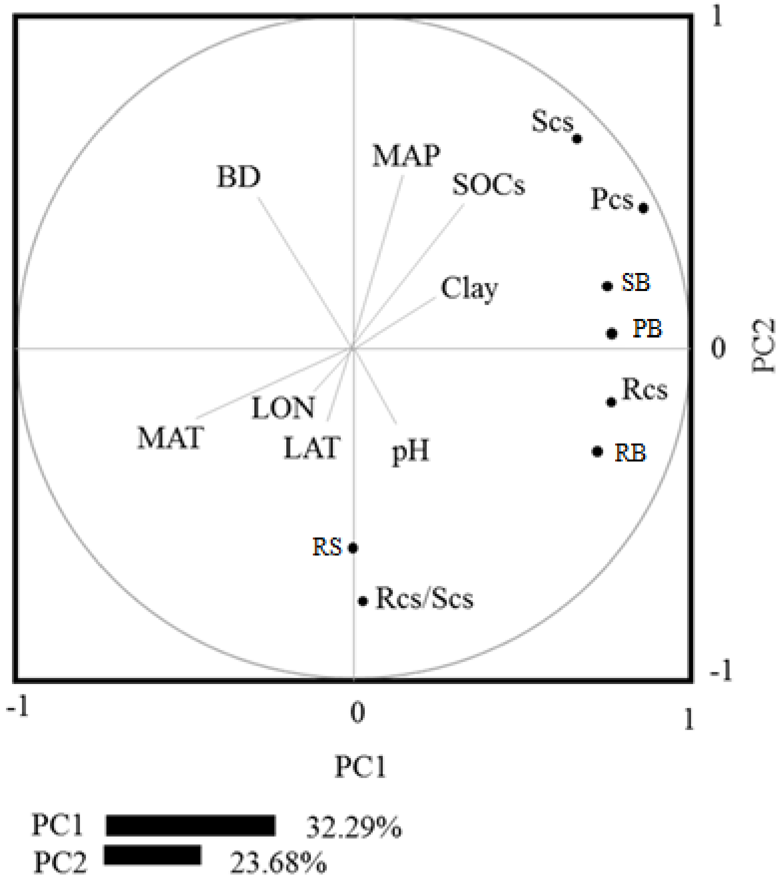

Fig 1.7 Principal component analyses (PCA) between plant carbon and biomass as variables for analysis and environmental factors as supplementary variables.

MAP=mean annual precipitation, MAT=mean annual temperature, BD=bulk density, PB=total plant biomass, RB=root biomass, SB=shoot biomass, RS=root:shoot biomass ratio, Pcs=total plant carbon stock, Rcs=root carbon stock, Scs=shoot carbon stock, Rcs/Scs=root:shoot carbon stock ratio, SOCs=soil organic carbon stock. See Tables 1.1 and 1.2 for definition of carbon and biomass variables and environmental factors.

Table 1.4 Pearson's correlations showing pair-wise relationship between plant and environmental parameters used in the study

Parameter[a]	**PB**	**SB**	**RB**	**Pcs**	**Scs**	**Rcs**	**RS**	**Rcs/Scs**	**MAP**	**MAT**	**SOCs**	**Clay**	**BD**	**pH**
Pb	1.00													
Sb	0.99*	1.00												
Rb	0.82*	0.79	1.00											
Pcs	0.91*	0.91*	0.70*	1.00										
Scs	0.84*	0.86*	0.57*	0.88*	1.00									
Rcs	0.59*	0.59*	0.65*	0.66*	0.39*	1.00								
RS	-0.13*	-0.25*	0.32*	-0.29*	-0.41*	0.11	1.00							
Rcs/Scs	-0.14*	-0.22*	0.14*	-0.14*	-0.44*	0.53*	0.59*	1.00						
MAP	0.11	0.14*	0.09	0.29*	0.43*	0.17*	-0.36*	-0.38*	1.00					
MAT	-0.29*	-0.23*	-0.12	-0.18*	-0.07	0.06	0.23*	0.13	0.38*	1.00				
SOCs	0.21*	0.26*	0.11	0.41*	0.55*	0.26*	-0.42*	-0.46*	0.32*	-0.22*	1.00			
Clay	-0.14*	-0.05	0.05	0.21*	0.17*	0.53*	-0.02	0.63*	0.40*	0.52*	0.14*	1.00		
BD	0.28*	0.28*	0.03	-0.26*	-0.23*	-0.17	0.16	-0.43*	-0.53*	0.45*	-0.65*	-0.32*	1.00	
pH	0.06	0.12	0.20*	-0.22*	-0.28*	-0.04	0.39*	0.23*	-0.42*	0.26*	-0.23*	-0.18*	0.36*	1.00

[a] See Tables 1.1 and 1.2 for description and units

*significance at p≤0.05

MAP=mean annual precipitation, MAT=mean annual temperature, BD=bulk density, Pb=total plant biomass, Rb=root biomass, Sb=shoot biomass, RS=root biomass:shoot biomass, Pcs=total plant carbon stock, Rcs=root carbon stock, Scs=shoot carbon stock, Rcs/Scs=root:shoot carbon stock ratio, SOCs=soil organic carbon stock

1.4 Discussion

1.4.1 Plant type effects on biomass allocation

The present study showed that oil and legume crops accumulated lower mean biomass compared to fibre crops, cereals and grasses (Fig 1.1a), confirming previous results (Ragaee et al. 2006; Shewry 2007). The low biomass in legumes and oil crops has previously been shown to be a result of the high energy required in the synthesis of protein products (Munier-Jolain and Salon 2005), and early leaf senescence (Gan et al. 2009). Perennial grasses accumulated higher biomass possibly because of their high efficiency in nutrient and water extraction rendered by an extensive root system (van Loocke 2012) and limited pest attacks (Lewandowski et al. 2003) compared to the other plants. Maize appeared to yield higher biomass as it maximizes light capture and carbon sequestration in biomass compared to the other cereals (Amanullah and Stewart 2013). Again, grasses had the highest root biomass because most of the grasses used in this study are perennial and use the roots as an energy reserve (Ghimire et al. 2013) (Fig 1.1c). In contrast, plants with an annual growth habit do not have the need to store energy and thus may have lower root biomass. Due to their high total and root biomass, grasses and cereals sequester more atmospheric C compared to the other crop types (Balesdent and Balabane 1996; Paustian et al. 2016). In some instances, grass accumulated higher root than shoot biomass (as expressed by RS above unity) as a result of their ability to cease shoot development, while maintaining root growth (Silvertown 2004). This ability does not exist among annual plants.

1.4.2 Plant type effects on C accumulation and allocation

The general decrease of total plant carbon stocks from grasses to cereals, fibre crops and to legumes (Fig 1.2a) was concomitant with decrease in biomass (Fig 1.1a). Grasses and cereals were shown to accumulate higher biomass, which increased their capacity to sequester more C (e.g. Anderson-Teixeira et al. 2013; Guzman and Al-kaisi 2010; Singh et al. 2014). The other plants showed limited C stocks as a direct result of low biomass production. Generally, all plants stored more carbon in the shoots showing that roots are relatively weaker C sinks compared to shoots (Fig 1.2d). Shoot C are higher than Rcs because C is only be exported to other sinks when the supply exceeds local demand (Ludewig and Flügge 2013). Due to their perenniality, ability to prolong root growth and extensive root system grasses have in some cases the ability to store more C in the roots compared to the shoot.

1.4.3 The impact of climate on plant biomass and C stocks

The variations in biomass production under different environments confirm previous studies investigating the effects of genotype and environmental interactions on biomass production (Anderson-Teixeira et al. 2013; Manna et al. 2005). Precipitation and temperature are among the most critical climatic factors for biomass production. The increase in biomass production from sub-tropical to tropical climates (Fig 1.5) is concordant with increase in with increase in precipitation and temperature (Pittelkow et al. 2015).

The increase in C stocks in a similar trend to biomass production from sub-tropical and temperate to tropical regions was concordant with improved plant productivity (e.g. Pittelkow et al. 2015). Sainju et al. (2005) attributed such a trend to increased production of assimilates through photosynthesis, which are mostly carbohydrates, and their subsequent translocation within the plant under adequate precipitation and high temperature. Temperature must be sufficient for plant growth and within the optimal range for biomass production (Llorens et al. 2003; Sánchez et al. 2014) and C sequestration. However, excessively high or low temperatures reduce biomass production, as temperature is a critical controlling factor of biological processes necessary in plant growth. Therefore, highest C sequestration would be expected to occur under tropical climates provided the respirational loss of C is compensated by the high biomass production.

1.4.4 The impact of soil conditions on plant biomass and C

There was a general decline in shoot and root biomass production under low soil pH confirming reports that acidic soil conditions lead to stunted growth in plants (Choudhury and Sharma 2014). For instance, the nodulation process, which is important for symbiosis in legumes, is inhibited by low pH causing low biomass accumulation (White and McNaughton 1997). Overall, the decrease in shoot and root biomass production under high clay content and low bulk density soils could be a result of compaction and poor drainage (e.g. Rich and Watt 2013). In contrast, the increase in root biomass associated with soil compaction in clayey soils with high bulk density may be a result of increased C allocation to the roots. According, to the ‘functional equilibrium’ plants increase allocation of resources to maintain development of the organ under stress as a way to counter the effects of the stress (Poorter and Nagel 2000). However, higher potential for C sequestration in plant biomass is attained when root and overall plant growth are not restricted by soil acidity and/or compaction.

1.4.5 The links between plant and soil C stocks

The increase of SOCs with plant C stocks (Table 1.4; Figs 1.3 and 1.4) points to a possible direct link. It is most likely that the high SOCs observed under grasses and cereals, particularly maize, were a consequence of high biomass and plant C stocks (e.g. Balesdent and Balabane 1996; Paustian et al. 2016). There was a trend showing high biomass and C stocks correlated with high SOCs although this trend was not evident in cotton. Therefore, to understand the link between crop type and SOCs techniques that are more comprehensive are required. Determining changes in SOCs using isotopic C tracing provides such a comprehensive tool to investigate short-term plant and soil C dynamics.

1.5 Conclusion

It was hypothesized that investigating the carbon allocation patterns between shoots and roots can provide insight into the link between plant C and SOCs and inform cultivar selection for C sequestration. Three main conclusions can be drawn from this meta-analysis of 389 trials world-wide:

1. Grass and cereals exhibited the highest potential for C sequestration because they showed high biomass production and C accumulation. There were no significant differences in C content within a species, which means that absolute biomass production was the major determinant of C sequestration.
2. On average plants allocated 76% of C stocks to shoots and only 24% to the roots; however, this was dependent on environmental conditions with higher root C under tropical climate.
3. Although grass, maize and soyabean exhibited high annual plant C stocks and SOCs, the plant C stocks did not always correspond to SOCs as observed for cotton. The relationship between plant and soil C stocks is subject to complex interactions among soil, plant and climatic factors.

These results provide a better understanding of biomass and C allocation within plants parts and potentially to the soils and show that biomass allocation rather than C concentration could be used as criteria for selection of crops with high C sequestration potential for breeding purposes.

However, there is need to compare different plants under similar pedo-climatic conditions using more accurate techniques such as isotopic C labeling to trace C fluxes between plants/soil system and the atmosphere. It is also important to consider investigating the variance components, heritability and genomic loci controlling key traits for drought tolerance and C sequestration in an identified species. Furthermore, once superior genotypes are identified they must be assessed for combining ability and to validate the gene action involved in the inheritance of the target traits and devise appropriate selection strategy for genetic advancement.

1.5.1 Heritability and variance of traits

Root to shoot ratios are highly influenced by the environment (Kumar et al. 2006), which means that their selection across environments is confounded by environmental variance. Kumar et al. (2006) alluded that environmental stresses, such as drought and heat, affect biomass allocation through their effects on plant growth and tillering capacity. Therefore, investigating agronomic traits related to biomass allocation may assist in indirect selection of biomass allocation for enhanced grain yield. Gowda et al. (2011) found that biomass partitioning in triticale was influenced mostly by plant height, while grain yield, days to heading, number of spikes per plant and thousand-grain weight had minor effects. However, the lack of adequate information on the heritability and genetic correlations between yield-related traits and biomass allocation limits development of ideotypes with optimum biomass partitioning for drought tolerance and C sequestration.

1.5.2 Genomic selection

The advent of next generation sequencing (NGS) and genotyping by sequencing (GBS) technologies has provided a means for discovering genomic regions controlling important traits (Korte and Farlow 2013). Micro-array based diversity array technology sequencing (DArTseq) derived single nucleotide polymorphisms (SNPs) have become increasingly important in genome-wide association studies (GWAS) and have been used extensively on genetic studies of wheat (e.g. Mwadzingeni et al. 2017; Sukumaran et al. 2018). DArTseq derived SNPs markers are reproducible genetic markers that provide a powerful means to identify genetic variation at large number of analogous genomic loci. This enables breeders to deduce population structures and genomic loci controlling traits through association mapping (Maccaferri et al. 2015). The scarcity of genetic markers and marker-trait associations for biomass allocation and related traits impedes the use of marker-assisted breeding for drought tolerance and C sequestration in wheat.

1.5.3 Combining ability

Fundamentally, gene action regulating heritability of traits can be inferred through combining ability analysis (Fasahat et al. 2016). Combining ability analysis broadly distinguishes the general combining ability (GCA) effect of parents and the specific combining ability (SCA) effect of the progenies. The GCA and SCA effects are associated with additive and non-additive gene action, respectively (Falconer 1967). Parents that exhibit good GCA will be useful for use in population development or maintenance as pure lines. Families with good SCA effect are useful for genetic advancement and development of pure line cultivars. The advent of powerful biometrical tools and mating designs has enabled the routine deduction of combining ability in breeding populations. The most commonly used mating designs to deduce combining ability include line x tester (Kempthorne 1957), diallel crossing (Griffing 1956) and North Carolina (Comstock and Robinson 1948). The choice of the mating design depends on the objectives of the breeding program. The diallel mating design is has been used extensively in wheat (Edwards et al. 1976; Khahani et al 2017). To our knowledge, many studies have evaluated combining ability for agronomic traits such as earliness, plant height and grain yield but there is a paucity of information on root to shoot biomass allocation.

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Appendix 1.1 References used in the meta-analysis showing locations, crops and climatic zones under which the studies were conducted

No.	Author	Country	Crop(s)	Region	Tillage
1	Al-Kaisi and Grote 2007	USA	Grass, Maize, Soyabean	Temperate	Conventional
2	Allmaras et al. 2004	USA	Maize	Temperate	
3	An et al. 2015	China	Maize	Temperate	
4	Bastia et al. 2013	India	Rice	Tropical	Conventional
5	Bolinder et al. 1997	Canada	Barley, Oats, Wheat	Temperate	Conventional
6	de Graaf et al. 2009	USA	Wheat	Sub-tropical	
7	de Moraes Sa et al. 2014	Brazil	Maize, Soyabean	Sub-tropical	Conventional, Minimum, No-till
8	Ferchaud et al. 2016	France	Grass	Temperate	No-till
9	Ferreira et al. 2012	Brazil	Maize, Soyabean, Wheat	Sub-tropical	No-till
10	Frank et al. 2004	USA	Grass	Temperate	
11	Gan et al. 2009	Canada	Brassica, Cotton, Lentil, Pea, Wheat	Temperate	Conventional
12	Ge et al. 2012	China	Rice	Sub-tropical	
13	Ghimire et al. 2013	USA	Alfa	Temperate	
14	Ghosh et al. 2006	India	Brassica, Groundnuts, Peas, Sunflower, Wheat	Tropical	Conventional, No-till
15	Guzman et al. 2010	USA	Grass, Maize, Soyabean	Temperate	
16	Gwenzi et al. 2009	Zimbabwe	Cotton, Wheat	Tropical	Conventional, Minimum, No-till
17	He et al. 2013	China	Grass	Temperate	
18	Kapkiyai et al. 1999	Kenya	Bean, Maize	Tropical	Conventional
19	Kauer et al. 2015	Estonia	Barley, Peas, Wheat	Temperate	Conventional
20	Khorramdel et al. 2013	Iran	Maize	Sub-tropical	Conventional, No-till
21	Kukal et al. 2009	India	Maize, Wheat	Sub-tropical	Conventional
22	Kundu et al. 2007	India	Soyabean, Wheat	Sub-tropical	Conventional
23	Kushwah et al. 2014	India	Maize, Millet, Rice, Sorghum, Soyabean	Tropical	
24	Lakaria et al. 2012	India	Soyabean	Tropical	Conventional
25	Lee et al. 2007	USA	Grass	Temperate	
26	Majumder et al. 2008	India	Rice	Tropical	Conventional
27	Makumba et al. 2007	Malawi	Maize	Tropical	Conventional
28	Mangalassery et al. 2014	India	Grass	Tropical	
29	Manna et al. 2005	India	Jute, Rice, Sorghum, Soyabean, Wheat	Tropical	Conventional
30	Mapfumo et al. 2002	Canada	Grass, Triticale	Temperate	
31	Mazzilli et al. 2015	Uruguay	Maize, Soyabean	Sub-tropical	Conventional, No-till
32	Meki et al. 2013	USA	Sorghum	Temperate	
33	Qian et al. 2010	USA	Grass		Minimum, No-till
34	Sainju et al. 2005	USA	Cotton, Sorghum	Temperate	No-till
35	Seben junior et al. 2014	Brazil	Brassica, Maize, Millet, Pea, Sorghum, Soyabean, Sunflower	Tropical	
36	Singh et al. 2014	India	Grass	Tropical	Conventional
37	Srinivasarao et al. 2012	India	Sorghum	Tropical	Conventional
38	Subedi et al. 2006	Canada	Wheat	Temperate	Minimum, Conventional
39	van Groenigen et al. 2011	Ireland	Wheat	Temperate	
40	Zan et al. 2001	Canada	Grass, Maize	Temperate	Conventional
41	Zhang et al. 2015	China	Maize	Sub-tropical	
42	Ziska and Teratnura 1992	USA	Rice		

Chapter 2 Selection of wheat genotypes for biomass allocation to improve drought tolerance and biomass allocation

Abstract

The biomass allocation pattern of plants is key in the cycle of elements such as carbon, water and nutrients with the greatest allocations to roots fostering the transfer of atmospheric carbon to soils through photosynthesis. Several studies have investigated the root to shoot ratio (RS) biomass of existing crops but variation within a crop species constitutes an important information gap for selecting genotypes aiming for improving drought tolerance and carbon sequestration. The objectives of this study were to evaluate biomass allocation between roots and shoots and agronomic performance of wheat in response to different soil water levels to select promising genotypes for breeding. Ninety-nine wheat genotypes and triticale (control) were evaluated under drought-stressed and non-stressed conditions in the field and greenhouse using a 10×10 alpha lattice design with two replications. The following phenotypic traits were collected: number of days to heading (DTH), number of productive tillers per plant (NPT), plant height (PH), days to maturity (DTM), spike length (SL), spikelets per spike (SPS), thousand kernel weight (TKW), root biomass (RB), shoot biomass (SB), root to shoot ratio (RS) and grain yield (GY). There was significant ($p<0.05$) genotypic variation for grain yield and biomass production. The highest grain yield of 247.3 g m^{-2} was recorded in the genotype LM52 and the least was in genotype Sossognon with 30 g m^{-2}. Shoot biomass ranged from 830g m^{-2} (genotype Arenza) to 437 g m^{-2} (LM57), whilst root biomass ranged between 140 g m^{-2} for LM15 and 603 g m^{-2} for triticale. Triticale also recorded the highest RS of 1.2, while the least was 0.30 for LM18. Overall, water stress reduced total biomass production by 35% and RS by 14%. Genotypic variation existed for all measured traits useful for improving drought tolerance, while the calculated RS values can improve accuracy in estimating C sequestration potential of wheat. Wheat genotypes LM26, LM47, BW140, LM70, LM48, BW152, LM75, BW162, LM71 and BW141 were selected for further development based on their high grain and biomass production, low drought sensitivity and genetic diversity.

Keywords: Agronomic traits, Biomass partitioning, Genotype by environment interaction, Grain yield, Root to shoot ratio, Water stress

2.1 Introduction

Evaluating biomass allocation to plant roots, shoots and economic traits in plants can help to find new pathways to enhance drought tolerance in crops but to assess the impact of crop biomass on several ecosystem functions such as carbon, water and nutrient cycles, which affect crop production. Up to 80% of soil C comes from root activity and turnover (Yang et al. 2012); consequently the allocation of C to roots has important consequences for transfer of C for mitigating against climate change. Soil organic matter, which constitutes the bulk of soil C, retains essential nutrients, improves water holding capacity and provides energy for soil living organisms, all of which enhance soil ecosystem functioning and crop production.

Biomass allocation between roots and shoots, expressed as root to shoot ratio (RS), is highly variable amongst plant species. An RS ratio above unity shows that production of root biomass exceeded that of above ground biomass. The RS ratios reported for annual cereal crops such as wheat are comparably lower than for perennial grasses. For example, Amanullah and Stewart (2013) reported RS ratios of 0.41 and 0.29 for sorghum and maize, respectively, while Yang et al. (2010) reported a mean of 0.25 for maize and wheat. Bolinder et al. (2002) reported RS ratios of up to 7 for forage grasses compared to a range of 0.1 to 0.5 cited for many annual crops including cereals and legumes (Mathew et al. 2017). There are also variations in RS within single species which can be attributed to genotypic differences.

Intra-specific variation in any trait results from genotypic differences among individuals in a given species. King et al. (2007) asserted that there is wide genotypic variation for biomass allocation. Fang et al. (2017) found that a RS ratio of 1.13 in a wheat landrace compared with 0.61 and 0.81 found in two modern cultivars. Similarly, Siddique et al. (1990) reported higher RS ratios ranging between 0.74 and 1.18 for obsolete varieties than modern cultivars of wheat which ranged from 0.72 to 0.84. Most wheat improvement programs focus on channeling more biomass towards economic traits such as grain and above ground biomass for food, feed and biofuel production without improving root systems leading to low RS in modern cultivars compared to landraces (Wasson et al. 2012) and consequently yields have stagnated due to poor root performance (White et al. 2015). However, variations do occur even in genotypically identical individuals due to environmental factors.

Biomass allocation is also affected by genotype x environment interaction showing environmental plasticity to soil properties, temperature and soil water availability (Pittelkow et al., 2015; Sánchez et al. 2014). Drought stress is known to reduce crop growth. For example, Perdomo et al. (2015) reported biomass reductions of 60% in maize and rice, and 90% in wheat. However, drought stress tends to increase RS ratios as reported by Tatar (2016) who found 7% higher ratio in wheat produced under 25% soil water content than wheat grown at 75% soil water content. However, Vanaja et al. (2011) reported a contrasting trend when they recorded 60 and 7% reduction in RS for maize and sunflower, respectively, as a result of 30% reduction in soil water content. Biomass allocation also vary with intensity and duration of drought stress (Farooq et al. 2009). Instantaneous and short-lived drought spells may not cause a significant shift in biomass allocation, while excessive drought stress beyond a threshold level causes plants to lose their biomass allocation regulatory ability completely (Xu et al. 2010). Conversely, Sharp and Davies (1989) indicated that soil water stress has greater negative impact on shoot than root growth which led to a reduction in RS.

Several, studies have investigated variations in RS between plant species but little is known about intra-specific variations. This constitutes an important information gap when selecting crop varieties for specific objectives (e.g. grain or biomass production, soil C sequestration, drought tolerance). Moreover, in the context of global warming (Ashraf and Fooled 2007), there is need to investigate intra-specific variation in response to drought stress. Therefore, the objectives of this study were to characterize intra-specific variations in RS ratios and agronomic performance of diverse wheat genotypes sourced from the International Maize and Wheat Improvement Centre (CIMMYT) and subjected to water stressed and non-stressed conditions. The results can be helpful to crop breeders in evaluating diversity in biomass allocation and agronomic performance, which is important for developing varieties with greater water use efficiency and drought tolerance for grain yield and C sequestration into soils.

2.2 Materials and Methods

2.2.1 Plant materials

One hundred genotypes, consisting of 97 drought and heat tolerant winter wheat (*Triticum aestivum* L., 2n=6x=42) accessions, 2 commercial winter wheat varieties from France and triticale

(*Triticosecale* Wittmack) were evaluated (Appendix 2.1). The drought and heat tolerant genotypes were obtained from CIMMYT. The CIMMYT genotypes were used owing to their genetic variability for rooting abilities and breeding history for drought tolerance. The commercial varieties and Triticale were used as comparative controls because they are known for their high rooting capacities. The French varieties, which are winter wheat genotypes, have twice their rooting capacity of wheat grown in warmer winters (Thorup-Kristensen et al. 2009), while Triticale has an aggressive root system inherited from rye (*Secale cereale*) (Solomon et al. 2007). The purpose of including triticale and the two commercial varieties was for their profuse rooting ability serving as a comparative controls to make inferences on whether their rooting ability can be related to drought tolerance under water limited condition. This may allow to select complementary genotypes for future crosses to improve rooting capacity and grain yield.

2.2.2 Greenhouse experiments

Two experiments were carried out in a greenhouse at the Controlled Environment Facility of the University of KwaZulu Natal Pietermaritzburg Campus. The first greenhouse experiment (GH1) was carried out in summer (October 2016 to February 2017), while the second one (GH2) was conducted in winter (May to September 2017) to represent two environments. The experiments were conducted using a 10×10 alpha lattice design with two replications. Ten seeds were sown in each pot and thinned to 8 plants per pot, 3 weeks after emergence. Ten pots were allocated per incomplete block and genotypes were randomly assigned to pots to minimize the experimental error associated with water discharge from the drip irrigation. The greenhouse provided shelter against rainfall and irrigation was provided via an automated drip irrigation system inserted directly into individual pots. Fertilizer was also applied through automated drip irrigation at a rate of 300 kg N ha^{-1} and 200 kg P_2O_5 ha^{-1}. The different water regimes were initiated 6 weeks after planting to ensure good establishment but also to ensure early exposure of all growth stages to drought. In the non-stress condition, the plants were watered to field capacity (FC) whenever average soil water content fell to 80% of FC, while in the water stress conditions volumetric soil water content was allowed to drop to 30% of FC before watering to FC. The soil water content was monitored by a soil moisture probe and weighing of the pots. The two watering treatments were maintained until maturity (~120 days).

2.2.3 Field experiment

The third experiment was carried out in the field at the University of KwaZulu Natal's Ukulinga Research farm (LAT: 29.667⁰ LON: 30.406⁰ and ALT: 811 m), to represent a third environment. The experiment was also set up using a 10×10 alpha lattice design with two replications. Long-term average temperature and rainfall for Ukulinga are 18ºC and 738mm, respectively. The average temperature during the growing period and soil properties are given in Table 2.1. The field was ploughed in May 2017 during the winter season where rainfall is minimal. The soil surface was covered by a custom made plastic, which acted as a mulch to prevent rain water from entering into the soil. Small holes of ~5cm diameter were drilled on the plastic mulch on the ridge for planting. The holes were progressively covered by the plant canopy after germination and over the growth period. Any rainwater that might possibly have trickled down the plants and dripped into the soil through the holes was negligible. Three seeds were planted per station at 10cm intra-row spacing and 30cm between rows soon after ploughing. Each row consisted of 10 genotypes and was treated as an incomplete block. Basal fertilizer composed of nitrogen (N), phosphorous (P) and potassium (K) was applied at a rate of 120:30:30 kg ha^{-1} (N:P:K). Other agronomic practices were as per normal wheat production practice in South Africa (DAFF 2010). Irrigation was applied through a drip irrigation system with the aim to maintain soil water content at FC in the well-watered regime. Under the drought stress treatment, irrigation was withheld 5 weeks after crop emergence until just before signs of permanent wilting were observed upon which irrigation was reinstated. This differs from the 80 and 30% FC soil water regimes maintained in the greenhouse because it is more difficult to determine field capacity and regulate soil water content appropriately under field conditions compared to a controlled greenhouse environment. During the field experiment, irrigation was withheld before anthesis to induce drought stress in a way that simulated *in situ* wheat production under field conditions. Amount of water applied and prevailing temperatures were recorded for the period to determine the extent of drought stress.

Table 2.1 Soil properties and mean temperatures for the environments used in this study

Property	First greenhouse experiment	Second greenhouse experiment	Field experiment
Bulk density	0.75	0.99	1.04
Phosporous (mg/L)	122	29.0	39.0
PotassiumK (mg/L)	289	412	241
Calcium (mg/L)	1906	1386	1453
Magnesium (mg/L)	404	504	369
Electrical Conductivity (cmol/L)	13.7	12.2	11.0
pH (KCl)	5.09	5.31	4.56
Organic carbon (%)	5.50	3.40	2.60
Nitrogen (%)	0.48	0.29	0.23
Clay (%)	16.0	33.0	28.0
Mean Temperature (°C)	25.7	20.1	16.6

pH (KCl)=pH measured on the potassium chloride scale

2.2.4 Data collection

Agronomic traits were recorded during the growth period. These traits include days to heading (DTH), recorded as the number of days from date of planting to the date when 50% of plants in a single plot had fully emerged spikes. Days to maturity (DTM) were recorded as the number of days from planting to the day when 50% of the plants were dry. The number of productive tillers (NPT) were counted per plant and plant height (PH) expressed in centimeters was measured as the average of three measurements from three main tillers from the soil surface to the tip of the spike excluding awns at maturity. Spike length was measured from the base of the spike to the tip excluding awns, averaged across five randomly selected spikes. Plant parts for each plot and pot were separated at maturity into grain, shoot and root. The above ground biomass was cut off at the soil surface to separate from below ground biomass. A 30×30×30cm sampling box was sunk to a depth of 60cm and all the roots within the soils volume were collected per genotype per plot. All root biomass for all the experiments was separated from the soil in a two-step procedure adapted from Hirte et al. (2018). The sampled soil volume (from a monolith box or a pot) was passed through a 2mm sieve and the remaining large roots were collected. The residue which passed through the sieve was washed under running water to dissolve the soil and sieved through a 0.5mm sieve to collect the fine roots. All roots were cleaned from the soil as much as possible. The separated plant parts were oven dried at 60°C for 72 hours to measure the dry weight. The weight was converted to gram per square meter (gm^{-2}) accordingly using the plant population of 128 and 134 plants per square meter for the greenhouse and field experiments, respectively. Root: shoot (RS) ratio and total biomass (PB) were computed after determining grain yield (GY), root biomass (RB), and shoot biomass (SB). Two hundred and fifty kernels of wheat for each genotype were weighed in grams and the weight was multiplied by 4 to obtain the thousand kernel weight (TKW). The dry shoot and root biomass were analyzed for carbon content by combustion in the carbon and nitrogen analyzer (LECO CN628) and C stocks were calculated as the product of C content and average biomass of the respective plant part.

2.2.5 Data analysis

Drought is more qualitative rather than a quantitative condition and is rather difficult to define, but in the context of crop production it is the shortage of water to support plant growth (Gulácsi and Kovács 2015). As such, the extent of drought in the field was determined by the Hydro-thermal Coefficient of Selyaninov (HTC) calculated following formula adapted from Evarte-Bundere and Evarts-Bunders (2012) based on Selyaninov (1928):

$$HTC = \frac{\sum x}{(\sum t) * 10}$$

where Σ x and Σ t are respectively sum of precipitations and temperatures in the period, when the temperature was above 10^{o} C whereby 1.0<HTC<2.0=sufficiently humid; 0.7<HTC<1.0=dry and 0.4<HTC<0.7=very dry.

Growth degree days (GDD) were calculated from daily average and base temperatures. The growth degree days were calculated as follows (Grzesiak 2001):

$$GDD = \sum_{n1+n2+\cdots .nn} [\frac{Tmax - Tmin}{2}] - Tb$$

where T_{max} and T_{min} are maximum and minimum daily temperatures, respectively; Tb=the base temperature (assumed to be 8.5 ^{o}C for wheat following Angus et al. 1981); n=the number of days when mean daily temperature was above base temperature.

Analysis of variance (ANOVA) was conducted using the lattice procedure using Genstat 18th edition (Payne et al. 2017). In addition, the means of genotypes and the different water regimes were separated by Fischers' unprotected least significant difference (LSD) at 0.05 significance level to quantify the effects of genotype, environment and water regime. The bivariate correlations among biomass and agronomic traits were analyzed by the Spearman rank correlations procedure, while multivariate correlations were analyzed by the principal component procedure. A multivariate procedure for hierarchical clustering was performed based on phenotypic data combined across water regimes and sites to group the genotypes for their similarity. A dendogram was derived from a Euclidean similarity matrix using the Unweighted Pair Group Method with Arithmetic mean algorithm (UPGMA). Drought sensitivity index (DSI) for each genotype was calculated from grain yield following Grzesiak (2001) using the following formula:

$$DSI = [1 - \left(\frac{Yds}{Yns}\right)] * DS^{-1}$$

$$DS = \frac{[\sum(H_2O_{ns}) - \sum(H_2O_{ds})]}{\sum(H_2O_{ns})}$$

Where Yds and Yns are yield of a particular genotype under drought-stressed and non-stressed conditions, respectively; DS is the intensity of the drought; $\Sigma(H_2O_{ns})$ and $\Sigma(H_2O_{ds})$ are total amount of water applied under non-stressed and drought-stressed treatments, respectively. Selection of genotypes for further development was based on hierarchical clustering, grain yield and biomass production and drought sensitivity index (DSI) in each cluster to capture as much diversity as possible, high performance and least reduction in grain yield due to drought.

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2.3 Results

2.3.1 General statistics

A hydro-thermal coefficient (HTC) of 1.49 indicated that the non-stressed treatment received sufficiently high amount of water compared to an HTC of 0.39 representing very dry conditions in the drought stress treatment (Table 2.2). Results show wide variability in genotype response to water and environmental conditions (Table 2.3). There was wide variability in rate of physiological development expressed as days to heading (39 to 138) and maturity (66 to 148). Root and shoot biomass also varied widely showing coefficient of variation (CV) of 79 and 110%, respectively. Biomass allocation between roots and shoots expressed as RS ratio ranged from 0.03 to 3.04. Grain yield varied from 9.9 to 4696 g m^{-2} showing a positive skewness, while total biomass ranged between 81.4 and 13529 g m^{-2}. Drought sensitivity index ranged from 0.03 to 0.9 with a mean of 0.45.

Table 2.2 Mean temperature, cumulative water applied and evapotranspiration during the growth period under field conditions

Date	Mean Temp oC	Non-stressed		Stressed	
		Cumulative water applied (mm)	Evapotranspiration	Cumulative water applied (mm)	Evapotranspiration
06-06-2017	12.36	123.33	64%	121.33	63%
15-06-2017	11.78	247.11	70%	235.22	65%
23-06-2017	11.52	363.36	66%	347.72	64%
30-06-2017	12.80	1396.93	42%	422.01	42%
07-07-2017	13.01	1499.79	61%	496.29	44%
14-07-2017	13.55	1594.08	45%	496.29	0%
28-07-2017	14.66	1654.50	31%	496.29	0%
04-08-2017	14.68	1779.36	62%	496.29	0%
11-08-2017	15.74	1961.79	87%	496.29	0%
18-08-2017	14.24	2206.50	139%	496.29	0%
25-08-2017	14.03	2462.08	113%	496.29	0%
01-09-2017	16.37	2777.93	139%	496.29	0%
08-09-2017	17.46	3079.36	90%	590.58	28%
15-09-2017	19.60	3439.36	130%	1074.87	175%
22-09-2017	19.61	3825.08	153%	1074.87	0%
29-09-2017	16.58	4084.22	62%	1076.29	1%
06-10-2017	17.70	4241.36	22%	1076.29	0%
13-10-2017	16.78	4338.50	0%	1076.29	0%
20-10-2017	19.17	4395.65	0%	1076.29	0%
Total	291.64	4396		1076	
HTC		1.49		0.37	

HTC= Hydro-thermal Coefficient of Selyaninov formula taken from Evarte-Bundere and Evarts-Bunders (2012) where 1.0<HTC<2.0=sufficiently humid; 0.7<HTC<1.0=dry and 0.4<HTC<0.7=very dry

Table 2.3 Summary statistics of biomass and agronomic traits measured in 100 genotypes across environments and water regimes

Statistic	**DTH**	**NPT**	**PH**	**DTM**	**SL**	**SB**	**RB**	**PB**	**RS**	**TKW**	**DSI**	**GY**
Mean	66	11	72	113	7.73	1061	259	1899	0.47	44.2	0.45	600
Median	64	10	69	108	8	551	249	1220	0.52	44.6	0.46	267
Minimum	39	1	23	66	2.5	26	6	81.4	0.03	18.2	0.03	9.94
Maximum	138	31	121	148	15	8658	1622	13529	3	64	0.9	4696
Quartile 1	53	8	59	101	5.17	114	75.7	272	0.15	40.8	0.29	93.9
Quartile 3	77	13	81	128	10	1829	375	3025	0.71	48.6	0.62	959
Standard deviation	13.8	4.06	18.9	16.9	2.8	1168	205	1895	0.31	6.97	0.22	695
Standard error of mean	0.4	0.12	0.55	0.5	0.08	33.9	5.93	54.9	0.01	0.25	0.01	20.5
Coefficient of variation (%)	21	37	26.5	15	36.1	110	79	99.8	66.5	15.8	47.2	116
Skewness	0.42	1.13	0.41	0.15	0.12	1.5	1.4	1.42	0.84	-1.46	-0.1	1.77
Kurtosis	0.13	1.33	-0.27	-0.89	-1.01	2.78	4.2	2.58	3.96	7.63	-0.9	3.94

DTH=number of days to 50% heading; NPT=number of productive tillers; PH=plant height; DTM=number of days to maturity; SL=spike length; SB=shoot biomass dry weight per m^{-2}; RB=root biomass dry weight per m^{-2}; PB=total plant biomass dry weight per m^{-2}; RS=root to shoot ratio; TKW=thousand kernel weight (g 1000^{-1} seed); DS=drought sensitivity index; GY=grain weight gm^{-2}

2.3.2 Genotypic variation in biomass allocation and agronomic performance

A combined analysis of variance revealed that genotype effects were significant ($p<0.05$) for agronomic traits and biomass (GY, PB, SB and RB) and RS (Table 2.4). In addition, genotypes interacted with water regimes with significant effect on GY and RS but non-significant for SB and RB. Genotype by environment interaction was significant for biomass (GY, PB, SB and RB) and RS, while the 3 way interaction was non-significant for shoot SB and RS. Further, genotype interactions with water regime and environment significantly influenced the complementary agronomic traits. Genotype by water regime interaction effect was significant for DTH, DTM and PH only, while genotype by environment interaction was significant for all the other agronomic traits except TKW (Table 2.4).

Table 2.4 Mean squares after combined analysis of variance for phenotypic traits of 100 wheat genotypes evaluated across the three test environments and two water regimes

Change	DF	DTH	NPT	PH	DTM	SL	SB	RB	RS	TKW	GY
Replication (Rep)	1	205	0.37	35.03	181	10.4	3190585	13931	0.07	98.6	1547987
Rep.Block	18	98.8	10.2	148.4	134	2.54**	236360	18521*	0.10*	60.7**	117122*
Genotype (Gen)	99	317***	13.9***	481***	175.27***	4.30***	556472***	52063***	0.06****	117***	101673**
Environment (Env.)	2	64335***	2987***	126114***	107577***	3382***	633806324***	14483166***	34.5***	9.14	214902285***
Water regime (WR)	1	1290***	2962***	44418***	39924***	420***	43087351***	2835547***	1.12***	3115***	43755727***
Gen.Env	192	107***	10.1***	99.8***	82.3***	1.94***	341187***	15459***	0.04***	38.8*	59370
Gen.WR	96	32.07	5.74	51.2	67.2***	0.87	167573	11016	0.03	28.36	43059
Env.WR	2	2449***	505***	7086***	348.2***	65.5***	12729698***	120902***	1.13***	1501***	486813***
Gen.Env.WR	192	28.61	8.19***	52.9*	52.9**	0.88	149771	9116	0.03*	29.79	58283
Residual	563	26.07	5.29	43.2	40.7	0.91	163807	10428	0.03	28.6	68604
Mean		65.5	11.0	71.5	113	7.74	1061	259	0.47	44.2	600
%CV		7.79	20.9	9.19	5.67	12.3	38.1	39.4	35.8	12.1	43.7
se		5.11	1.30	4.57	6.38	0.95	105	32.1	0.07	2.35	51.9
GCV		71.4	74.4	79.2	52.9	67.0	55.1	72.6	52.9	53.7	43.1
PCV		72.8	78.6	80.0	53.3	69.0	57.6	75.1	83.8	54.7	49.5

DF=degrees of freedom, DTH=days to heading, NPT=number of productive tillers, PH=plant height, DTM=days to maturity, SL=spike length, SB=shoot biomass weight, RB=root biomass weight, RS=root to shoot ratio, TKW=thousand-grain weight, GY=grain yield, CV=coefficient of variation, se=standard error, GCV=genetic coefficient of variation, PCV=phenotypic coefficient of variation, *, ** and ***=significance level at <0.05, <0.01 and <0.001, respectively.

2.3.3 Cluster analysis

The dendogram resulting from the UPGMA revealed two major distinct clusters of the 100 genotypes based on their similarity in agronomic performance (Fig 2.1). Member genotypes for each cluster are presented in Table 2.5. The first cluster comprised of 97 genotypes that were further divided into subgroups A and B at 0.95 similarity. They were all from CIMMYT heat and drought tolerant genotypes except LM70, which was a local line. The sub-cluster A was further divided into 4 clusters, which was comprised of genotypes such as LM26, BW141, BW140, LM70 and LM48. Sub-cluster B was further divided into 2 clusters with genotypes such as BW152, LM47, LM75, BW162 and LM71. Cluster 2 comprised of three genotypes, Triticale, Sossognon and Arenza. After clustering, the genotypes were ranked based on the total biomass production under drought-stressed condition and their drought sensitivity index. The best performing genotypes with high biomass production and low DSI in each cluster were selected for further study.

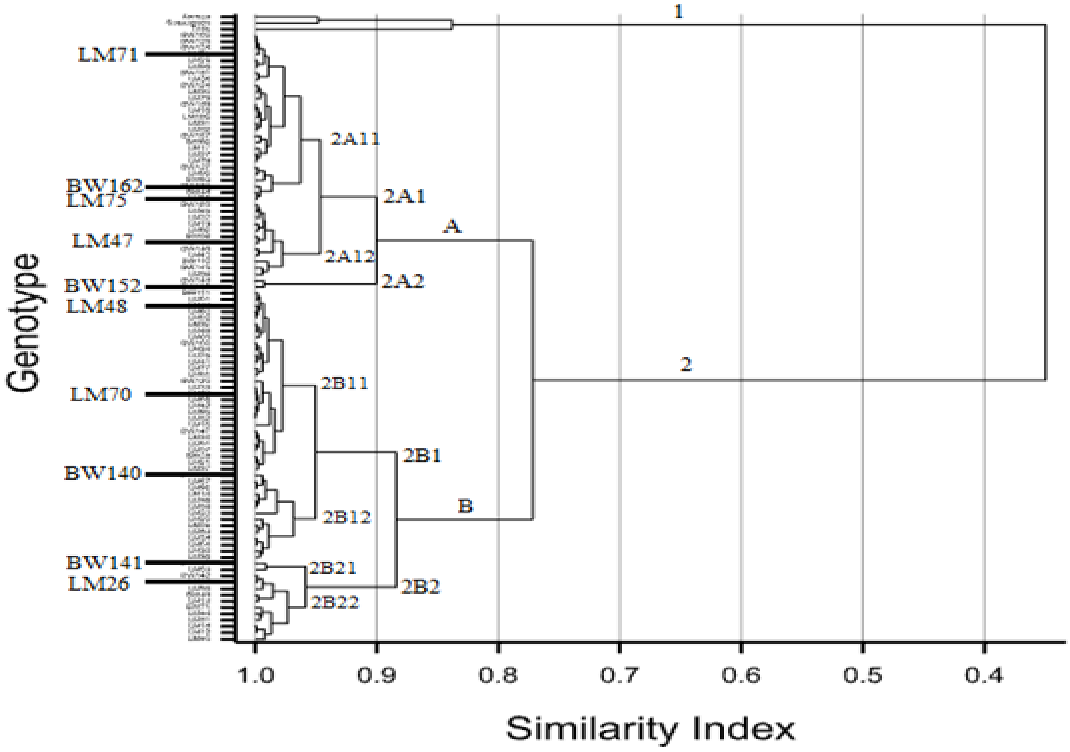

Fig 2.1 Dendogram showing the clustering of 100 genotypes evaluated across stress and non-stressed conditions. The 10 highlighted genotypes were selected for high biomass, low DSI and diversity under drought-stressed conditions

Table 2.5 Clustering of the 100 genotypes based on phenotypic similarity across the test environments and drought-stressed and non-stressed conditions

Cluster	No. of Genotypes	Name of genotypes and DSI*					Selected genotypes
A11	11	BW142 (0.52)	LM26 (0.22)	LM99 (0.52)	BW49 (0.48)		LM26
		LM12 (0.51)	BW71 (0.37)	LM44 (0.52)	LM51 (0.41)		
		LM18 (0.42)	LM72 (0.33)	LM40 (0.56)			
A12	2	BW141 (0.22)	LM50 (0.41)				BW141
A22	29	LM37 (0.38)	LM21 (0.54)	BW28 (0.48)	LM97 (0.49)	LM91 (0.40)	LM70 LM48
		LM35 (0.56)	BW147 (0.34)	LM15 (0.49)	LM82 (0.53)	LM60 (0.43)	
		LM42 (0.44)	LM56 (0.42)	LM70 (0.27)	LM39 (0.48)	BW120 (0.43)	
		LM81 (0.53)	LM77 (0.50)	LM41 (0.52)	LM25 (0.52)	LM24 (0.49)	
		BW150 (0.51)	LM85 (0.39)	LM49 (0.38)	LM32 (0.46)	LM93 (0.42)	
		LM83 (0.51)	LM48 (0.28)	LM01 (0.46)	BW111 (0.44)		
A21	14	LM38 (0.44)	LM33 (0.42)	LM54 (0.46)	LM28 (0.62)	LM84 (0.52)	BW140
		LM59 (0.53)	LM20 (0.53)	LM23 (0.40)	LM86 (0.56)	LM55 (0.61)	
		LM14 (0.42)	LM98 (0.47)	LM57 (0.51)	BW140 (0.20)		
B1	2	BW148 (0.46)	BW152 (0.27)				BW152
B21	12	BW103 (0.51)	LM46 (0.42)	LM22 (0.38)	LM19 (0.36)		LM47
		LM80 (0.55)	BW58 (0.47)	LM47 (0.27)	BW145 (0.54)		
		LM43 (0.52)	BW116 (0.46)	BW149 (0.59)	LM96 (0.49)		
B22	27	LM79 (0.45)	BW157 (0.50)	LM16 (0.46)	BW124 (0.42)		LM71 LM75 BW162
		LM27 (0.44)	LM52 (0.64)	BW159 (0.55)	LM36 (0.49)		
		LM17 (0.47)	LM31 (0.44)	LM76 (0.29)	BW151 (0.51)		
		BW80 (0.51)	LM100 (0.43)	LM30 (0.44)	LM58 (0.47)		
		LM29 (0.41)	LM71 (0.29)	BW128 (0.47)	BW129 (0.51)		
		BW100 (0.40)	LM75 (0.29)	BW48 (0.43)	BW162 (0.27)		
		BW63 (0.45)	LM90 (0.38)	BW127 (0.52)			
C	3	Triticale (0.47)	Sossognon	Arenza			

*numbers in parentheses indicate the drought sensitivity index (DSI)

2.3.4 Impact of water regime on biomass and agronomic performance

The performance of the 100 genotypes under each soil water regime across the different environments were recorded. Drought stress reduced the number of days to heading and maturity compared to non-stress condition (Table 2.6). Mean DTH was 65 days when plants were subjected to drought stress compared to 66 under non-stress condition. Similarly, days to maturity were reduced to 107 under drought stress relative to 118 observed under non-stressed condition. Plant height showed significant differences between the contrasting soil water regimes with a mean of 78 cm under non-stress condition compared to 65 cm under drought-stressed conditions (Table 2.6).

Mean shoot biomass was 1262 g m^{-2} under non-stressed condition (Table 2.6). Genotype BW152 exhibited the highest shoot biomass of 1260 g m^{-2} under stressed condition, while LM30 had the highest reduction (61%) in shoot biomass due to drought stress. On average, shoot biomass was reduced by 28% under drought stress relative to non-stressed condition (Fig 2.2a). On average, root biomass was 308 g m^{-2} under non-stressed compared to 209 g m^{-2} attained under drought stress conditions (Table 2.6). LM71 produced the lowest root biomass of 218 gm^{-2} under non-stressed condition, while LM54 had the lowest under drought stress conditions. Overall, drought stress significantly reduced root biomass by 23% compared to non-stressed condition (Fig 2.2b). Biomass allocation between roots and shoot (RS) was not significantly different between stressed and non-stressed conditions (Table 2.6). Under non-stressed condition, RS ranged between 0.39 and 1.48 compared to 0.35 and 0.68 under drought-stressed condition. Drought stress reduced mean RS ratios across genotypes by 40% compared to non-stressed condition (Fig 2.2c).

The mean drought sensitivity index was 0.45, while the top 10 ranked genotypes exhibited DSI below 0.30 (Table 2.6). The mean grain yield under non-stress condition was 756 g m^{-2} compared to 444gm-2 under drought stress condition. The genotypes exhibited wide variation in grain accumulation under both soil water regimes. Under non-stressed condition, grain yield ranged from 479 to 1212 gm-2 compared to 198 to 870 gm-2 recorded under drought stress condition. On average, drought stress reduced grain yield by 35% across the different soil water regimes (Fig 2.2d).

Table 2.6 Mean values for biomass and agronomic traits of 100 genotypes showing the top 10 and bottom 5 ranked genotypes across environments, ranked according to total biomass under drought stress conditions

		DTH		**NPT**		**PH**		**DTM**		**RB**		**SB**		**RS**		**SL**		**TKW**		**GY**		**DSI**
	Genotype	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	
Top 10 genotypes	BW140	56	57	8	11	67	77	104	116	249	344	964	1181	0.48	0.49	8	9	46	51	631	761	0.2
	BW141	55	57	9	10	68	77	97	109	218	369	1111	2426	0.45	0.5	8	8	42	45	844	1213	0.22
	LM26	66	66	8	13	57	71	105	119	263	255	936	965	0.5	0.46	8	8	36	35	692	962	0.22
	BW152	65	66	11	13	67	81	104	117	272	272	1260	1694	0.5	0.48	8	8	51	52	959	1278	0.27
	LM47	58	58	8	12	65	76	97	113	249	453	1060	1266	0.42	0.6	7	8	39	43	630	957	0.27
	BW162	64	61	11	14	66	78	109	118	186	325	1109	1291	0.42	0.45	7	8	41	47	760	1040	0.27
	LM70	65	64	9	12	63	74	107	115	290	429	1077	1682	0.49	0.57	8	8	39	44	888	1126	0.27
	LM75	72	67	9	13	68	86	112	121	210	264	1050	1424	0.38	0.5	8	9	49	55	763	1048	0.28
	LM48	61	64	8	13	67	81	99	119	255	306	1042	1378	0.35	0.48	8	9	40	43	566	724	0.28
	LM71	63	69	10	17	61	73	107	119	214	218	987	994	0.43	0.41	7	7	42	51	898	1211	0.29
Bottom 5 genotypes	BW100	68	65	11	16	59	66	111	118	159	310	665	1168	0.44	0.49	6	6	41	41	293	501	0.4
	LM30	48	55	11	12	69	82	91	108	249	295	540	1370	0.64	0.49	8	9	43	48	418	930	0.44
	LM54	66	66	9	12	60	79	110	121	132	425	778	1739	0.32	0.57	7	7	44	47	214	792	0.46
	BW142	70	75	8	13	64	74	114	120	177	342	636	1468	0.41	0.47	6	8	43	47	299	828	0.52
	LM28	72	68	9	13	62	76	111	119	187	277	710	1337	0.4	0.44	7	8	40	48	269	692	0.62
	Mean	65	66	9	13	65	78	107	118	209	308	855	1262	0.43	0.5	7	8	42	46	435	789	0.45
	SE	8	8	1	1	2.3	2.3	10	10	34	34	61.5	61.5	0.06	0.06	0.3	0.3	6	6	45	45	0.2
	LSD (5%)	16	16	1.9	1.9	4.5	4.5	19	19	66	66	119.1	119.1	0.12	0.12	0.7	0.7	6.3	6.3	85	85	0.17
	CV (%)	6.2	6.2	21	21	7.8	7.8	4.3	4.3	42	42	28	28	31	31	10.8	10.8	13	13	35	35	47

DTH=number of days to 50% heading; DTM=number of days to maturity; NPT=number of productive tillers; PH=plant height cm; SL=spike length; SB=shoot biomass dry weight gm^{-2}; RB=root biomass dry weight gm^{-2}; RS=root to shoot ratio; TKW=thousand kernel weight g1000^{-1} seeds; GY=grain weight gm^{-2}; DSI=drought sensitivity index; DS=drought-stressed treatment; NS=non-stressed treatment; SE=standard error; LSD=least significant difference at 0.05; CV=coefficient of variation

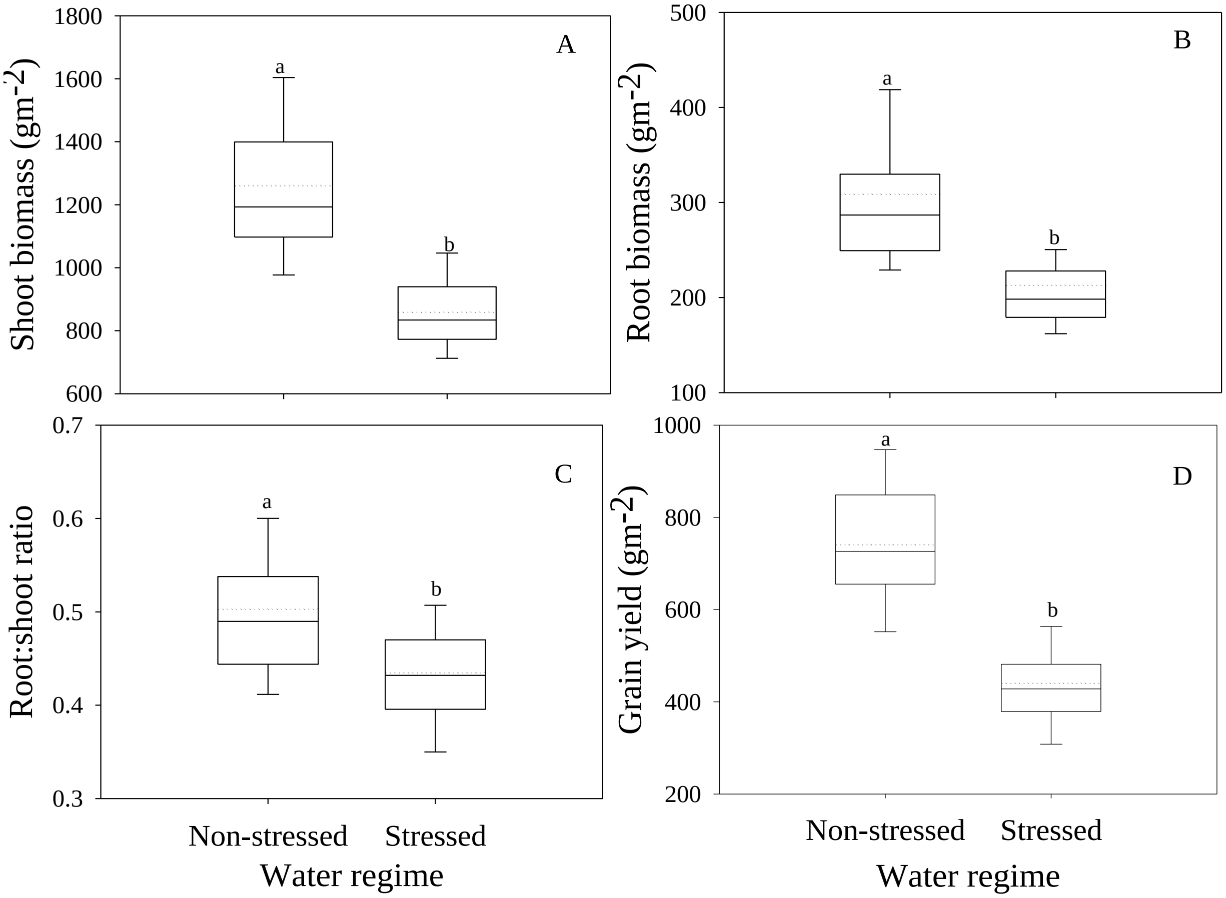

Fig 2.2 Boxplots showing differences in means of A) grain yield B) shoot C) root and D) RS means of 100 wheat genotypes evaluated under soil water stress and non-stress conditions across environments.

Dotted line represents mean value and different letters above whiskers show significant differences at the 0.05 probability level

2.3.5 Principal component analysis

Under non-stressed conditions, two principal components (PCs) were identified with Eigenvalues >1. The first PC was the most important as it accounted for 78% of the total variation. This PC well correlated with DTM, PB, SB, PH, SL and DTH (Table 2.7). Root to shoot ratio (RS) and TKW were negatively associated with PC1. The second PC only accounted for 8% of the variation. Under drought stress condition, five PCs with Eigen values >1 were identified (Table 2.7). The five PCs explained a cumulative 83% of the total variation observed in the data (Table 2.7). The first PC accounted for 32% of the total variation, which was contributed mostly by the biomass variables (GY, RB and SB). All traits showed positive correlations with PC1 except DTH and DTM. The second PC accounted for 17% of the variation and can be regarded as an axis of phenology as it was strongly correlated to DTM and DTH. The other three PCs accounted for the remaining 12, 11.6 and 10% of the variation. The third PC was closely related to SL, PH and SPS, while PC4 and 5 showed weaker correlations with the remaining variables.

Table 2.7 Matrix showing principal component scores and variance of traits measured in 100 genotypes across environments under drought-stressed and non-stressed condition

Stressed													
PC	DTH	NPT	PH	DTM	RB	SB	RS	SL	TKW	GY	Eigen Value	% of Variance	Cumulative %
1	-0.07	0.13	0.13	-0.03	0.19	0.2	0.08	0.12	0.04	0.22	3.86	32.18	32.18
2	0.38	-0.09	-0.02	0.35	0.22	0.17	0.16	-0.25	-0.002	-0.02	2.05	17.05	49.23
3	0.05	-0.35	0.32	0.25	0.09	-0.12	0.19	0.35	0.21	-0.22	1.46	12.17	61.4
4	0.15	-0.09	0.24	0.17	-0.35	0.26	-0.58	0.01	0.24	0.08	1.39	11.61	73.01
5	0.15	0.07	-0.25	0.25	-0.05	0.15	-0.169	0.24	-0.63	-0.11	1.24	10.37	83.38
Non stressed													
PC	DTH	NPT	PH	DTM	RB	SB	RS	SL	TKW	GY	Eigen Value	% of Variance	Cumulative %
1	0.1	0.09	0.1	0.1	0.08	0.1	-0.1	0.1	-0.04	0.1	9.34	77.8	77.8
2	0.08	-0.15	0.07	-0.01	-0.15	0.1	-0.01	-0.35	-0.02	0.08	1	8.05	85.9

PC=principal component; DTH=number of days to 50% heading; DTM=number of days to maturity; NPT=number of productive tillers; PH=plant height cm; SL=spike length; SB=shoot biomass dry weight gm^{-2}; RB=root biomass dry weight gm^{-2}; PB=total plant biomass dry weight gm^{-2}; RS=root to shoot ratio; TKW=thousand kernel weight g1000^{-1} seeds; GY=grain weight gm^{-2}

2.3.6 Correlation analysis

The correlation coefficients (r) among the variables are presented in Table 2.8 based on combined data from the different test environments. Under non-stressed condition, agronomic traits which were moderately correlated with RS were PH (r=-0.29, $p<0.05$), SL (r=-0.22, $p<0.05$) and KPS (r=-0.25, $p<0.05$) (Table 2.8, top diagonal). DTH (r=-0.22, $p<0.001$), NPT (r=0.14, $p<0.05$), TPP (r=0.20, $p<0.01$) and TKW (r=0.20, $p<0.001$) were associated with GY. Grain yield (GY) was strongly correlated ($p<0.001$) SB (r=0.54) and moderately with RB (r=0.27) (Table 2.8, top diagonal). GY and SB did not exhibit significant correlations with RS, whereas RB was strongly correlated with RS (r=0.78, $p<0.05$). Drought sensitivity index (DSI) showed non-significant correlations with any of the traits under non-stressed conditions.

Under drought stress condition, RB was moderately correlated with agronomic traits PH (r=0.26, $p<0.001$), NPT (r=0.25, $p<0.001$), SL (r=0.16, $p<0.001$) and KPS (r=0.21, $p<0.001$). Grain yield also exhibited moderate but stronger correlations with traits such as DTH (r=-0.26; $p<.001$) and NPT (r=0.44, $p<.001$) compared to the non-stressed condition. Only TKW exhibited weaker correlation with grain yield (r=0.16, $p<.001$) compared to the correlation observed under non-stressed condition. Biomass allocation under drought-stressed condition showed that grain yield exhibited stronger and highly significant ($p<.001$) correlations with SB (r=0.62), RB (r=0.46) and RS (r=0.12, $p<0.05$) (Table 2.8, bottom diagonal). Root to shoot ratio (RS) also exhibited 3% stronger correlations with RB (r=0.81, $p<0.05$) under drought stress. Shoot biomass (SB) exhibited insignificant associations with RS under drought stress. Shoot and root biomass were significantly correlated (r=0.55, $p<0.001$) but their association was 4% weaker compared to their association under non-stress conditions. Drought sensitive index exhibited significant and positive correlations with DTH, DTM NPT and PH, while its association with RS was negative.

Table 2.8 Pearson's correlation coefficients (r) showing the correlations of phenotypic traits of 100 genotypes evaluated across environments under water stress (below diagonal) and non-stress (above diagonal) conditions

	Non-stressed conditions										
Traits	**DTH**	**DTM**	**NPT**	**PH**	**RB**	**SB**	**RS**	**SL**	**TKW**	**DSI**	**GY**
DTH		0.42***	-0.001	0.27***	0.03	0.03	-0.001	0.01	-0.14	-0.01	-0.22***
DTM	0.59***		0.13	0.25***	0.18**	0.35***	-0.07	0.01	-0.08	-0.04	-0.08
NPT	-0.21***	-0.27***		-0.03	0.13	0.19**	0.04	-0.05	-0.002	0.04	0.14*
PH	-0.1	0.04	0.07		-0.12	0.17*	-0.29***	0.28***	0.24***	0	0.09
RB	0.04	0.13	0.25***	0.26***		0.55***	0.78***	0.16*	0.12	0.1	0.27***
SB	0.1	0.20***	0.31***	0.33***	0.59***		0.004	0.15*	0.07	0.04	0.54***
RS	0.003	0.05	0.06	0.08	0.81***	-0.02		0.05	0.11	0.09	-0.03
SL	-0.34***	-0.14*	0.11	0.31***	-0.12	0.08	-0.22***		0.11	-0.02	0.08
TKW	-0.06	-0.03	-0.09	0.40***	0.04	0.04	0.03	0.07		0.08	0.27***
DSI	0.65***	0.64***	0.50***	0.61***	0.53	0.61	-0.50***	0.61	0.17*		0.1
GY	-0.26***	-0.23***	0.44***	0.29***	0.46***	0.62***	0.12*	0.21***	0.16***	-0.26	
	Stressed conditions										

DTH=number of days to 50% heading; DTM=number of days to maturity; NPT=number of productive tillers; PH=plant height cm; RB=root biomass dry weight gm^{-2}; SB=shoot biomass dry weight gm^{-2}; RS=root to shoot ratio; SL=spike length; TKW=thousand kernel weight g1000^{-1} seeds; DSI=drought sensitivity index; GY=grain weight gm^{-2}; Above diagonal=correlations under non stress treatment, below diagonal=correlations under water stress. *, **, ***=level of significance at 0.05, 0.01 and ≤0.001

2.3.7 Plant and root carbon stocks

The selected genotypes were analyzed for their carbon content. C stocks were estimated as the product of C content and biomass (Fig 2.3A). The total plant carbon stock varied from 1100 g C m^{-2} (genotype LM26) to 1700 g C m^{-2} (BW162). Four genotypes including X, Y, Z and W exhibited significantly ($p<0.05$) higher C stocks. Six genotypes: BW152, LM26, BW141, LM71, LM48 and LM47 had the lowest plant C stock without showing significant differences. Root C stocks followed a similar trend to that of total C stocks. Root C varied from 330 g C m^{-2} recorded in genotype LM26 to 650 g C m^{-2} in BW162 (Fig 2.3B). Genotypes BW140 and BW162 exhibited significantly ($p<0.05$) higher root C stocks than the rest of the test genotypes.

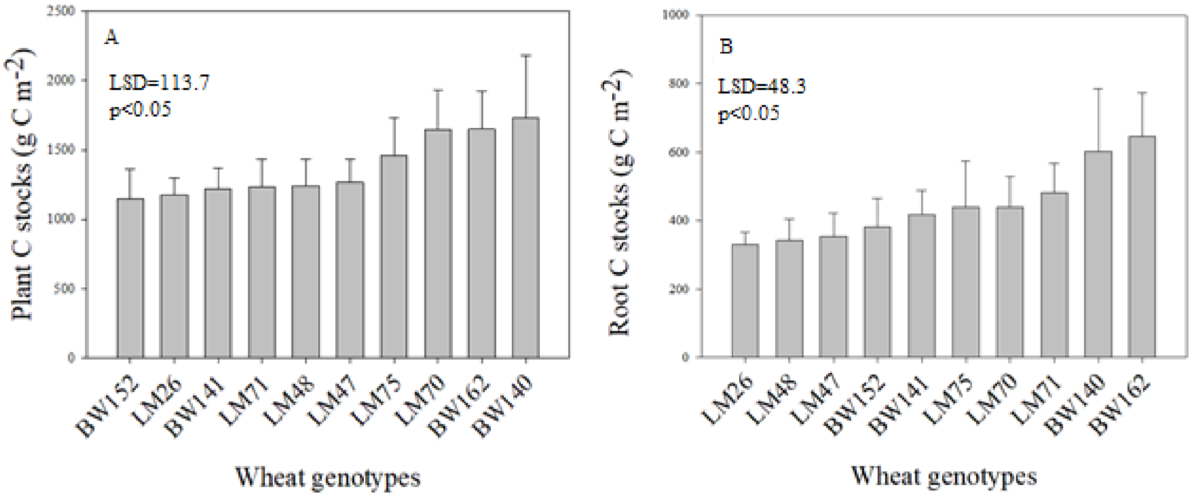

Fig 2.3 Total (A) and root (B) plant carbon stocks displayed by 10 genetically diverse wheat genotypes selected for high biomass and grain yield production under drought-stressed and non-stressed conditions in the greenhouse and field environments.

LSD=least significant difference, p=probability value, C=carbon, g=grams, m=metre

2.4 Discussion

2.4.1 Genotypic variation in biomass and agronomic performance

The 100 genotypes accumulated different biomass, which they allocated in variable proportions between roots and shoots ($p<0.05$) (Tables 2.3, 2.4 and 2.6). This is in agreement with Akman et al. (2017), who reported significant genotypic variation among 47 wheat genotypes evaluated under field conditions in Turkey. Differences in performance among genotypes reflects genetic diversity (Bhutta et al. 2006), which is a consequence of variable genetic background of the genotypes. Twenty-one genotypes were from the drought tolerant nursery, while 75 originated from the heat tolerant nursery of CIMMYT (Appendix 2.1) resulting in differences in their agronomic performance. The heat tolerant lines such as BW152, LM47, BW141, BW162 had higher shoot biomass compared to the drought tolerant genotypes such as LM98, LM71 and LM75 showing their ability to tolerate heat stress conferred an advantage in above ground biomass production. The range of root biomass found in this study differed from the range of 97 to 1176 g m^{-2} reported by Waines (2012) under greenhouse condition, but confirms the existence of genotypic variation. The biomass production by triticale could be a result of its higher solar radiation conversion efficiency of 3.2 g MJ^{-1} compared to 2.0 g MJ^{-1} exhibited by wheat under similar conditions (Estrada-Campuzano et al. 2012). Triticale is also well known to combine an aggressive rooting capability of one of its parents, rye (Solomon et al. 2007).

Biomass allocation between roots and shoots expressed as RS ratios also varied significantly among the 100 genotypes with values of 0.03 to 3.04 (Tables 2.3 and 2.4), which encompasses a range of 1.00 to 1.36 reported in wheat varieties by Fang et al., (2017). The high RS exhibited by the heat tolerant genotypes suggests their ability to maintain productivity even under combined drought and heat stress conditions that allowed them to allocate more biomass towards root development compared to the drought tolerant genotypes. Such genotypes with heat tolerance and high biomass accumulation are more relevant for sub-Saharan Africa where drought and heat stresses often occur simultaneously.

Cluster analysis grouped the genotypes into distinct groups based on their phenotypic similarity (Table 2.5). Phenotypic differences are important as they simultaneously reflect the influence of genes and environmental factors. The clustering of the genotypes into distinct clusters highlights variation in the pedigree of genotypes from the different nurseries (heat and drought tolerant) because clusters reflect relatedness in the genetic background (Sorkheh et al. 2007; Mofokeng et al. 2014). The wide genotypic variation in the germplasm opens opportunities for wheat improvement by selecting the best performing genotypes from different clusters to preserve genetic diversity that is critical for breeding (Nevo and Chen 2010).

Genotypes in clusters A21, A22 and B21 had above average DSI, showing that they were more drought sensitive compared with genotypes in the other clusters. Selection based on DSI only allows for the selection of genotypes based on their grain yield potential (Abraha et al. 2017). Therefore it was essential to cluster the genotypes and also to consider their overall biomass production. The low DSI and biomass productivity exhibited by the top-ranked genotypes (Table 2.6) such as BW140, BW141, LM26, BW152 and LM47 shows that they are both highly productive under optimal conditions and incur the least reduction under water scarce conditions. Similarly, Abraha et al. (2017) performed hierarchical clustering on tef genotypes and found differences in DSI among the clusters, which enabled them to select for drought tolerance.

2.4.2 The impact of water regime and environmental conditions on trait expression

Water regime had significant ($p<0.05$) impact on biomass traits (RB, SB, RS and GY) and other agronomic traits (Table 2.4), similar to other studies (e.g. Fang et al. 2017; Mwadzingeni et al. 2017). Yield ranged from 235 to 568 g m^{-2} under drought stress and from 550 to 988 g m^{-2} under non-stress conditions with an overall 40% significant reduction (averaged across genotypes) (Fig 3.4d), which was relatively comparable to 26% yield reduction reported by Foulkes et al. (2007). The higher impact of drought stress on biomass production revealed that drought stress imposed earlier during pre-anthesis vegetative stages impacts on all biomass and growth parameters, whereas other studies focused on terminal drought imposed at anthesis or post-anthesis (eg. Foulkes et al. 2007; Mwadzingeni et al. 2017). However, the changes in ranking of genotypes in biomass accumulation across water regimes disagreed with Foulkes et al. (2007) who asserted that genotypes with high performance under optimal soil water conditions still perform well under drought stress.

Biomass allocation to roots (RS) was reduced by 14% due to drought stress (Table 2.6 and Fig 2.2c) unlike previous studies which supported the optimal partitioning theory of increased RS under drought (Eziz et al. 2017; Poorter et al. 2012). The present findings agree with Vanaja et al. (2011) who also found that drought reduced RS ratios by 7% in sunflower and 60% in maize. The reason for decreased RS ratios can be explained by the fact that the prolonged drought stress may have exceeded a threshold level causing the plant to completely lose its biomass allocation regulatory ability (Xu et al. 2010). Nonetheless, the higher impact of drought on biomass production (>30%) compared to 14% on RS ratio agreed with previous report by Lopez-Castaneda and Richards (1994) who indicated that drought stress has more impact on dry matter production than its allocation to organs.

The ANOVA revealed that water regime, environment and their interaction with genotype had significant impact ($p<0.05$) on other agronomic traits such as plant height, tillering ability, kernel numbers and weight and number of days to heading and maturity (Table 2.4). Plant height reflects biomass investment in vegetative growth and is related to soil water availability as represented by a decline of 17% due to drought stress (Table 2.6). Vegetative growth is paramount to total biomass production. Reduced plant height, low number of productive tillers, low number of kernels and low weight of the kernels all contributed to significant reduction in total biomass. Plants faced with water stress shorten their developmental period in order to escape the effects of drought stress (Edae et al. 2014). Days to heading were not significantly different between stressed and non-stressed conditions, which was discordant with many researchers. However, under drought stress genotypes took 65 days compared to 66 days under non-stress condition, which points to the effect that genotypes shortened their development duration. Similarly, genotypes matured earlier (107 days) under drought stress relative to non-stressed condition (118 days) indicating accelerated development due to water deficit. Inadvertently, grain yield components (tillering, kernel numbers and weight) were all reduced under drought because they are expressly related to vegetative and reproduction, which are also severely reduced under stress. Genotypes performed differently under greenhouse and field conditions. Plants were taller, flowered later, had a higher number of tillers, spikes and kernels, had heavier thousand-grain weight and matured later under field conditions compared with greenhouse due to the aforementioned expression of potential.

2.4.3 Associations between biomass and agronomic traits under contrasting water regimes

The principal component (PC) analysis higher PC scores under drought stress compared to non-stressed conditions, showing that drought stress affected the relationship among traits (Table 2.7). Similarly, Mwadzingeni et al. (2017) found differences in trait relationship explained by PC under different water regimes. Correlations among biomass variables (RB, SB and GY)), drought sensitivity index (DSI) and roots to shoots ratio (RS) under drought stress were stronger compared to their respective correlations under non-stressed conditions (Table 2.8) in concurrence with other reports which also found that drought stress conditions strengthened correlations among variables (Pauli et al. 2016; Zhao et al. 2018). Under both drought stress and non-stress conditions, variations in yield were largely explained by changes in shoot biomass ($r= 0.62$, $P < 0.001$, drought stressed; $r= 0.54$, $P < 0.001$, non-stressed conditions) than any other parameter. This was in agreement with Dodig et al. (2012) and Sareen et al. (2014), who both reported moderate to high correlations ($r > 0.3$) between grain yield and biomass production under stressed conditions. Reynolds et al. (2009) reckoned that the association between grain yield and shoot biomass is vital since future yield improvement in wheat will be accountable to increase in above ground biomass rather than shifts in biomass partitioning. This presents a conflict in efforts to increase soil C input via root biomass, while attempting to maintain or increase yield potential simultaneously.

Root biomass and biomass allocation between roots and shoots (RS) were more important under drought-stressed conditions, as shown by higher principal component scores compared to non-stressed PC scores (Table 2.7), because under stressed conditions root biomass is pivotal in accessing water resources (Poorter et al. 2012; Tatar 2016; Eziz et al. 2017). Correlations between grain yield and root biomass were 70% stronger under drought stress (r=0.46, p<0.001) compared to non-stressed conditions (r=0.27, p<0.001) showing that root biomass assumes a more important role when water resources are limiting, in agreement with the optimal partitioning theory (Brouwer 1962). In concurrence, Atta et al. (2013) attributed 45% of variance in grain yield to root traits. Similarly, Fang et al. (2017) reported significant correlation of 0.24 between root biomass and grain yield under irrigation and even stronger correlation of 0.78 under lower soil water availability in a wheat rain-fed system.

Root biomass also showed 3% stronger correlation with total biomass (*r*=0.63, p<0.001) under drought stress compared to non-stressed conditions (*r*=0.61, p<0.001) showing that root biomass accounts for more variation in the total productivity of a genotype when drought stress is exerted. The 7% weaker correlations between shoot and root biomass under drought stress compared to their association under non-stress conditions points to the fact that there is a shift in biomass allocation towards roots at the expense of shoot when plants are subjected to drought stress. The significant association of RS with grain yield (*r*=0.12, p<0.05) under drought stress agreed with the proposition that improved water accessibility and use efficiency due to increased allocation of biomass to roots sustains crop productivity under drought prone environments (White et al. 2015; Zhang et al. 2013).

The association of DSI with DTH, DTM, NPT and RS were significant under drought stress (Table 2.8). For instance, the positive association of DTH and DTM with DSI suggests that late heading and long season genotypes can be susceptible to drought stress. This can be related to the ability by early heading and maturing genotypes to escape terminal drought stress and such genotypes will be more appropriate for regions where subsoil moisture depletes exponentially towards end of growth season (Haque et al. 2016). There is indication that increased in RS reduces drought susceptibility shown by the negative association between RS and DSI. This agrees with the report of Zhang et al. (2013).

The variable correlations among agronomic traits DTH, DTM, PH, NPT, TPP, SL, KPS and TKW (Table 2.8) under different soil water regimes revealed that agronomic traits affect yield differently depending on the prevailing water availability. For example, Slafer et al. (2014) found that heavier grain weight under non-stressed conditions contributed to higher yield despite a decline in number of kernels per spike. The inverse proportionality between grain weight and number of grains per spike (*r* =-0.37; p<0.001) was confirmed in our results under non-stressed and (*r* =-0.08; p>0.005) under drought stress conditions. The negative association between days to heading and grain yield (r=-0.26, p <0.001) under drought stress and (*r*=-0.22, p<0.001) under non-stress agree with assertions that early heading confers advantage for higher grain yield (Blum 2010). Further, the significant difference for days to maturity under drought stress suggests that there could be drought escape mechanism among genotypes.

The positive association between plant height and biomass variables (RB and SB) points to the interdependence of rooting ability and accumulation of reserves. Plants with high root biomass have higher potential for nutrient and water uptake, which avails resources for stem elongation and biomass accumulation in above ground parts. Ahmed et al. (2007) found that shorter plants had lower yield and total biomass productivity, while Miralles and Slafer (1995) opined that improvement in biomass production would be difficult in shorter plants because of their reduced radiation interception. The positive correlation between RB and SB with days to heading shows that the phenological cycle is an important determinant of biomass accumulation as it demarcates cessation of vegetative growth and onset of reproductive growth stage. Vegetative and reproductive phases promote biomass accumulation in non-grain components and grains, respectively. Biomass allocation (RS) exhibited fewer significant correlations with agronomic traits with only PH (r=-0.29, $p<0.05$), SL (r=-0.22, $p<0.05$) and KPS (r=-0.25, $p<0.05$) under non-stress condition. The negative correlation between RS and PH (r=-0.29, $p<0.05$) suggests that RS in taller plants was low, not necessarily as a result of reduced root biomass production, but due to accumulation of more biomass above ground in taller compared to shorter plants.

2.4.4 Plant carbon stocks

The differences in plant carbon accumulation among top 10 performing genotypes shows that there is possibility to develop high yielding cultivars that also accumulate higher C stocks, especially in the roots. The C will eventually be stabilized in the soil since root debris are commonly left in the field. This can be complemented with agronomic practices such as conservation agriculture to help to restore soil C stocks and improve soil health, with positive feedback on crop production. Based on assertion by Martens et al. (2009) that wheat can deposit up to 70% of its total below ground carbon into the soil, the selected wheat genotypes can deposit between 2.31 (calculated in genotype LM26) and 4.5 tons C hectare^{-2} (BW162) based on their root C stocks. These values are higher than 0.84 to 2.69 tons C hectare^{-1} reported by Lu et al. (2018). The calculations in this study do not account for respiration, which would significantly reduce the amount of C that would ultimately be stabilized in the soil. Assuming a 20% loss of C due to respiration (Sauerbeck and Johnen 1976), the range of C that could potentially be stabilized in the soil will be 1.8 to 3.6 tons

C hectare^{-1}. However, there are other factors such as soil type and microbial activity that are paramount to C sequestration in agricultural soils.

2.5 Conclusion

Wide intra-specific variation in biomass production and its allocation to roots and shoots was found among the 100 genotypes of wheat, showing that these are vital genetic resources for development of drought tolerant and enhanced C sequestering varieties. The results from correlation and diversity analyses show that it is possible to simultaneously select for high grain yield and root biomass production to satisfy both food production and C sequestration needs. The study also concluded that RS is inadequate as a sole predictor for drought tolerance, biomass productivity or soil C input by wheat due to the low correlations between RS and biomass variables (GY and SB) under both water regimes. The reduction in RS due to drought stress contradicted with widely accepted optimal partitioning theory, but supports the theory that prolonged drought stress can lead to the collapse of biomass allocation regulatory ability in plants. These results provide valuable information for investigating C sequestration potential and revealing the genetic basis of drought tolerance in wheat. Meanwhile, 10 genotypes, highlighted in Table 2.5, were identified and selected for their diversity, high yield and total biomass production under drought stress conditions. These genotypes will be used in genetic studies and combining ability analyses of biomass allocation, yield and yield components, which will lay the foundation for breeding C efficient, drought tolerant and high yielding varieties.

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Appendix 2.1 List of genotypes and their pedigree used in the study

ENTRY CODE	PEDIGREE
	Genotypes from CIMMYT Heat Stress Tolerance Nursery
LM01	ACHTAR*3//KANZ/KS85-8-5/4/MILAN/KAUZ//PRINIA/3/BAV92/5/MILAN/KAUZ//PRINIA/3/BAV92
LM12	SOKOLL/ROLF07
LM14	MILAN/KAUZ//PRINIA/3/BAV92/4/WBLL1*2/KUKUNA
LM15	RL6043/4*NAC//PASTOR/3/BAV92/4/ATTILA/BAV92//PASTOR
LM16	PASTOR*2/BAV92/3/FRET2/KUKUNA//FRET2
LM17	ESDA/KKTS
LM18	GOUBARA-1/2*SOKOLL
LM19	SOKOLL*2/4/CHEN/AEGILOPS SQUARROSA (TAUS)//FCT/3/STAR
LM20	PBW343
LM21	PRL/2*PASTOR
LM22	MUNAL #1
LM23	QUAIU
LM24	WBLL1*2/BRAMBLING
LM25	WHEAR//2*PRL/2*PASTOR
LM26	ATTILA*2/PBW65//TAM200/TUI
LM27	YUNMAI 48//2*WBLL1*2/KURUKU
LM28	ATTILA/3*BCN//BAV92/3/TILHI/4/SHA7/VEE#5//ARIV92
LM29	PRL/2*PASTOR*2//SKAUZ/BAV92
LM30	C80.1/3*BATAVIA//2*WBLL1/3/ATTILA/3*BCN*2//BAV92/4/WBLL1*2/KURUKU
LM31	ATTILA*2/HUITES//FINSI/3/ATTILA*2/PBW65
LM32	ATTILA*2//CHIL/BUC*2/3/KUKUNA
LM33	ATTILA*2/PBW65//KACHU
LM35	WBLL1//UP2338*2/VIVITSI
LM36	WBLL1*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/KACHU
LM37	KACHU/SAUAL
LM38	SAUAL/3/MILAN/S87230//BAV92
LM39	ATTILA/3*BCN//BAV92/3/TILHI/5/BAV92/3/PRL/SARA//TSI/VEE#5/4/CROC_1/AE.SQUARROSA (224)//2*OPATA
LM40	WBLL1*2/VIVITSI/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*JANZ
LM41	C80.1/3*BATAVIA//2*WBLL1/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES
LM42	TRCH/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES
LM43	ROLF07*2/6/PVN//CAR422/ANA/5/BOW/CROW//BUC/PVN/3/YR/4/TRAP#1
LM44	ROLF07/TUKURU/5/WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ
LM46	FRET2/KUKUNA//FRET2/3/PARUS/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
LM47	FRET2/KUKUNA//FRET2/3/YANAC/4/FRET2/KIRITATI
LM48	FRET2/KUKUNA//FRET2/3/PASTOR//HXL7573/2*BAU/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
LM49	TRCH/SRTU//KACHU
LM50	HUW234+LR34/PRINIA*2//SNLG
LM51	HUW234+LR34/PRINIA*2//YANAC
LM52	HUW234+LR34/PRINIA*2//WHEAR
LM54	PBW343*2/KUKUNA*2//KITE
LM55	PBW343*2/KUKUNA//PARUS/3/PBW343*2/KUKUNA
LM56	PBW343*2/KUKUNA*2//YANAC
LM57	PBW343*2/KUKUNA//SRTU/3/PBW343*2/KHVAKI
LM58	ATTILA*2/PBW65/6/PVN//CAR422/ANA/5/BOW/CROW//BUC/PVN/3/YR/4/TRAP#1/7/ATTILA/2*PASTOR
LM59	FRET2/KUKUNA//FRET2/3/WHEAR/4/FRET2/TUKURU//FRET2
LM60	ALD/CEP75630//CEP75234/PT7219/3/BUC/BJY/4/CBRD/5/TNMU/PF85487/6/PBW343*2/KUKUNA/7/CNO79//PF7035 4/MUS/3/PASTOR/4/BAV92
	Genotypes from CIMMYT Drought Stress Tolerance Nursery
LM71	BABAX/3/PRL/SARA//TSI/VEE#5/4/CROC_1/AE.SQUARROSA (224)//2*OPATA
LM72	BABAX/3/PRL/SARA//TSI/VEE#5/4/WBLL1
LM75	BUC/MN72253//PASTOR
LM76	MILAN/KAUZ//PRINIA/3/BABAX
LM77	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*FRAME
LM79	CROC_1/AE.SQUARROSA (205)//BORL95/3/KENNEDY
LM80	CROC_1/AE.SQUARROSA (205)//KAUZ/3/SLVS
LM81	CROC_1/AE.SQUARROSA (224)//2*OPATA/3/2*RAC655
LM82	HD30/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI
LM83	PASTOR/3/VEE#5//DOVE/BUC
LM84	SRN/AE.SQUARROSA (358)//MILAN/SHA7
LM85	SW94.60002/4/KAUZ*2//DOVE/BUC/3/KAUZ/5/SW91-12331
LM86	CHAM 6
LM90	CROC_1/AE.SQUARROSA (205)//BORL95/3/KENNEDY-2
LM91	FRTL/CMH83.2517
LM93	PASTOR/FLORKWA.1//PASTOR

Appendix 2.1 continued

ENTRY CODE	**PEDIGREE**
LM96	ALTAR 84/AE.SQ//2*OPATA/3/PIFED
LM97	KRICHAUFF/2*PASTOR
LM98	KABY//2*ALUBUC/BAYA
LM99	ALTAR 84/AEGILOPS SQUARROSA (TAUS)//OCI/3/VEE/MJI//2*TUI
LM100	SW89.5277/BORL95//SKAUZ
	Genotypes from CIMMYT BW Program Heat Stress Tolerance Nursery
BW28	CMSA05Y01011T-040M-040ZTP0Y-040ZTM-040SY-14ZTM-03Y-0B
BW48	CMSA04M00346S-040ZTP0Y-040ZTM-040SY-27ZTM-04Y-0B
BW49	CMSA04M00346S-040ZTP0Y-040ZTM-040SY-28ZTM-01Y-0B
BW58	CMSA04M00067S-040ZTB-040ZTY-040ZTM-040SY-2ZTM-02Y-0B
BW63	CMSA04M01020T-050Y-040ZTP0M-040ZTY-040ZTM-040SY-5ZTM-03Y-0B
BW71	CMSA05Y00325S-040ZTP0Y-040ZTM-040SY-7ZTM-01Y-0B
BW103	CMSS05B00581S-099Y-099M-099Y-099ZTM-2WGY-0B
BW111	CMSS05B00663S-099Y-099M-099Y-099ZTM-13WGY-0B
BW116	CMSS05B00742S-099Y-099M-099Y-099ZTM-5WGY-0B
BW124	CGSS05B00153T-099TOPY-099M-099NJ-22WGY-0B
BW127	CGSS05B00162T-099TOPY-099M-099Y-099ZTM-15WGY-0B
BW128	CGSS05B00162T-099TOPY-099M-099NJ-13WGY-0B
BW129	CGSS05B00162T-099TOPY-099M-099NJ-099NJ-7WGY-0B
BW141	CGSS05B00243T-099TOPY-099M-099NJ-099NJ-1WGY-0B
BW142	CGSS05B00243T-099TOPY-099M-099NJ-099NJ-2WGY-0B
BW145	CGSS05B00253T-099TOPY-099M-099Y-099ZTM-8WGY-0B
BW147	CGSS05B00256T-099TOPY-099M-099NJ-099NJ-5WGY-0B
BW148	CGSS05B00258T-099TOPY-099M-099Y-099ZTM-3WGY-0B
BW149	CGSS05B00258T-099TOPY-099M-099Y-099ZTM-11WGY-0B
BW150	CGSS05B00258T-099TOPY-099M-099Y-099ZTM-12WGY-0B
BW151	CGSS05B00258T-099TOPY-099M-099Y-099ZTM-13WGY-0B
BW152	CGSS05B00258T-099TOPY-099M-099NJ-1WGY-0B
BW157	CGSS05B00261T-099TOPY-099M-099NJ-099NJ-8WGY-0B
BW159	CGSS05B00290T-099TOPY-099M-099NJ-099NJ-7WGY-0B
BW162	CGSS05B00304T-099TOPY-099M-099NJ-099NJ-3WGY-0B
	Local Checks
LM70	Check
BW80	Check
BW100	Check
BW120	Check
BW140	Check
	Temperate checks
Arenza	Check
Sossognon	Check
Triticale	Check

Chapter 3 Variance components and heritability of traits related to root: shoot biomass allocation and drought tolerance in wheat

Abstract

Enhanced root growth in plants is fundamental to improve soil water exploration and drought tolerance. Understanding of the variance components and heritability of root biomass allocation is key to design suitable breeding strategies and to enhance the response to selection. This study aimed to determine variance components and heritability of biomass allocation and related traits in 99 genotypes of wheat (*Triticum aestivum* L.) and one triticale (X. *Triticosecale* Wittmack) under drought-stressed and non-stressed conditions in the field and greenhouse using a 10 × 10 alpha lattice design. Days to heading (DTH), days to maturity (DTM), number of tillers (NPT), plant height (PH), spike lenghth (SL) shoot and root biomass (SB, RB), root to shoot ratio (RS), thousand kernel weight (TKW) and grain yield (GY) were recorded. Analyses of variance, variance components, heritability and genetic correlations were computed. Significant ($p<0.05$) genetic and environmental variation were observed all the traits except for spike length. Drought stress increased heritability of RS from 28 to 47% and GY from 17 to 55%. The correlations between RS with PH, NPT, SL, SB and GY were weaker under drought-stress ($r\leq-0.50$; $p<0.05$) compared to non-stressed conditions, suggesting that lower root biomass allocation under drought stress compromises wheat productivity. The negative association between GY and RS ($r=-0.41$ and -0.33; $p<0.05$), low heritability (<42%) and high environmental variance (>70%) for RS observed in this population constitute several bottlenecks for improving yield and root mass simultaneously. However, indirect selection for DTH, PH, RB, and TKW, could help optimize RS and simultaneously improve drought tolerance and yield under drought-stressed conditions.

Keywords: Correlation, Genetic variance, Heritability, Root-to-shoot ratio, Water stress, Wheat

3.1 Introduction

Biomass allocation plays a major role in determining the yield and drought tolerance of crops. Genotypes allocate biomass differently between roots and shoots (Weiner 2004) and there are indications that drought tolerance can be improved via traits such as root length and biomass allocation (Paustian et al. 2016; Griffiths and Paul 2017). There is a need to assess the genetic variation and heritability of biomass allocation and agronomic traits as a prerequisite for developing cultivars with enhanced drought tolerance in wheat (*Triticum aestivum* L.) which is increasingly being grown under drier conditions due to global warming.

Biomass allocation has been more widely reported in studies on tree and forestry breeding than in annual crops such as wheat. However, these reports cannot be reliable for reference to biomass allocation in cereals due to the huge interspecific differences. In studies carried out on annual cereal crops, the heritability of biomass allocation was inferred from related variables such as harvest index, which is a ratio of grain yield to total biomass. For example, Rattey et al. (2009) reported low to moderate heritability ($0.27<H>0.49$) of harvest index in Seri/Babax recombinant inbred lines (RIL) of wheat across diverse environments, while Ahmad et al. (2017) found higher heritability of 0.74 for the same trait in drought-stressed wheat. In sorghum, heritability of biomass accumulation can be inferred from structural fibre accumulation, which had a moderate heritability of 0.5 (Brenton et al. 2016). Bo et al. (2013) identified quantitative trait loci (QTL) responsible for biomass allocation in soybean and reported that its heritability could be lower than 10%. In contrast, Busemeyer et al. (2013) reported higher heritability, ranging between 0.78 and 0.84, for biomass allocation in triticale, a species well known for aggressive rooting and biomass production. By inference, low to moderate heritabilities of harvest index and structural fibre accumulation suggest that biomass allocation could be a complex trait.

Indirect selection via yield related traits could be more effective in selecting genotypes with desirable biomass allocation compared to direct selection for high root to shoot ratios, for a number of reasons. Root to shoot ratios are highly influenced by the environment (Kumar et al. 2006), which means that their selection across environments is confounded by environmental variance. Kumar et al. (2006) alluded that environmental stresses, such as drought and heat, affect biomass allocation through their effects on plant growth and tillering capacity. Therefore, investigating agronomic traits related to biomass allocation may assist in indirect selection of biomass allocation for enhanced grain yield. There is the possibility of using indirect selection for biomass allocation. Gowda et al. (2011) found that biomass partitioning in triticale was influenced mostly by plant height, while grain yield, days to heading, number of spikes per plant and thousand-grain weight had minor effects. However, the lack of adequate information on the heritability and genetic correlations between yield-related traits and biomass allocation limits development of ideotypes with optimum biomass partitioning for drought tolerance. Root to shoot ratios are often used as indicators of relative biomass allocation to below and above ground biomass (Edwards et al. 2016). However, they are not easy to analyze and their use can lead to inaccurate inferences (Curran-Everett 2013). Therefore, it is important that selection criteria are not restricted to ratios only but include a wide range of other traits. Therefore, the objectives of this study were to measure variance components and heritability of biomass allocation and grain yield related traits among 100 genotypes of bread wheat and triticale (*Triticosecale* Wittmack) as a preliminary step towards optimizing biomass partitioning.

3.2 Materials and Methods

3.2.1 Plant materials

A hundred genotypes, consisting of 97 drought and heat tolerant wheat (*Triticum aestivum* L., 2n=6x=42) accessions obtained from the International Maize and Wheat Improvement Centre (CIMMYT), two commercial wheat cultivars and one triticale were used in this study (Chapter 2, Appendix 2.1). Their genetic diversity allowed genetic variation analysis and estimation of variance components. Triticale was included in the study as a control given its aggressive root system (Solomon et al. 2007), high biomass productivity compared to other crops (Pronyk and Mazza 2011) and its potential to diversify crop rotations in cereal production systems (Gowda et al. 2011).

3.2.2 Site description and set up for greenhouse experiments

Two greenhouse experiments were carried out at the Controlled Environment Facility of the University of KwaZulu Natal. The first greenhouse experiment was carried out between October 2016 and February 2017, while the second was conducted in May till September 2017. The humidity in both experiments was maintained between 55 and 65% with minimum day length of

11 hours without supplemental light. The experiments were carried out in different seasons to ensure the repeatability of the study and hence to increase the accuracy in estimating trait heritability similar to Herzig et al. (2018). Mean temperatures and soil properties are presented in Chapter 2 (Table 2.1). The second experiment was based on field sampled soils. The two greenhouse experiments represented two distinct environments. The experiments were conducted using a 10 × 10 alpha lattice design with two replications. Ten seeds of a genotype were sown in each 25-centimetre diameter plastic pot containing five litres of soil to make a total of 200 pots per water regime. The number of plants per pot was thinned to eight three weeks after emergence, providing about 128 plants per square meter. Fertilizer was applied through automated drip irrigation at a rate of 300 kg N ha^{-1} and 200 kg P_2O_5 ha^{-1}. The plants were allowed to establish for 6 weeks to an even stand before initiating different water treatments. The field capacity (FC) of the soil was determined according to Grewal et al. (1990). Calculations of the amounts of water applied were specific to a particular soil based on its field capacity. This ensured that drought stress was equally induced despite different soils used. In the non-stressed regime, the plants were watered to field capacity (FC) whenever average soil moisture content fell to 80% of FC. The volumetric soil moisture content was allowed to drop to 30% of the FC in the drought-stressed treatment before watering back to FC. The soil moisture content was monitored using a soil moisture probe inserted in the middle of the pot. The two watering treatments were maintained until maturity (~120 days).

3.2.3 Site description and set up for field experiment

The field experiment was set up at the University of KwaZulu-Natal’s Ukulinga research farm (LAT: 29.667 LON: 30.406 and ALT: 811m). Long-term average annual temperature and rainfall for Ukulinga are 18 °C and 738 mm, respectively. The mean temperature during the growing period and soil properties are listed in Chapter 2, Table 2.1. The field was ploughed to the depth of 30cm and a plastic mulch was used to exclude rainwater. Planting was in May 2017. Three seeds were planted per hole at 15cm in row spacing and 45cm between rows and later thinned to two plants per station. Each plot was a 0.8m long single row per genotype. Basal fertilizer was applied at a rate of 120:30:30 kg ha^{-1} (N:P:K). Other agronomic practices were as per standard wheat production practice in South Africa (DAFF 2010). Different water treatments were imposed at 6 weeks after emergence. The field capacity of the soil was deduced from digital moisture sensors and tensiometers inserted at 30 and 60 cm depths at several points in the field. Irrigation water was applied through a drip irrigation system to maintain adequate soil moisture (average 80% of field capacity) in the non-stressed treatment. Under the drought stress treatment, water was withheld until the soil moisture content fell to 30% of field capacity. Afterwards, it was replenished to field capacity. Moisture sensors and tensiometers monitored soil moisture content constantly. The greenhouse and field moisture regimes were different because natural drought rarely occurs uniformly across environments and seasons and it also enabled the separation of genetic and environmental variance components for estimating heritability.

3.2.4 Data collection and analyses

The following agronomic traits were recorded: days to heading (DTH) were recorded as the number of days from planting to the date when 50% of plants in a plot had fully emerged spikes. The number of productive tillers (NPT) were counted per plant and plant height (PH) was measured at maturity as the average of three measurements made from the soil surface to the tip of the spike, excluding awns. Days to maturity (DTM) were recorded as the number of days from planting to the day when 50% of the plants were dry. The spike length (SL) was expressed as the average length of three heads per plot measured from the base to the tip, excluding awns. Plants were cut at the soil surface to separate roots from shoots. All root biomass was sampled at maturity. In the greenhouse, roots were sampled from the entire soil in the pot. In the field, 30×30×30cm monolith sampling box was used to sample the roots to the depth of 60cm. All root biomass for all the experiments was separated from the soil in a two-step procedure adapted from Hirte et al. (2018). The sampled soil volume (from a monolith box or a pot) was passed through a 2mm sieve and the remaining large roots were collected. The residue which passed through the sieve was washed under running water to dissolve the soil and sieved through a 0.5mm sieve to collect the fine roots. All roots were cleaned from the soil as much as possible. The grain yield (GY), shoot biomass (SB) and root (RB) biomass were determined after the roots, shoots and grains were dried in an oven with forced air circulation at 60°C for 72 hours. All biomass variables were normalized to grams per square meter based on the population density of 128 and 134 plants m^{-2} in the greenhouse and field experiments, respectively. Root: shoot (RS) ratios were computed from RB and SB. Thousand-kernel weight (TKW) was determined by weighing a random sample of 1000 seeds.

The relationship between root and shoot biomass were tested for isometry according to Curran-Everett (2013). Afterwards, root biomass was regressed on shoot biomass to explain biomass partitioning using the relationship

$$Y = a + \beta X$$

where Y is the shoot biomass, a is a constant, β is the slope gradient and X is the root biomass. The data was tested for normality using the Shapiro-Wilk test and subjected to the analysis of variance (ANOVA) using the general linear model procedure for unbalanced designs in the Genstat 18th edition (Payne et al. 2017). The genotypic (GCV) and phenotypic (PCV) coefficients of variation were calculated following Burton (1952) using variance components of combined ANOVA. Variance components were calculated by the general linear model (GLM) procedure in Genstat 18th edition (Payne et al. 2017) for the separate water regimes. Environment and water regimes were considered to have fixed effects, while genotype effects were treated as random following Edwards et al. (2016). The term "environments" refers to the different experiments which were conducted under distinctively specific soil properties, relative humidity and temperature conditions. The inclusion of different environments allows for an effective evaluation of quantitative traits (Gillespie and Turelli 1989; Herzig et al. 2018). Variances below zero were adjusted to zero according to Robinson et al. (1955), while the expected mean squares (EMS) were calculated following Shimelis and Shiringani (2010) as presented in Table 3.1.

Table 3.1 Partial analysis of variance and expected mean squares for 100 genotypes evaluated in three environments

Source of variation	DF	Expected mean squares
Genotype (g)	(g-1)	$\delta^2e+r\delta^2gs+rs\delta^2g$
Environment (s)	(s-1)	-
G*S	(g-1)(s-1)	$\delta^2e+r\delta^2gs$
Residual (e)	s(g-1)(r-1)	δ^2e

δ^2e=residual variance, δ^2gs=genotype X environment interaction variance, δ^2g=genotypic variance, r=number of replications per site, g=genotype, s=number of environment, e=error

Genetic correlations under each water regime were estimated following the formula presented in Sharma (1998) and their significance was tested using the Student t-test (Steel and Torrie 1960).

Broad sense heritability (H) estimates under each water regime were calculated from the phenotypic variance (δ^2p) and the genotypic variance (δ^2g) according to Allard (1999) as;

$$H = \frac{\delta^2 g}{\delta^2 p}$$

Where $\delta^2 p = \delta^2 g + \delta^2 gs/s + \delta^2 e/rs$

Where δ^2p=phenotypic variance, δ^2g=genotypic variance, δ^2gs=genotype × environment interaction variance, δ^2e=residual variance, while r=number of replications and s=number of environments.

3.3 Results

3.3.1 Relationship between root and shoot biomass

The relationship between root and shoot biomass exhibited an isometric relationship with the regression line passing through the origin showing that root to shoot ratios were not constant across the genotypes (Fig 3.1).

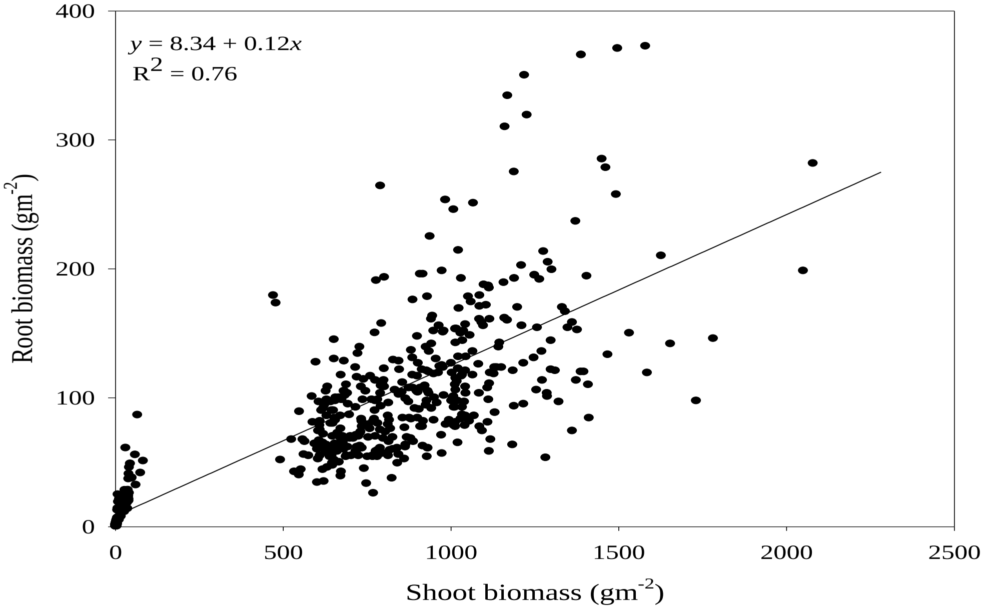

Fig 3.1 The isometric relationship between root and shoot biomass for all genotypes evaluated under stress and non-stress conditions

3.3.2 Genotype and environmental influence on trait variability

Single environment analysis of variance (ANOVA) revealed significant differences across the three experiments so a combined ANOVA was conducted to test the consistency of genotype responses and estimate variance components. The ANOVA revealed that the genotype, water regime and environment interaction significantly ($p<0.05$) affected NPT, PH, DTM and RS (Table 3.2). The effects of the genotype × water regime interaction were significant for DTM only, while the genotype × environment interaction significantly affected all traits except GY. Individually, genotype, water regime and environment effects were significant for all traits, except TKW. Most traits exhibited small differences between their GCV and PCV estimates (Table 3.2). GY and RS had the lowest GCV estimates, of 43.1 and 51.9%, respectively. Root to shoot ratio had the highest discrepancy of more than 30% between the GCV and PCV estimates, followed by GY with a discrepancy of 6.4%. RS also recorded high PCV values above 83% compared to GY, DTM, TKW and SB, which had PCV<55%. There was a significant variability in season means as expressed by differences between trait means of the greenhouse experiments.

Table 3.2 Mean squares after combined analysis of variance for phenotypic traits of 100 wheat genotypes evaluated across the three test environments and two water regimes

Change	DF	DTH	NPT	PH	DTM	SL	SB	RB	RS	TKW	GY
Replication (Rep)	1	205	0.37	35.03	181	10.4	3190585	13931	0.07	98.6	1547987
Rep(Block)	18	98.8	10.2	148.4	134	2.54**	236360	18521*	0.10*	60.7**	117122*
Genotype (Gen)	99	317***	13.9***	481***	175.27***	4.30***	556472***	52063***	0.06****	117***	101673**
Environment (Env.)	2	64335***	2987***	126114***	107577***	3382***	633806324***	14483166***	34.5***	9.14	214902285***
Water regime (WR)	1	1290***	2962***	44418***	39924***	420***	43087351***	2835547***	1.12***	3115***	43755727***
Gen.Env	192	107***	10.1***	99.8***	82.3***	1.94***	341187***	15459***	0.04***	38.8*	59370
Gen.WR	96	32.07	5.74	51.2	67.2***	0.87	167573	11016	0.03	28.36	43059
Env.WR	2	2449***	505***	7086***	348.2***	65.5***	12729698***	120902***	1.13***	1501***	486813***
Gen.Env.WR	192	28.61	8.19***	52.9*	52.9**	0.88	149771	9116	0.03*	29.79	58283
Residual	563	26.07	5.29	43.2	40.7	0.91	163807	10428	0.03	28.6	68604
Mean		65.5	11	71.5	113	7.74	1061	259	0.47	44.2	600
%CV		7.79	20.9	9.19	5.67	12.3	38.1	39.4	35.8	12.1	43.7
se		5.11	1.3	4.57	6.38	0.95	105	32.1	0.07	2.35	51.9
Skewness		0.029	0.037	-0.011	0.03	-0.03	0.025	0.09	-0.01	0.048	0.014
Kurtosis		-0.027	-0.048	-0.087	-0.028	-0.066	-0.083	-0.083	-0.084	-0.125	-0.085
GCV		71.4	74.4	79.2	52.9	67	55.1	72.6	51.9	53.7	43.1
PCV		72.8	78.6	80	53.3	69	57.6	75.1	83.8	54.7	49.5

DF=degrees of freedom, DTH=days to heading, NPT=number of productive tillers, PH=plant height, DTM=days to maturity, SL=spike length, SB=shoot biomass weight, RB=root biomass weight, RS=root to shoot ratio, TKW=thousand-grain weight, GY=grain yield, CV=coefficient of variation, se=standard error, GCV=genetic coefficient of variation, PCV=phenotypic coefficient of variation, *, ** and ***=significance level at <0.001, <0.01 and <0.05, respectively.

3.3.3 Variance components and heritability of traits

Generally, traits exhibited higher genotypic variance and heritability estimates under non-stressed conditions. For instance, genotypic variance for RS decreased from 45.5% in non-stressed conditions to 30.5% under drought (Table 3.3). The estimated heritability values for traits under non-stressed conditions ranged between 46.6 and 92.5% compared with 17.1 to 88.6% under drought. GY and RS exhibited the highest reduction in heritability due to drought stress of 18.6 and 37.4 %, respectively.

3.3.4 Associations among traits across water regimes and environments

Genetic correlation coefficients were generally high and significant for most traits under both water regimes (Table 3.4). The highest genetic correlation under non-stressed conditions was between GY and DTH ($r=0.72$; $p<0.001$), while most correlations were generally above 0.3 (Table 3.4, above diagonal). TKW exhibited the lowest genetic correlation below 0.04 with PH, DTM and SL under non-stress conditions. Root to shoot ratio exhibited negative genetic correlations with most traits. Under non-stressed conditions, RS showed significant ($p<0.001$) genetic correlations ranging between $-0.33< r >-0.59$ with DTH, PH, SL, DTM, SB and GY (Table 3.4, above diagonal), while drought stress reduced the same correlations to a range between $-0.14< r >-0.48$ ($p<0.001$) (Table 3.4, below diagonal). RB and RS were weakly associated under drought-stress conditions ($r=0.29$; $p<0.001$), yet they were strongly correlated ($r=0.61$; $p<0.001$) under non-stress conditions (Table 3.4, below diagonal).

Table 3.3 Variance components and heritability of traits for 100 genotypes grown under non-stressed and stressed conditions

Non-stressed conditions	DTH		NPT		PH		DTM		SL		SB		RB		RS		TKW		GY	
Component	Var	%	Var	%	Var	%	Var	%	Var	%	Var	%	Var	%	Var	%	Var	%	Var	%
Genotype (Gen)	1006	78.9	154	83.4	1238	72.8	742	69.5	27.3	68.9	869758	27.4	83796	59.4	0.22	45.5	2048	97.1	220188	24.6
Environment (Env)	199	15.6	13.2	7.16	362	21.3	254	23.8	7.28	18.4	2080675	65.5	42262	30.0	0.09	19.3	3.98	0.19	549676	61.5
Gen*Env	20.1	1.58	2.48	1.34	14.9	0.88	5.95	0.56	0.32	0.81	18748	0.59	409	0.29	0.00	0.59	6.14	0.29	2797	0.31
Residual	50.1	3.93	14.8	8.05	85.3	5.02	66.1	6.19	4.71	11.9	206815	6.51	14485	10.3	0.17	34.6	51.1	2.42	121598	13.6
Total	1275	100	184	100	1700	100	1068	100	39.6	100	3175996	100	140953	100	0.48	100	2109.09	100	894259	100
Phenotypic	1088		177		1371		843		34.5		1186230		105660		0.47		2126.58		403517	
Heritability%	92.5		87.0		90.3		88.0		79.2		73.3		79.3		46.6		96.3		54.5	

Stressed conditions	DTH		NPT		PH		DTM		SL		SB		RB		RS		TKW		GY	
Component	Var	%	Var	%	Var	%	Var	%	Var	%	Var	%	Var	%	Var	%	Var	%	Var	%
Genotype (Gen)	1115	79.3	67.7	80.7	907	65.1	1099	68.3	28.5	55.6	386290	21.8	36389	47.6	0.16	30.5	785.6	91.8	28161	3.95
Environment (Env)	147	10.4	5.10	6.08	316	22.7	303	18.8	10.5	20.5	1189595	67.3	31879	41.7	0.09	16.6	3.12	0.36	584219	82.0
Gen*Env	20.7	1.47	1.35	1.61	19.9	1.43	60.3	3.75	4.21	8.19	62639	3.54	1546	2.02	0.01	1.01	0.00	0.00	12029	1.69
Residual	124	8.83	9.78	11.7	150	10.8	147	9.11	8.12	15.8	130104	7.36	6582	8.62	0.28	52.0	67.2	7.85	88428	12.4
Total	1406	100	83.9	100	1393	100	1609	100	51.4	100	1768627	100	76396	100	0.53	100	856	100	712837	100
Phenotypic	1308		82.8		1139		1339		42.1		602325		46777		0.58		886		164813	
Heritability%	85.2		81.7		79.6		82.1		67.8		64.1		77.8		28.0		88.6		17.1	

DTH=days to heading, NPT=number of productive tillers, PH=plant height, DTM=days to maturity, SL=spike length, SB=shoot biomass weight, RB=root biomass weight, RS=root to shoot ratio, TKW=thousand-kernel weight, GY=grain yield, var=variance

Table 3.4 Genetic correlations among traits measured on 100 genotypes across environments and water regimes

Non-stressed conditions										
	DTH	NPT	PH	DTM	SL	SB	RB	RS	TKW	GY
DTH		0.15**	0.69*	0.81***	0.34	0.36**	0.56*	-0.55**	-0.26**	0.72***
NPT	0.17*		0.42**	0.13	0.28	0.55***	0.33*	-0.59***	-0.84*	0.34**
PH	0.21**	0.28**		0.68**	0.77**	0.28***	0.63**	-0.48**	-0.04	0.40***
DTM	0.48***	0.30**	0.36*		0.61***	0.36**	0.62**	-0.56***	-0.04	0.14*
SL	0.27	0.09	0.81*	0.41**		0.67***	0.34**	-0.19**	-0.03	0.40**
SB	0.22**	0.40**	0.72*	0.22**	0.85**		0.23**	-0.59**	-0.31**	0.71***
RB	0.12*	0.13**	0.41*	0.06	0.59**	0.65**		0.61***	-0.14	0.41**
RS	-0.62**	-0.31***	-0.39**	-0.68**	-0.14	-0.47***	0.29**		0.10	-0.33**
TKW	0.05	-0.22	0.30	0.12	0.11**	0.25**	0.16*	-0.12		0.04
GY	0.27**	0.55***	0.81***	0.42***	0.84***	0.55***	0.57***	-0.41***	0.33***	
Stressed conditions										

DTH=days to heading, NPT=number of productive tillers, PH=plant height, DTM=days to maturity, SL=spike length, SB=shoot biomass weight, RB=root biomass weight, RS=root to shoot ratio, TKW=thousand-kernel weight, GY=grain yield, *, ** and ***=significance level at <0.001, <0.01 and <0.05, respectively

3.4 Discussion

3.4.1 Validity of using root to shoot ratios

Root and shoot biomass exhibited an isometric relationship (Fig 3.1), which shows that the root to shoot ratios conformed to basic statistical assumptions and could be used to draw logical inferences according to Snedecor (1946). The regression analysis confirmed the preferential allocation of biomass to shoots, concomitant with its higher C sink capacity (Ludewig and Flügge 2013).

3.4.2 Genotypic and environmental variation

The ANOVA revealed significant effects of genotypes on most traits (Table 3.2) confirming that the population was genetically diverse. This is important as crop improvement depends on the relative importance of these genotypic effects (Aparicio et al. 2015). The higher PCV compared with GCV estimates for traits is an indicator of the presence of other contributing factors to the total variation. The environment and water availability had substantial contributions to the total phenotypic variation, similar to Liu et al. (2015). Significantly lower trait means of greenhouse experiments conducted in summer indicate the impact of combined heat and drought stresses. Similarly, Subira et al. (2016) reported year to year variability in root and above ground biomass in durum wheat evaluated across multiple environments. Low PCV and GCV values (<55%) for GY and RS suggest that their exhibited genetic variation may not be sufficient for significant improvement. New germplasm has to be introduced to widen the genetic base for significant future breeding gains in these traits. Wider differences between PCV and GCV for SB and GY compared to RB showed that the effects of external factors were comparatively stronger on SB and GY. Sharp and Davies (1989) asserted that the impact of water stress was higher in above ground compared to below ground biomass. The significant effects of the environment and water regime on most traits highlight the importance of agronomic practice and site selection for wheat production. However, the interactions may lead to crossover performance of genotypes, which complicates breeding (Yan and Hunt 2001).

3.4.3 Variance and heritability of traits

Generally, higher genotypic variance and heritability estimates for traits under non-stressed conditions (Table 3.3) signify that genotypes expressed higher genetic potential under more favorable growing conditions. Water is an important substrate for many processes that are critical for plant growth (Sanjari and Yazdansepas 2010). However, low to moderate genotypic variance, ranging between 21.8 and 59.4% for SB, RB and RS (Table 3.3) across water regimes, suggests that a substantial proportion of variation was attributable to other factors. This was closer to findings by Rattey et al. (2009) who observed higher environmental than genotypic variance for plant height, total biomass, harvest index and grain yield. This implies that the environment effects may be a hindrance to efforts to improve biomass allocation in wheat even when there is adequate genetic variation in the population.

Heritability values ranged from 46.6 to 96.3% under non-stressed conditions compared with 17.1 to 88.6% under drought (Table 3.3), similar to Ceccarelli, (1989) who observed that even for traits that generally exhibit moderate heritability across environments, their heritability is reduced by exposure to abiotic stress. Plant height was under strong genetic control even under variable conditions, with high heritability (H>79%) and genetic variance (δ_g^2>65%) estimates. Tian et al. (2017) and Liu et al. (2017b) reported that PH has high heritability (above 80%) and is controlled by major QTL such as *Rht* allele, which could explain the stability of its heritability under contrasting environments. The heritability values for DTH, NPT, SL and TKW reported in this study were high and similar to the 65 to 96% range reported by Liu et al. (2017a). Traits with high and stable heritability can be effectively selected for under both conditions (Dalal et al. 2017). Grain yield related traits are less complex and have considerably higher heritability than the grain yield itself, which makes them suitable for indirect selection for grain yield. Low heritability values for SB under both water regimes could be a consequence of wide and frequent fluctuations in the ambient atmosphere. In comparison, heritability values for RB were higher than SB because the soil has a buffering effect and fluctuates less frequently and within a narrow range (Davies and Zhang 1991). Ekanayake et al. (1985) also found root biomass to have high heritability (60%), which could facilitate rapid improvement of root biomass in wheat if selection efficiency in early generations is improved. However, the total root biomass is not the only important trait. Other root traits such as root system architecture, length and the proportion of fine to large roots are also important for drought tolerance (Ekanayake et al. 1985; Lopes and Reynolds 2010).

The heritability for the root to shoot allocation (46.6%) and grain yield (54.5%) were the lowest compared to other traits, under both watering regimes possibly as an indication of their complexity.

Grain yield is known to be a complex trait and its heritability is reduced drastically under stress (Eid 2009). Traits with low heritability are usually controlled by many genes with minor effects (Tsegaye et al. 2012). This will make direct selection for optimal biomass allocation less effective. Heritability of RS and GY is reduced considerably to 28 and 17.1%, respectively (Table 3.3) and their selection response may be lower under drought-stressed conditions since the calculated heritabilities include additive and dominance effects that are highly variable under different environments (Govindaraj et al. 2016). The environmental impact on RS resulted in large differences between PCV and GCV values (Table 3.2) and low heritability estimates (Table 3.3) under both water regimes. This is line with Aparicio et al. (2015) who attributed biomass allocation patterns to environmental changes. The heritability estimates calculated for RS were lower than 78 to 84% in triticale reported by Busemeyer et al. (2013). Saeed and Khalil (2017) reported a heritability value of 35% for grain yield, which is higher than those found in this study. Any improvement of traits with low heritability and GCV values (such as RS and GY) through conventional means will require numerous selection cycles, increasing the cost of developing drought tolerant cultivars.

3.4.4 Genetic correlations among traits

Breeding strategies aimed at enhancing biomass allocation to roots in this wheat population must be pursued with due diligence to avoid compromising the above ground traits that are more favorably correlated to grain yield because RS exhibited negative correlations with all traits except RB (Table 3.4). The size of the root system and its effect on yield is more complex and contradicting results have been found. The results of this study concurred with Fang et al. (2017) and Ehdaie et al. (2012) who asserted that a greater root mass may be more beneficial under water limited environments. In contrast, Zhu and Zhang (2013) argued that larger root systems reduced the amount of assimilates available for grain production. While this study advocates for a higher root biomass, a yield tradeoff is undesirable for food security. It would therefore be advantageous to break this undesirable association and separately improve both the below ground traits and grain yield. This will be possible if below and above ground traits have common as well as distinct underlying genomic loci (Richards et al. 2010). A negative correlation between PH and RS under both water regimes indicates that taller plants in this population had low RS ratios although it may not necessarily mean that they had low root biomass. Similarly, Bai et al. (2013) found a negative correlation of r=-0.33 between plant height and root to shoot ratio in wheat double haploids. The low RS in taller plants may be a result of a faster accumulation rate of the above-ground biomass compared to the below-ground biomass when water availability improves (Smith et al. 2005). It is imperative to select for shorter plants with high RS because a reduction in height may increase the availability of assimilates for the above-ground parts, including grain yield (Song et al. 2009). By inference, negative correlations between RS and most of the above-ground variables could be emanating from the competition for resources between the above and below ground traits, as has been widely reported (e.g. Benincasa et al. 2017; White et al. 2016).

Under drought, RB exhibited stronger positive correlations with RS, PH, SB and GY demonstrating that the size of the root system was pivotal in crop biomass and grain yield when moisture is limiting, which agreed with reports elsewhere (e.g. Ehdaie et al. 2012; Liu et al. 2017b). Similarly, Subira et al. (2016) found strong and positive correlations (r>0.45) among shoot, root and total biomass parameters in tall and semi-dwarf wheat cultivars. The establishment of a large root system may set precedence for efficient soil water and nutrient mobilization required for a higher above ground biomass accumulation.

Plant height is very critical in the above-ground biomass accumulation as shown by positive correlation between PH and SB under non-stressed (r=0.28; p<0.05) and drought-stressed (r=0.72; p<0.05) conditions. These correlations were higher than 0.46 reported by Bai et al (2013) between PH and shoot dry weight in wheat double haploids. Nevertheless, these genetic correlations are helpful in formulating selection criteria specific to this population for developing high yielding genotypes that can also potentially sequester higher amounts of C to the soil compared to the current genotypes.

3.5 Conclusion

The study demonstrated that biomass allocation in wheat is a complex trait and that increasing the RS ratio may compromise GY. In the current population, direct selection for root to shoot biomass allocation pattern will be ineffective to improve drought tolerance in wheat because of its low heritability, high environmental variance and negative association with grain yield. Alternatively, selecting for DTH, PH, RB and TKW will improve drought tolerance and grain yield. In the future, it is important to identify the genomic regions associated with the latter traits to enable marker assisted breeding and breaking unfavorable linkages between negatively correlated traits.

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Chapter 4 Genome-wide association study of drought tolerance and biomass allocation in wheat

Abstract

Genetic analysis of biomass allocation to roots, shoots and grain is important for improving drought tolerance and carbon sequestration capacity of wheat. Genome wide association studies (GWAS) are important in discerning population structure and marker-trait association for marker-assisted breeding. The objectives of this study were to deduce the population structure and genome-wide marker-trait association of yield and yield components and biomass allocation in wheat under drought-stressed and non-stressed conditions. A population including 99 wheat (*Triticum aestivum* L.) and triticale (*Triticosecale* Wittmack) genotypes were phenotyped using the following traits: the number of days to heading (DTH), number of days to maturity (DTM), plant height (PH), spike length (SL), shoot biomass (SB), root biomass (RB), root to shoot ratio (RS), number of kernels per spike (KPS), thousand seed weight (TSW) and grain yield (GY). The test genotypes were genotyped using 28356 DArTseq derived single nucleotide polymorphism (SNPs) markers and subjected to genetic analyses. Population structure analysis revealed seven clusters with a mean polymorphic information content of 0.42, showing a high degree of diversity. A total of 54 significant marker-trait associations (MTAs) were identified. Twenty-one of the MTAs were detected under drought-stressed condition and 11% were on the genomic loci where quantitative trait loci (QTLs) for GY and RB were previously identified, while the remainder are new events providing information on biomass allocation. There were four genetic markers, two each under drought-stressed and non-stressed conditions with pleiotropic effects on RB and SB that may possibly serve as a means for simultaneous selection. Significant MTAs observed in this study will be useful in devising strategies for marker-assisted breeding to improve drought tolerance and to enhance C sequestration capacity of wheat.

Keywords: Biomass allocation, Carbon sequestration, Drought tolerance, Genetic control, Population structure

4.1 Introduction

Wheat is a commodity crop with global harvested area of about 210 million hectares (Portmann et al. 2010). It serves as a food crop for over 2.5 billion people worldwide (Pfeifer et al. 2014). Wheat production and productivity is challenged by numerous biotic and abiotic stresses. Among the major abiotic constraints to wheat production is recurrent drought which is driven by climate change. In addition, the inherently low-fertile soils in sub-Sahara Africa exacerbate the impact of drought stress and hence yield losses in wheat production (Mapfumo et al. 2017). Altering biomass allocation pattern with a focus to improve the root system in modern wheat cultivars has been proposed as a method to improve its drought resilience (White et al. 2015).

One mechanism by which plants respond to environmental stresses is to adjust their biomass allocation (Poorter and Nagel 2000). Therefore, drought tolerance and carbon (C) sequestration potential of crops can be improved by exploiting this plasticity. Enhancing biomass allocation to roots will improve drought tolerance by increasing moisture extraction capability, while promoting soil C input via root exudation and decomposition (Baldock and Skjemstad 2000). However, simultaneous improvement for drought tolerance and C sequestration has not been pursued in crop breeding programs (Paustian et al. 2016), particularly in cereals such as wheat where high grain yield is the primary breeding goal. In addition, there has been less studies on genetic analysis of roots because they are difficult to phenotype (Osmont et al. 2007). More importantly, progress in breeding for drought tolerance or C sequestration is slow because these traits have low heritability due to their polygenic effect. Discovering the underlying genetic markers for the root, shoot and grain biomass will enable marker-assisted selection to improve selection efficiency (Collard and Mackill 2008) and to accelerate development of cultivars with optimal biomass allocation for drought tolerance and C sequestration.

The advent of next generation sequencing (NGS) and genotyping by sequencing (GBS) technologies has provided a means for examining genetic diversity and discovering novel markers (Korte and Farlow 2013). Micro-array based diversity array technology sequencing (DArTseq) derived from single nucleotide polymorphisms (SNPs) have become increasingly important in genome-wide association studies (GWAS) (Qiu et al. 2012). DArTseq derived SNPs have been used extensively on genetic studies of wheat (e.g. Liu et al. 2017; Mwadzingeni et al. 2017;

Sukumaran et al. 2018). These markers are reproducible and provide a powerful means to identify genetic variation at large number of analogous genomic loci. This enables breeders to deduce population structures and genomic loci controlling economic traits through association mapping (Maccaferri et al. 2015).

It is important to deduce associations between markers and traits for accelerated breeding. Association mapping has been employed successfully to elucidate complex traits in wheat using SNPs (Charmet et al. 2009), SSR markers (Breseghello and Sorrells 2006) and DArTs (Crossa et al. 2007). There has been limited GWAS on root traits in wheat compared with other phenotypic traits. Edae et al. (2014) deduced 7 sub-populations in a population structure analysis and detected stable quantitative trait loci (QTLs) for harvest index (located on chromosomes 1A and 5A) in wheat under contrasting water regimes. The QTLs for harvest index may be related to biomass allocation between roots and shoots.

The scarcity of genetic markers and marker-trait associations for biomass allocation and related traits impedes the use of marker assisted selection (MAS) in developing breeding populations for drought tolerance and C sequestration in wheat. Hence, studies on biomass allocation to roots, shoots and grains are required to identify reliable and stable markers. Therefore, the objective of this study was to deduce the population structure and marker-trait associations in a diverse population of wheat genotypes in order to improve selection efficiency and enhanced breeding for drought tolerance and C sequestration capacity.

4.2 Materials and Methods

4.2.1 The germplasm

A sample of 100 genotypes consisting of 99 bread wheat (*Triticum aestivum* L., 2n=6x=42) genotypes and one triticale (*Triticosecale* Wittmack) accession were evaluated. The bread wheat genotypes included 95 drought and heat tolerant genotypes obtained from the International Maize and Wheat Improvement Center (CIMMYT). The test genotypes were purposefully selected for their genetic divergence and breeding history for drought tolerance. The remainder were two local checks, two commercial cultivars adapted to temperate climates and triticale. The temperate commercial cultivars and triticale were included in the study to widen the genetic diversity for rooting ability (Solomon et al. 2007). The commercial varieties have twice the rooting capacity of wheat grown in warmer winters (Thorup-Kristensen et al. 2009). The details of the germplasm are presented in Chapter 2 (Appendix 2.1).

4.2.2 Phenotyping

Data were collected involving three experiments under drought-stressed and non-stressed conditions. The three experiments corresponded to three test environments that included two greenhouse and one field trials. The field and greenhouse experiments were conducted at University of KwaZulu-Natal (UKZN) between 2016 and 2018. The field trial was conducted at Ukulinga Farm of the UKZN (LAT: 29.667 LON: 30.406 and ALT: 811m). In the first greenhouse experiment the 99 genotypes were sown in October in 2016, while the second experiment was established in May 2017. Both trials were conducted using a 10×10 alpha lattice design with 2 replications. The field experiment was established in May 2017 following a similar design. The following phenotypic traits were assessed: the number of days to heading (DTH), number of days to maturity (DTM), plant height (PH), spike length (SL), shoot biomass (SB), root biomass (RB), root to shoot ratio (RS), number of kernels per spike (KPS), thousand seed weight (TSW) and grain yield (GY). Details on trait measurements and units are provided in Chapter 2, section 2.2.4.

General statistics including means, standard error, and coefficient of variation for the phenotypic data were derived in Genstat 18th (Payne et al. 2017). Further, the data were subjected to the Shapiro-Wilk test for normality before analysis of variance in Genstat 18th edition (Payne et al. 2017). The associations among the phenotypic traits was tested using Pearson correlations. Broad sense heritability (H^2) estimates were calculated from phenotypic variance ($\sigma^2{}_p$) and the genotypic variance ($\sigma^2{}_g$) according to Allard (1999) as follows:

$$H = \frac{\delta^2 \mathrm{g}}{\delta^2 p}$$

Where $\delta^2 p = \delta^2\mathrm{g} + \delta^2\mathrm{ge}/\mathrm{e} + \delta^2\mathrm{e}/\mathrm{re}$

Where δ^2p=phenotypic variance, δ^2g=genotypic variance, δ^2ge=genotype × environment interaction variance, δ^2e=residual variance, while r=number of replications and e=number of environments. The inclusion of three environments allows for an effective evaluation of quantitative traits (Gillespie and Turelli 1989; Herzig et al. 2018).

4.2.3 Genotyping

Hundred genotypes comprising of 99 bread wheat and one triticale accession were sequenced. Briefly, genomic DNA was extracted from leaves of 3-week old seedlings. The DNA was extracted using CTAB method (Huang et al. 2000). After extraction, the nucleic acid concentration and purity of the DNA was checked using a NanoDrop 2000 spectrophotometer (ND- 2000 V3.5, NanoDrop Technologies, Inc.) before being shipped to Diversity Arrays Technology (DArT) Pty Ltd, Australia (http://www.diversityarrays.com/dart-mapsequences) for whole genome sequencing on DArTseq platform. Whole-genome genotyping for the 99 wheat genotypes was carried out on the platform developed by Cruz et al. (2013) using 28,356 DArT markers. The markers were integrated into a linkage map by inferring marker order and position from the consensus DArT map. The mean polymorphic information of the silico DArTs was 0.16 and ranged between 0.0 and 0.50 with a reproducibility index of 0.93.

4.2.4 DArTseq SNP filtering

DArTseq SNP derived markers were filtered by imputation to remove bad SNPs with > 5% missing data as previously described by Mwadzingeni et al. (2017). All the individuals were genotyped using 28,356 silico DArT markers assigned to 21 chromosomes. A total of 16,362 DArT markers were used in the analysis after imputing missing values.

4.2.5 Population structure

The allele frequency, alleles per locus and expected heterozygosity were calculated to estimate genetic variation in the 99 accessions. Genetic distances were calculated among the genotypes. Genetic clusters were established based on the neighboring joining method, while the population structure was constructed using the Bayesian clustering procedure in STRUCTURE version 2.3 software following Pritchard et al. (2000). A 10,000 burn-in period and 10,000 Markov Chain Monte Carlo (MCMC) iterations were used to derive the population structure based on 16,362 DArTseq-derived SNP markers distributed across the wheat genome. The K-value was set between 1 and 10 to generate the number of subpopulations in the accessions and reduce the risk of false associations (Gupta et al. 2014). The best K-value for estimating a suitable population size for the dataset was determined by the K-value with the highest likelihood. The DArT markers were coded as a binary value with 1=present and 0=absent after imputation.

4.2.6 Determination of linkage disequilibrium

The GAPIT program in R software was used to conduct linkage disequilibrium analysis following Lipka et al. (2012). Linkage disequilibrium was based on the 16,362 DArT markers whose positions were specific out of 26, 226 polymorphic markers. The squared allele frequency correlations R^2 at p-values <0.001 for each pair of loci were considered to estimate significant linkage disequilibrium.

4.2.7 Association mapping

A total of 16,362 DArTseq derived SNP markers were used to determined marker traits associations in the population. The association mapping was conducted on biomass allocation traits involving RB, SB, GY and RS using a mixed linear model (MLM) method that factors in both population structure and kinship (Gupta et al. 2014) in the GAPIT program of the R software (Lipka et al. 2012). DArT markers were considered as significant by considering each trait individually at a critical value of 1%, which was deemed to be highly stringent and reduces the risk of false marker-trait associations (MTAs) (Gupta et al. 2014).

4.3 Results

4.3.1 Phenotyping variation across genotypes and water regimes

The 3-way interaction involving the levels of the following three factors: genotypes, water regime and test environment was highly significant ($p<0.05$) for NPT, PH, DTM and RS as revealed by the analysis of variance (ANOVA) (Table 4.1). The number of days to maturity (DTM) was significantly ($p<0.05$) affected by the interaction between genotype and water regime. The genotype × test environment interaction effects were significant ($p<0.05$) on all traits except GY. Individually, the genotype, water regime and test environment effects significantly ($p<0.05$) affected all traits except TKW.

Table 4.1 Mean squares after combined analysis of variance for phenotypic traits of 99 wheat genotypes and a triticale accession evaluated across three test environments under drought-stressed and non-stressed conditions

SOV	DF	DTH	NPT	PH	DTM	SL	SB	RB	RS	TKW	GY
Replication (Rep)	1	205	0.37	35.03	181	10.4	3190585	13931	0.07	98.6	1547987
Rep(Block)	18	98.8	10.2	148.4	134	2.54**	236360	18521*	0.10*	60.7**	117122*
Genotype (Gen)	99	317***	13.9***	481***	175.27***	4.30***	556472***	52063***	0.06****	117***	101673**
Environment (Env.)	2	64335***	2987***	126114***	107577***	3382***	633806324***	14483166***	34.5***	9.14	214902285***
Water regime (WR)	1	1290***	2962***	44418***	39924***	420***	43087351***	2835547***	1.12***	3115***	43755727***
Gen.Env	192	107***	10.1***	99.8***	82.3***	1.94***	341187***	15459***	0.04***	38.8*	59370
Gen.WR	96	32.07	5.74	51.2	67.2***	0.87	167573	11016	0.03	28.36	43059
Env.WR	2	2449***	505***	7086***	348.2***	65.5***	12729698***	120902***	1.13***	1501***	486813***
Gen.Env.WR	192	28.61	8.19***	52.9*	52.9**	0.88	149771	9116	0.03*	29.79	58283
Residual	563	26.07	5.29	43.2	40.7	0.91	163807	10428	0.03	28.6	68604
Mean		65.5	11	71.5	113	7.74	1061	259	0.47	44.2	600
%CV		7.79	20.9	9.19	5.67	12.3	38.1	39.4	35.8	12.1	43.7
se		5.11	1.3	4.57	6.38	0.95	105	32.1	0.07	2.35	51.9
Skewness		0.029	0.037	-0.011	0.03	-0.03	0.025	0.09	-0.01	0.048	0.014
Kurtosis		-0.027	-0.048	-0.087	-0.028	-0.066	-0.083	-0.083	-0.084	-0.125	-0.085
GCV		71.4	74.4	79.2	52.9	67	55.1	72.6	51.9	53.7	43.1
PCV		72.8	78.6	80	53.3	69	57.6	75.1	83.8	54.7	49.5

SOV=source of variation, DF=degrees of freedom, DTH=days to heading, NPT=number of productive tillers, PH=plant height, DTM=days to maturity, SL=spike length, SB=shoot biomass weight, RB=root biomass weight, RS=root to shoot ratio, TKW=thousand-grain weight, GY=grain yield, CV=coefficient of variation, se=standard error, GCV=genetic coefficient of variation, PCV=phenotypic coefficient of variation, *, ** and ***=significance level at <0.05, <0.01 and <0.001, respectively.

Mean phenotypic data obtained from three screening experiments was used in this study. The summary statistics including mean, minimum, maximum, quartile 1 (Q1) and quartile 3 (Q3), standard deviation and coefficient of variation for four biomass allocation traits (GY, RB, SB and RS) of the 99 genotypes evaluated under two water conditions are presented in Table 4.2. Root biomass ranged between 75 and 903 g m^{-2} under non-stressed, while it ranged from 64 to 669 g m^{-2} under drought-stressed conditions. A 32% reduction in mean RB due to drought stress was observed. The lowest shoot biomass was 109 g m^{-2} obtained under stressed condition, while the highest was 1244 g m^{-2} with improved water availability. Root to shoot ratios varied between 0.14 in wheat genotype LM39 and 1.45 recorded in triticale. Grain yield declined by 48% under drought-stressed condition. The variance components and heritability for the phenotypic traits are presented in Chapter 3, Section 3.2.3. The traits exhibited varied heritability values. Lower heritability values were calculated for GY (17%) and RS (28%) compared with RB (78%) and SB (64%) under drought-stressed condition. The genetic correlations between the biomass allocation traits were significant (Chapter 3, section 3.3.4), confirming that the germplasm was suitable for use in a genome wide association study involving yield and yield components.

Table 4.2 Summary statistics of biomass and agronomic traits measured in 100 genotypes evaluated in three environments under drought-stressed and non-stressed conditions

	Non-stressed				Drought-stressed			
Statistic	RB (gm^{-2})	SB (gm^{-2})	RS	GY (gm^{-2})	RB (gm^{-2})	SB (gm^{-2})	RS	GY (gm^{-2})
Mean	308	1244	0.50	789	210	876	0.44	409
SEM	8.78	52.22	0.01	26.92	7.38	41.2	0.01	28.5
Median	320	1248	0.51	755	226	896	0.43	456
Minimum	75.3	165	0.14	121	64.3	109	0.11	79.0
Maximum	903	4731	1.45	2633	669	3347	1.20	2779
Q1	145	295	0.27	205	83.9	262	0.24	109
Q3	462	2275	0.71	1310	329	1708	0.66	988
Std. Dev.	215	1277	0.32	649	180	1002	0.29	683
Skewness	-0.06	0.07	0.07	0.37	-0.25	-0.50	-0.17	-0.24
Kurtosis	-0.37	-0.57	-0.17	-0.65	0.08	0.16	-0.13	-0.25
Heritability%	79.3	73.3	46.6	54.5	77.8	64.1	28.0	17.1

RB=root biomass dry weight per m^{-2}; SB=shoot biomass dry weight per m^{-2}; RS=root to shoot ratio; GY=grain weight gm^{-2}; SEM=standard error of mean, Std. Dev. =standard deviation

4.3.2 Population structure analysis

The 99 wheat genotypes and a triticale in the population were grouped into 2 clusters and 7 sub-clusters (Fig 4.1) at the highest mean ln likelihood of -629992.6 at K=7. Cluster 2 had the largest membership with 24.6% of population, while the smallest was Cluster 7 with only 3% (Table 4.3). Clusters 5 and 7 exhibited the highest level of heterozygosity providing within group mean distance of 0.41, while the rest of the groups averaged below 0.17. The mean fixation index (Fst) ranged between 0.01 and 0.85 among the clusters. Cluster 7 was comprised of triticale, the 2 temperate cultivars and one CIMMYT accession (LM39) from the heat nursery, showing the widest heterozygosity. Clusters 1, 2 and 6 comprised of drought and heat tolerant accessions (Table 4.3).

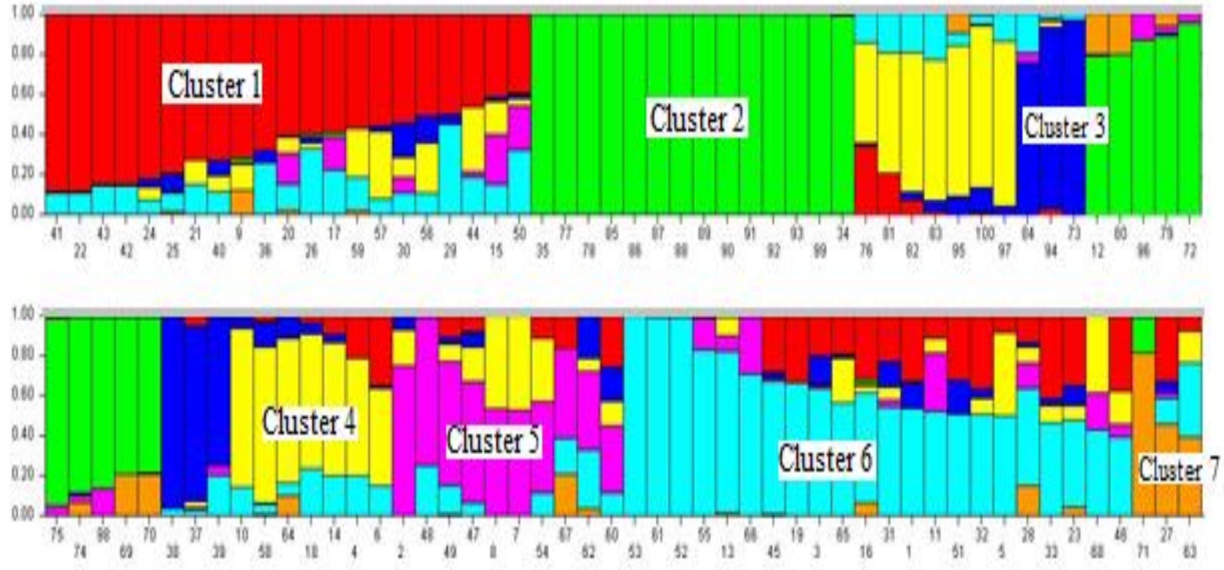

Fig 4.1 Population structure of 99 wheat genotypes and a triticale accession based on 16,362 DArTseq derived SNP markers. The different colored segment estimate proportion of membership of each genotype to the respective clusters. See Table 4.3 for codes of genotypes.

Table 4.3 Genetic clusters and their member genotypes, proportion of membership, expected heterozygosity and the mean values of Fst observed from structure analysis of 99 wheat genotypes and a triticale accession

Cluster	*⸸Genotypes	% Membership	Expected heterozygosity	Mean fixation index
1	LM 55 (41), LM33 (22), LM57 (43), LM36 (24), LM37 (25), LM32 (21) LM54 (40), LM20 (9), LM49 (36), LM31 (20), LM38 (26), LM28 (17) LM85 (59), LM83 (57), LM42 (30), LM82 (56), LM41 (29), LM58 (44) LM26 (15), LM75 (50)	0.199	0.2499	0.4371
2	LM48 (35), BW124 (77), BW127 (78), BW147 (85), BW148 (86), BW149 (87) BW150 (88), BW151 (89), BW152 (90), BW157 (91), BW159 (92), BW162 (93) BW71 (99), LM47 (34), LM23 (12), BW129 (80), BW49 (96), BW128 (79) BW100 (72), BW116 (75), BW111 (74), BW63 (98), LM93 (63)	0.230	0.1747	0.6785
3	BW28 (94), BW103 (73), BW145 (84), LM51 (38), LM50 (37), LM52 (39)	0.061	0.1667	0.7085
4	BW120 (76), BW140 (81), BW141 (82), BW142 (83), BW48 (95), BW80 (100) BW58 (97), LM21 (10), LM84 (58), LM96 (64), LM29 (18), LM25 (14) LM15 (4), LM17 (6)	0.140	0.1734	0.6557
5	LM12 (2), LM71 (48), LM72 (49), LM70 (47), LM19 (8), LM18 (7) LM80 (54), LM99 (67), LM91 (62), LM86 (60)	0.103	0.4112	0.0113
6	LM79 (53), LM90 (61), LM77 (52), LM81 (55), LM24 (13), LM98 (66) LM59 (45), LM30 (19), LM14 (3), LM97 (65), LM27 (16), LM43 (31) LM01 (1), LM22 (11), LM76 (51), LM44 (32), LM16 (5), LM40 (28) LM46 (33), LM35 (23), LM100 (68), LM60 (46)	0.223	0.153	0.656
7	Triticale (71), Arenza (69), Sossogn (70), LM39 (27)	0.040	0.4146	0.85

*number in parenthesis refers to the code used in generating population structure in Fig 4.1; ⸸the description of genotypes is provided in Chapter 3 (Appendix 3.1)

4.3.3 Linkage disequilibrium and genetic distance among genotypes

A total of 10,064 loci pairs within a physical distance extending up to 163831bp were detected by linkage disequilibrium analysis. Only 9.02% of the loci pairs were deemed to be significant at $P < 0.001$, while 0.43% were in complete disequilibrium with $R^2=1$. Linkage disequilibrium was observed to weaken between distant pairs of markers and there was a negative correlation (r=-0.14) between LD and physical distance.

The genetic distance among the different populations was estimated with 16,362 DArTseq derived SNP markers where clusters 3, 4 and 6 exhibited the longest genetic distances of more than 0.30 from Cluster 2 (Table 4.4). Cluster 7 exhibited the shortest genetic distances below 0.10 to all the other clusters except Cluster 2.

Table 4.4 Genetic distances between different clusters obtained from structure analysis of 99 wheat genotypes and a triticale accession

Clusters	1	2	3	4	5	6	7
1	-						
2	0.2919	-					
3	0.1617	0.3271	-				
4	0.1581	0.3319	0.1843	-			
5	0.0651	0.188	0.0971	0.0907	-		
6	0.1575	0.3407	0.1857	0.1888	0.1001	-	
7	0.0638	0.186	0.0962	0.0905	0.0001	0.0994	-

4.3.4 Marker-trait associations under different water regimes

Biomass allocation traits (RB, SB, RS and GY) were subjected to GWAS using the 16,362 DArTseq derived SNP markers. Fifty-four marker traits associations (MTAs) were identified at a stringent P value <0.001 revealing candidate loci for each trait. Manhattan plots for RB, SB, RS and GY were generated in GWAS to show the significant associations (−log (p-value)>3) (Figs 4.2 and 4.3). A quantile-quantile (Q-Q) plot was constructed for each trait under each water regime to confirm their normal distribution (Appendices 4.1 and 4.2). A total of 10 significant ($P<0.001$) MTAs were detected for RB under non-stressed conditions, while only 4 were detected under stressed conditions (Table 4.5). A total of 27 significant ($P < 0.001$) MTAs were detected for SB with 14 being found under stressed condition. Similarly, markers associated with RS were evenly distributed between non-stressed and drought-stressed conditions, with 4 and 5 significant ($P < 0.001$) MTAs, respectively. There were non-significant MTAs for GY under stressed condition, while 8 were detected under non-stress condition. There were four pleiotropic loci which were common for RB and SB. Two of them were detected on chromosome 1B under non-stress, while two were detected on chromosomes 2B and 3B under stressed condition.

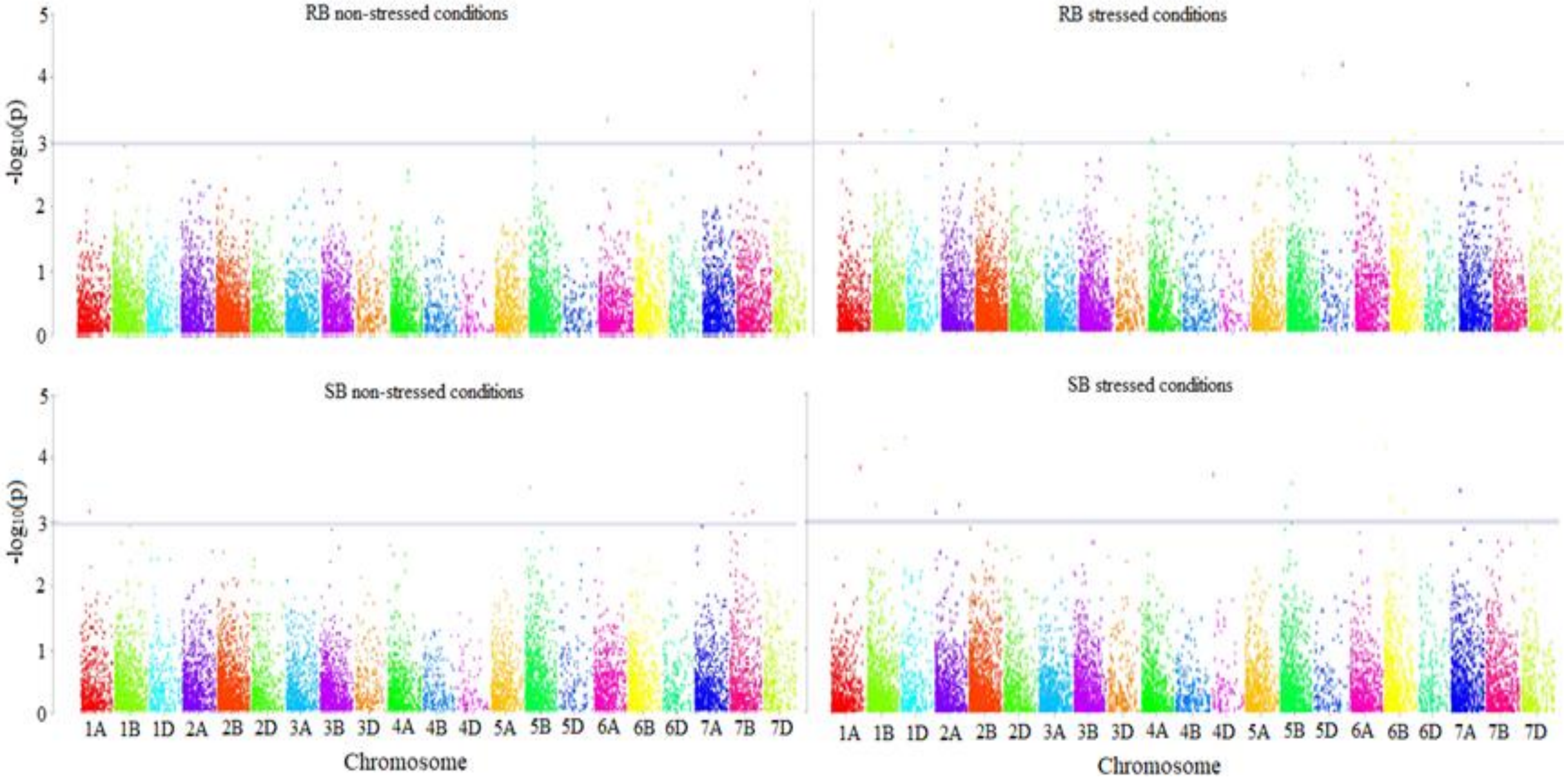

Fig 4.2 Manhattan plots showing the significant marker-trait association involving 99 wheat genotypes and a triticale accession evaluated under drought-stressed and non-stressed conditions for root biomass (RB) and shoot biomass (SB). Only marker-trait associations significant at $p<0.001$ were considered.

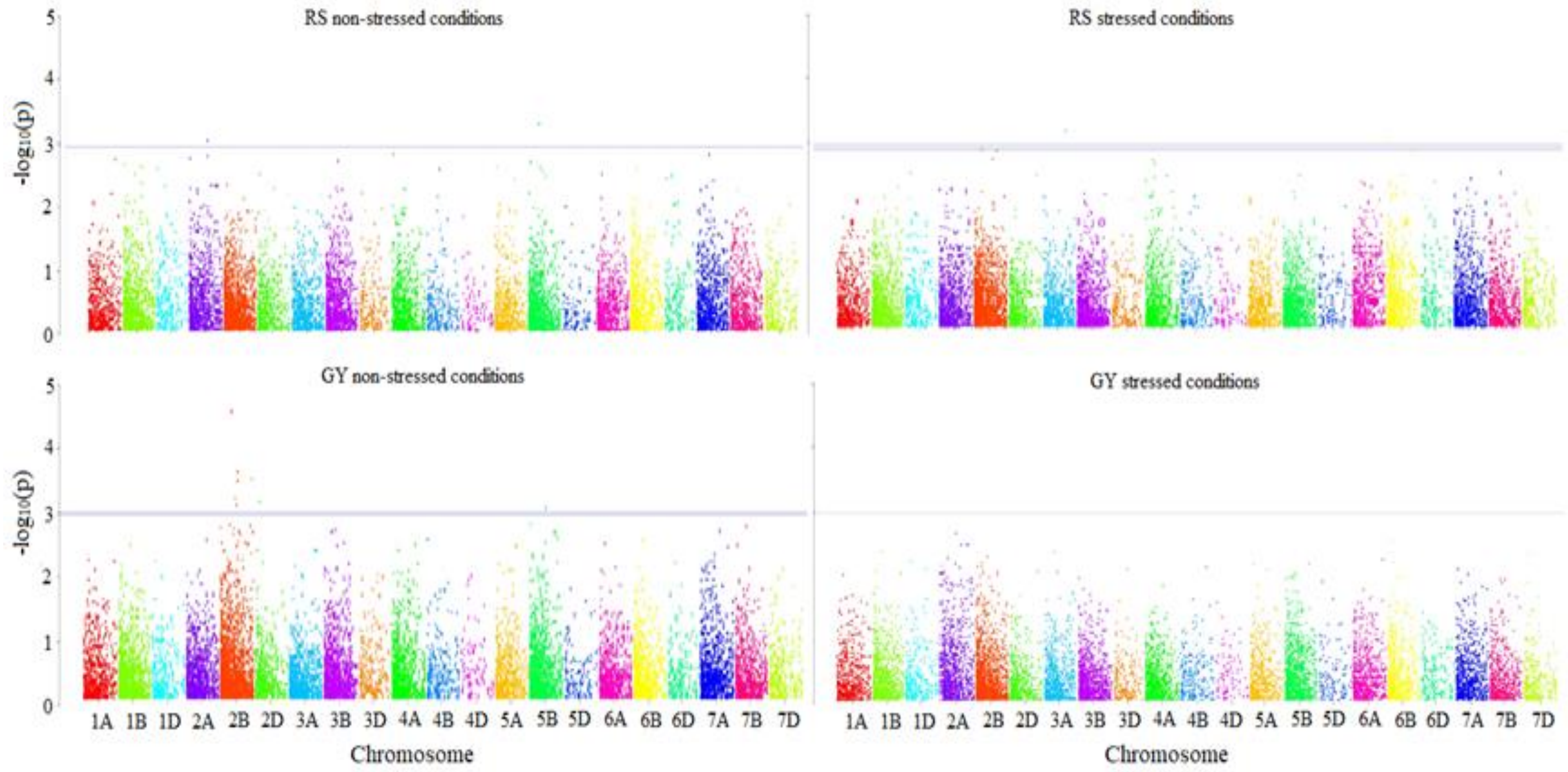

Fig 4.3 Manhattan plots showing the significant marker-trait association involving 99 wheat genotypes and a triticale accession evaluated under drought-stressed and non-stressed conditions for root to shoot ratio (RS) and grain yield (GY). Only marker-trait associations significant at $p<0.001$ were considered.

Table 4.5 Identities of DArTseq derived SNP markers with high association with biomass allocation traits of 99 wheat genotypes and a triticale accession evaluated under non-stressed and drought-stressed conditions.

<table>
<tr><th></th><th colspan="3">Non-stressed</th><th colspan="3">Drought stressed</th></tr>
<tr><th>Trait</th><th>Marker</th><th>P value</th><th>R²</th><th>Marker</th><th>P. value</th><th>R²</th></tr>
<tr><td>Grain yield (GY)</td><td>1B|096.919529820|3951105|3951105</td><td>0.001</td><td>0.110</td><td></td><td></td><td></td></tr>
<tr><td></td><td>4D|046.560672660|1042586|1042586</td><td>0.000</td><td>0.136</td><td></td><td></td><td></td></tr>
<tr><td></td><td>4D|052.445309860|1105329|1105329</td><td>0.000</td><td>0.141</td><td></td><td></td><td></td></tr>
<tr><td></td><td>4D|055.949793250|1251584|1251584</td><td>0.000</td><td>0.197</td><td></td><td></td><td></td></tr>
<tr><td></td><td>4D|074.910798920|1022538|1022538</td><td>0.000</td><td>0.134</td><td></td><td></td><td></td></tr>
<tr><td></td><td>4D|074.910798920|1022538|1022538</td><td>0.001</td><td>0.119</td><td></td><td></td><td></td></tr>
<tr><td></td><td>4D|092.694020460|1078101|1078101</td><td>0.001</td><td>0.112</td><td></td><td></td><td></td></tr>
<tr><td></td><td>5B|139.016174800|988795|988795</td><td>0.001</td><td>0.115</td><td></td><td></td><td></td></tr>
<tr><td>Root biomass (RB)</td><td>1B|040.630655570|3570152|3570152</td><td>0.001</td><td>0.172</td><td>1B|105.042716600|980658|980658</td><td>0.000</td><td>0.235</td></tr>
<tr><td></td><td>1B|149.556316800|1003008|1003008</td><td>0.001</td><td>0.166</td><td>1D|068.349058130|1745081|1745081</td><td>0.000</td><td>0.243</td></tr>
<tr><td></td><td>2A|122.189982600|1088391|1088391</td><td>0.000</td><td>0.188</td><td>2B|078.880456780|1863039|1863039</td><td>0.000</td><td>0.226</td></tr>
<tr><td></td><td>2B|028.016500700|1136582|1136582</td><td>0.001</td><td>0.161</td><td>3B|097.589047780|1025605|1025605</td><td>0.000</td><td>0.260</td></tr>
<tr><td></td><td>3A|022.609241590|5412055|5412055</td><td>0.001</td><td>0.166</td><td></td><td></td><td></td></tr>
<tr><td></td><td>3A|050.873426070|1068273|1068273</td><td>0.000</td><td>0.226</td><td></td><td></td><td></td></tr>
<tr><td></td><td>3A|057.991003790|1027581|1027581</td><td>0.001</td><td>0.176</td><td></td><td></td><td></td></tr>
<tr><td></td><td>3A|108.276835900|3022046|3022046</td><td>0.000</td><td>0.205</td><td></td><td></td><td></td></tr>
<tr><td></td><td>3A|125.207584900|4439815|4439815</td><td>0.001</td><td>0.164</td><td></td><td></td><td></td></tr>
<tr><td></td><td>5B|078.008889670|1091711|1091711</td><td>0.001</td><td>0.158</td><td></td><td></td><td></td></tr>
<tr><td>Shoot biomass (SB)</td><td>1B|040.630655570|3570152|3570152</td><td>0.000</td><td>0.126</td><td>1B|039.453178300|1343256|1343256</td><td>0.001</td><td>0.241</td></tr>
<tr><td></td><td>1B|149.556316800|1003008|1003008</td><td>0.000</td><td>0.127</td><td>1B|102.790287400|1279080|1279080</td><td>0.001</td><td>0.228</td></tr>
<tr><td></td><td>1B|160.880942600|1021859|1021859</td><td>0.001</td><td>0.087</td><td>1B|216.169951100|3064575|3064575</td><td>0.000</td><td>0.257</td></tr>
<tr><td></td><td>2B|067.471137370|1020329|1020329</td><td>0.001</td><td>0.093</td><td>2A|114.031067400|5411386|5411386</td><td>0.000</td><td>0.246</td></tr>
<tr><td></td><td>3A|006.435082769|3533170|3533170</td><td>0.001</td><td>0.105</td><td>2B|049.308495820|985214|985214</td><td>0.001</td><td>0.242</td></tr>
<tr><td></td><td>3A|061.934234200|985496|985496</td><td>0.001</td><td>0.103</td><td>2B|061.073137960|3023643|3023643</td><td>0.000</td><td>0.284</td></tr>
<tr><td></td><td>3A|082.028313990|1043620|1043620</td><td>0.001</td><td>0.092</td><td>2B|076.621010660|1090560|1090560</td><td>0.001</td><td>0.237</td></tr>
<tr><td></td><td>3A|106.335698900|1121298|1121298</td><td>0.001</td><td>0.102</td><td>2B|078.880456780|1863039|1863039</td><td>0.000</td><td>0.251</td></tr>
<tr><td></td><td>3A|117.219677800|1383547|1383547</td><td>0.000</td><td>0.130</td><td>3B|019.126953840|1104851|1104851</td><td>0.000</td><td>0.291</td></tr>
<tr><td></td><td>3A|117.343605600|1017215|1017215</td><td>0.001</td><td>0.085</td><td>3B|026.842033450|981169|981169</td><td>0.001</td><td>0.242</td></tr>
<tr><td></td><td>3A|122.894696300|3025853|3025853</td><td>0.001</td><td>0.087</td><td>3B|053.297285010|989932|989932</td><td>0.001</td><td>0.236</td></tr>
<tr><td></td><td>4A|115.895065100|1130584|1130584</td><td>0.001</td><td>0.105</td><td>3B|097.589047780|1025605|1025605</td><td>0.000</td><td>0.282</td></tr>
<tr><td></td><td>7A|016.280220860|3957571|3957571</td><td>0.001</td><td>0.090</td><td>5B|053.839150260|3534049|3534049</td><td>0.000</td><td>0.269</td></tr>
<tr><td></td><td></td><td></td><td></td><td>7D|070.635822220|1082016|1082016</td><td>0.000</td><td>0.263</td></tr>
<tr><td>Root to shoot ratio (RS)</td><td>1B|148.725447200|1007365|1007365</td><td>0.000</td><td>0.152</td><td>2B|026.534676770|4993300|4993300</td><td>0.001</td><td>0.103</td></tr>
<tr><td></td><td>3B|029.893695730|1113797|1113797</td><td>0.001</td><td>0.131</td><td>2B|026.707409100|1071081|1071081</td><td>0.001</td><td>0.111</td></tr>
<tr><td></td><td>3B|057.720958320|2262365|2262365</td><td>0.001</td><td>0.138</td><td>4A|133.900438100|1126379|1126379</td><td>0.001</td><td>0.105</td></tr>
<tr><td></td><td>7B|089.123102830|1061631|1061631</td><td>0.001</td><td>0.126</td><td>4A|133.900438100|1126379|1126379</td><td>0.001</td><td>0.104</td></tr>
<tr><td></td><td></td><td></td><td></td><td>6A|002.349181058|3028610|3028610</td><td>0.001</td><td>0.121</td></tr>
</table>

P value=the level of significance, only $p \leq 0.001$ was considered; R^2=marker-trait correlation

4.4 Discussion

4.4.1 Phenotypic variability of germplasm and environmental response

Understanding biomass allocation in wheat could provide an opportunity and alternative approach to developing drought tolerant cultivars that can also sequester more C for soil remediation. The 99 wheat genotypes evaluated in this study exhibited wide genetic variation for biomass accumulation and allocation to roots, shoot and grain (Tables 4.1 and 4.2). This allowed GWAS to be successfully conducted. The wide genetic variation was expected since the population included genotypes from CIMMYT, local accessions and temperate adapted cultivars. Biomass accumulation in roots, shoot and grains was significantly reduced by 32, 30 and 48%, respectively, under drought stress confirming that biomass accumulation has phenotypic plasticity. This plasticity could be exploited to improve wheat response to water scarcity (Dalal et al. 2017). The traits exhibited different levels of heritability, with RB (78%) and SB (64%) having higher heritability estimates than RS (28%) and GY (17%). However, the lower heritability estimates observed under stressed conditions could reduce selection efficiency (Dalal et al. 2017) and may impact negatively on QTL detection (Tian et al. 2015).

4.4.2 The structure of the population structure and linkage disequilibrium

The Bayesian method of clustering was used because it eliminates distortions in populations that may have large clusters of closely related members (Maccaferri et al. 2005; Pritchard et al. 2000). Simulation models were carried out on the 100 genotypes with K parameter set between 1 and 10 with the highest log likelihood occurring at K=7. This confirms that the population could be clustered into 7 clusters. The K-value at which the highest log likelihood occurs confirms the number of appropriate clusters for that population (Gupta et al. 2014; Mogga et al. 2018; Mwadzingeni et al. 2017). The mean fixation indices (Fst) associated with Clusters 1, 2, 3, 4, 5, 6 and 7 were 0.44, 0.68, 0.71, 0.66, 0.01, 0.66 and 0.85, respectively. This suggest that there was potentially high level of differentiation between the clusters although within cluster variation was marginal with low heterozygosity estimates (0.17 to 0.41). However, these F_{st} values must be used cautiously in analyzing diversity or differentiation (Jost 2008). The resultant population structure and genetic distances between pairs of clusters observed in this study also confirmed the existence of some level of genetic relatedness. The differentiation between the clusters is a result of systematic breeding in CIMMYT’s wheat genetic resources. However, the low within cluster variation could be a result of common parentage because a substantial number of lines used in this study shared one or more parents leading to some groups being dominated by closely related individuals. The low within cluster variation can be traced to common parents within each cluster. For instance, 13 out of 23 bread wheat genotypes including genotypes BW124, BW147, BW151 and BW159 in Cluster 2 shared a common parent CGSS05B00258T-099TOPY. Parent CROC_1/AE.SQUARROSA was common for 3 genotypes LM79, LM81 and LM90 in Cluster 6, while WBLL1 and ROLF07 were common parents for some genotypes in Cluster 6. The use of common parents is a standard practice in developing modern wheat cultivars in which a small number of elite varieties exhibiting desirable traits are repeatedly crossed to fix the alleles (Hao et al. 2011). Cluster 7 exhibited the highest heterozygosity and differentiation among members because its members were more divergent with triticale, temperate winter cultivars and a drought tolerant accession. The overall mean polymorphic information content (PIC) of 0.42 obtained in this study points to a considerable degree of diversity within the population. The PIC was comparable to those reported by other studies (Lopes et al. 2015; Tadesse et al. 2015). Some recent studies on genetic basis of root growth used bi-parental populations (Ma et al. 2018; Zhai et al. 2018), which restricts the inference of their results to highly specific genetic pedigrees, while the use of a diverse population, such as the one used in this study, can provide more valuable inference (Vos-Fels et al. 2017). The observed markers in LD and markers with non-significant LD is not unique given that other studies reported them as a possible admixtures of genotypes (Neumann et al 2011; Nakamura et al. 2015).

4.4.3 The detected marker-trait associations

The GWAS analysis identified a total of 54 significant markers associated with the biomass allocation traits assessed. Of these, 13 were detected on chromosomes 2A, 3B, 5B and 7B. Other studies have previously detected significant markers for root and shoot biomass on these chromosomes (Vos-Fels et al. 2017; Beyer et al. 2018). Vos-Fels et al. (2017) found extremely rare haplotype variants that increased root growth on chromosome 5B, while Beyer et al. (2018) reported significant SNPs on 1A, 2A, 3B, 5B, 6A, and 7B for root dry weight with the major two QTLs being on 1A and 5B. The remaining significant SNPs detected in this study which have not been reported previously could be novel alleles important in biomass allocation patterns in wheat. The use of phenotypic data collected on mature plants used in this study is more useful since selection at early stages may not reflect trait performance at later growth stages, particularly, for traits that are relevant for drought tolerance and C sequestration.

A pleiotropic locus is associated with and affects the expression of more than one phenotypic trait. Four significant pleiotropic loci were detected for root and shoot biomass, showing that root and shoot biomass has common and distinct genomic loci. Root and shoot biomass shared an association region on chromosomes 1B, 2B and 3B which suggest that this could be the basis for their high genetic correlation as reported by Mathew et al. (2018). The detection of common SNPs for root and shoot biomass provide an opportunity for effective simultaneous improvement using the overlapping markers. There is concern that increasing below ground biomass might negatively affect other economic traits due to linkage drag associated with unfavorable pleiotropy. Richards et al. (2010) asserted that simultaneous improvement of root and above ground traits will only be possible if they have common and distinct genomic loci that can be manipulated independently or simultaneously. Vos-Fels et al. (2017) suggested that unfavorable linkage drag between negatively correlated traits can be overcome by identifying rare recombinant genes. The lack of observed MTAs for GY under stressed conditions was consistent with Edae et al. (2014) and Mwadzingeni et al. (2017) who also found reduced number of MTAs for GY under drought stress in wheat. Grain yield is highly influenced by genotype by environment interaction, which could have negatively impacted the ability to detect its markers under stressed conditions. The identified MTAs under non-stressed conditions are useful for future marker-assisted selection.

4.5 Conclusions

The use of a diverse population which included 99 bread wheat genotypes with different pedigrees and a triticale accession allowed for detection of 54 MTAs for biomass allocation to roots, shoots and grain yield. The identified markers such as 1B|040.630655570|3570152|3570152 and 5B|078.008889670|1091711|1091711 for root biomass and 3B|029.893695730|1113797|1113797 and 7B|089.123102830|1061631|1061631 for RS can be used in marker-assisted selection to improve the root system of wheat, which will increase their inclusion in breeding for drought tolerance and C sequestration. The four markers 1B|040.630655570|3570152|3570152, 1B|149.556316800|1003008|1003008, 2B|078.880456780|1863039|1863039 and 3B|097.589047780|1025605|1025605, which exhibited pleiotropy effects for root and shoot biomass will enable simultaneous selection for above and below ground biomass and indicate that drought tolerance and C sequestration are tightly linked. The identified MTAs on chromosomes 1B, 2B, 3A, 4D and 7A that have not been previously reported could provide novel genes for breeding. This study provides a foundation for marker-assisted breeding for drought tolerance and C sequestration in wheat. Further studies using complementary techniques would be required to corroborate the identified markers.

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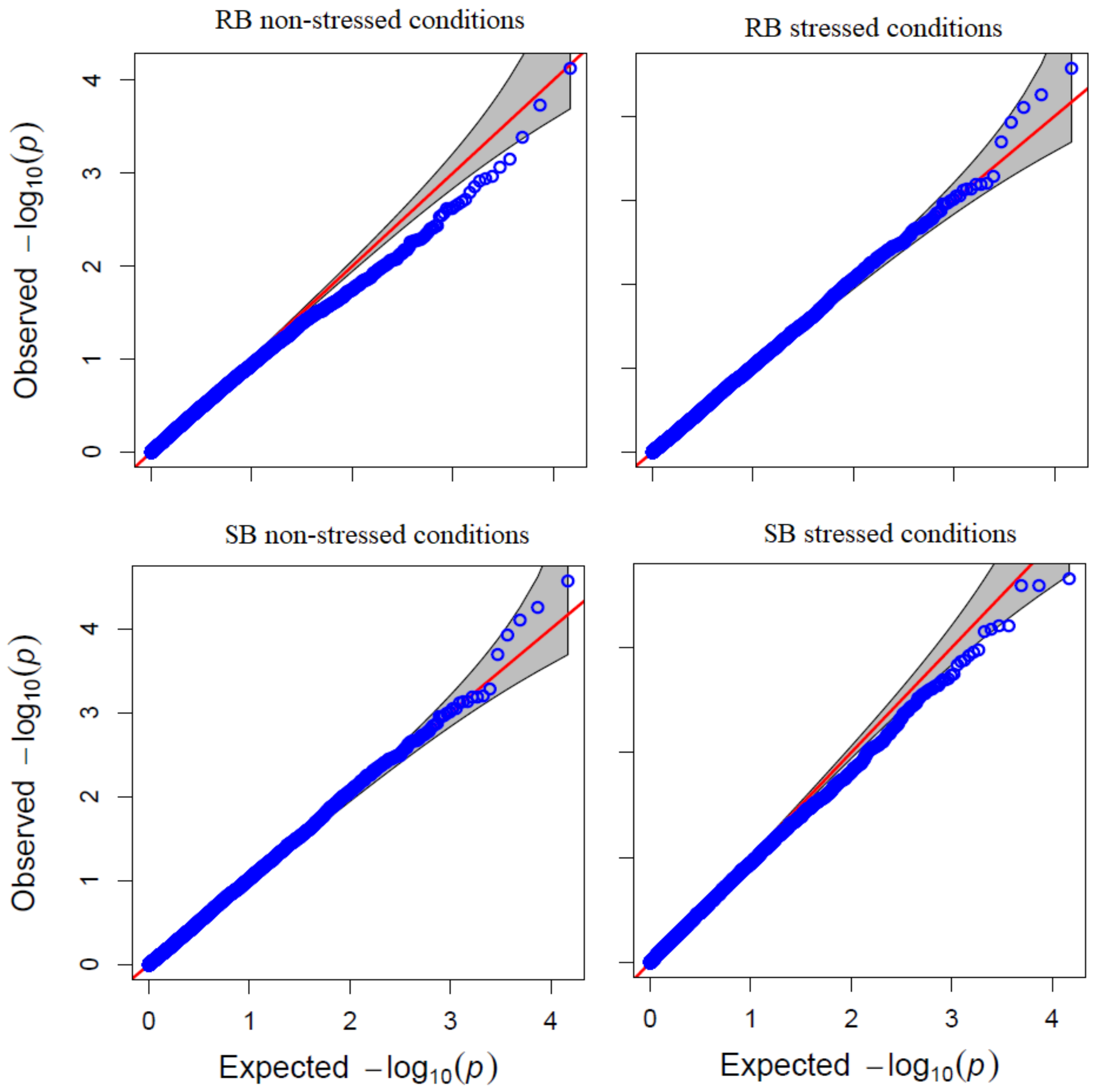
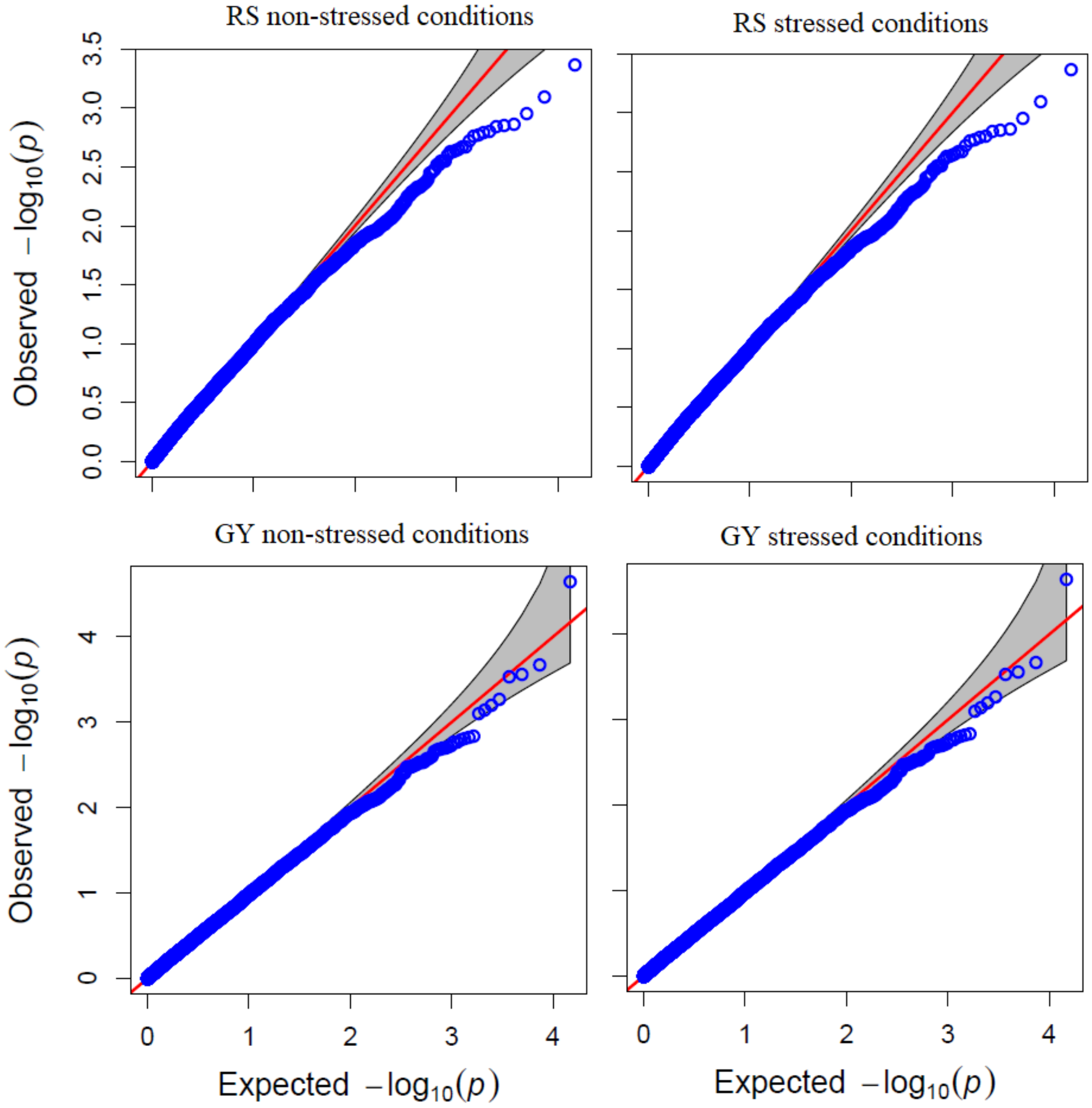


Appendix 4.1 Quantile-Quantile plots indicating the normality of data for root biomass (RB) and shoot biomass (SB) recorded in 99 wheat genotypes and a triticale accession under drought-stressed and non-stressed conditions



Appendix 4.2 Quantile-Quantile plots indicating the normality of data for root to shoot ratios (RS) and grain yield (GY) recorded in 99 wheat genotypes and a triticale accession under drought-stressed and non-stressed conditions

Chapter 5 Combining ability of selected wheat genotypes for drought tolerance and biomass allocation

Abstract

Selection of genotypes with complementary traits and good combining ability are pre-requisites in breeding wheat for drought tolerance and carbon sequestration ability through well-balanced biomass allocation. The aim of this study was to determine the combining ability of selected wheat genotypes for grain yield (GY) and related traits and biomass allocation under drought-stressed (DS) and non-stressed (NS) conditions. Ten parental genotypes were selected and crossed using a half diallel mating design and F_1 families generated. Parents and crosses were evaluated using a randomized complete block design with 2 replications under controlled environment condition. Significant ($p<0.05$) genotype by water regime interaction effect was recorded for root biomass (RB), shoot biomass (SB), root to shoot biomass allocation (RS) and GY. Root and shoot biomass were reduced by 48 and 37%, respectively due to drought stress hindering biomass allocation patterns and hence C sequestration potential of the tested genotypes. Further, drought stress reduced RS and GY by 18 and 28%, respectively compared with the non-stressed treatment. Analysis of variance showed that both general combining ability (GCA) and specific combining ability (SCA) effects were significant ($p<0.05$) conditioning the inheritance of grain yield and related traits and biomass allocation. Non-additive gene effects were more important controlling the inheritance of the measured traits under drought-stressed and non-stressed conditions. Parental genotypes LM48, LM70, LM75, BW140 and BW162 had significant and positive GCA effects for root biomass and GY under both testing conditions. These are recommended for recurrent selection programs to improve the respective traits. The crosses BW141×LM48 and LM47×LM75 were good specific combiners for biomass allocation and GY under drought stress, while LM26×LM70 and LM26×LM71 were good combiners under non-stressed condition. These families were selected for advanced breeding to develop pure line cultivars. Overall, the preliminary results suggest that simultaneous improvement of grain yield and root biomass can be realized to improve drought tolerance and C sequestration ability in wheat.

Key words: Biomass allocation, Combining ability, Grain yield, *Triticum aestivum* L.

5.1 Introduction

Bread wheat (*Triticum aestivum* L., 2n=6x=42) is the third most important cereal crop globally after maize and rice. It is cultivated over 240 million hectares (Portmann et al. 2010) under diverse agro-ecologies (Shiferaw et al. 2013). However, its production and productivity is affected by an array of biotic and abiotic constraints. Recurrent drought and poor soil fertility are the key abiotic constraints to wheat productivity. The spread, intensity and frequency of drought stress has increased due to the effects of climate change, while inherent and human induced soil nutrient deficiencies are widespread in sub-Saharan Africa (SSA) (Zougmoré et al. 2018).

The Consultative Group on International Agricultural Research (CGIAR) through The International Maize and Wheat Improvement Center (CIMMYT), International Centre for Agricultural Research in Dry Areas (ICARDA) and various national breeding programs are engaged in wheat improvement for the drier regions. Developing wheat cultivars adapted for drier environments is particularly important for regions such as SSA where wheat is mainly cultivated under dry-land conditions dependent on residual soil moisture (Haque et al. 2016). There is a need to develop wheat cultivars with deeper and denser root systems for enhanced resilience against recurrent droughts. Drought resilient wheat cultivars with improved root systems and optimal biomass allocation will have better water and nutrient use efficiency and, hence, increased productivity.

Declining soil fertility is a major problem to sustainable wheat production in SSA (Masso et al. 2017). Various studies elaborated the vital contribution of plant residues to soil carbon (C) deposition and ultimately in enhancing soil fertility (De Deyn et al. 2008). About 80% of soil C is reportedly originated from plant roots (Yang et al. 2012). Fostering C input into the soil by crops such as wheat will contribute to improved soil productivity given that organic C is the major constituent of soil organic matter, a key element in nutrient cycling. Consequently, there is a need to develop wheat cultivars that are drought resilient and able to deposit residual C into the soil for optimal crop production.

Simultaneous improvement for drought tolerance and C sequestration ability in wheat requires selection of promising genotypes with balanced biomass allocation pattern. The biomass allocation pattern in the currently grown modern wheat cultivars is not optimized for C sequestration ability. But these cultivars are bred for high grain yield under intensive management systems. This makes them highly susceptible to drought and poor soil fertility stresses under low management systems. Increasing biomass allocation to the root system stimulates the translocation of larger amounts of assimilates below ground (Kell 2011; Lynch and Wojciechowski 2015), which will eventually be stabilized in the soil as organic carbon. High root biomass production promotes high organic matter residue retention for soil reclamation. Plants regulate biomass and C allocation between above and below ground parts to acclimatize to the environment (Poorter et al. 2012). Such phenotypic plasticity can be exploited to foster more biomass allocation to roots and ultimately to increase soil C input. Biomass allocation pattern influences drought tolerance in wheat by determining the absolute size, depth and density of the root system (Haque et al. 2016). There is limited study on the underlying gene action and heritability of drought tolerance and C sequestration ability in wheat to initiate suitable breeding strategies. Selection of complementary breeding parents with high root biomass, root to shoot ratios and grain yield and subsequent knowledge on the inheritance of these traits under drought-stressed and non-stressed conditions is key for breeding. Good combiner parents and crosses are fundamental to select superior progeny and to deduce a suitable breeding strategy.

Gene action conditioning the heritability of traits can be inferred through combining ability analysis (Fasahat et al. 2016). Two types of combining ability effects are broadly distinguished, the general combining ability (GCA) effect of parents, and the specific combining ability (SCA) effect of the progenies. The GCA and SCA effects are associated with additive and non-additive gene action, respectively (Falconer 1967). Parents that exhibit good GCA effect will be useful in population development or maintenance of pure lines. Families with good SCA effect are useful for genetic advancement and development of pure line cultivars. The advent of powerful biometrical tools and mating designs have enabled the routine deduction of combining ability in breeding populations. The most commonly used mating designs to deduce combining ability include the North Carolina Designs (Comstock and Robinson 1948), diallels (Griffing 1956) and the line x tester design (Kempthorne 1957). The choice of the mating design depends on the objectives of the breeding program. The diallel mating design has been extensively used in genetic analysis of agronomic traits such as earliness, plant height and grain yield in wheat (Edwards et al. 1976; Subhani and Chowdhry 2000; Khahani et al. 2017). To our knowledge, there is a paucity of information on combining ability analysis and gene effects controlling root to shoot biomass allocation and C sequestration ability in wheat. Therefore, the objective of this study was to determine the combining ability of selected wheat genotypes for biomass allocation including root and shoot biomass and grain yield and yield-related traits. This information may serve as a guide to identify best performing parents and crosses for cultivar development with optimized biomass allocation for drought tolerance and C sequestration ability.

5.2 Materials and Methods

5.2.1 Site description

The experiment was conducted under controlled environment condition at the University of KwaZulu-Natal during October 2017 to November 2018.

5.2.2 Plant materials

The study used 10 parental genotypes, BW140, BW141, BW152, BW162, LM26, LM47, LM48, LM70, LM71 and LM75 (Table 5.1). The genotypes were selected from an initial population of 100 entries based on their low drought sensitivity indices (DSI) and genetic diversity analyses as presented in Chapter 2 (Table 2.5).

Table 5.1 List of parental genotypes used in generating crosses evaluated in this study

ENTRY CODE	**PEDIGREE**	**Drought sensitivity index**
BW140	Check	0.20
BW141	CGSS05B00243T-099TOPY-099M-099NJ-099NJ-1WGY-0B	0.22
BW152	CGSS05B00258T-099TOPY-099M-099NJ-1WGY-0B	0.27
BW162	CGSS05B00304T-099TOPY-099M-099NJ-099NJ-3WGY-0B	0.27
LM26	ATTILA*2/PBW65//TAM200/TUI	0.22
LM47	FRET2/KUKUNA//FRET2/3/YANAC/4/FRET2/KIRITATI	0.27
LM48	FRET2/KUKUNA//FRET2/3/PASTOR//HXL7573/2*BAU/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	0.28
LM70	Check	0.27
LM71	BABAX/3/PRL/SARA//TSI/VEE#5/4/CROC_1/AE.SQUARROSA (224)//2*OPATA	0.29
LM75	BUC/MN72253//PASTOR	0.28

5.2.3 Crosses

Crosses were initiated with 10 selected parents using a half-diallel mating design (Table 5.2). Crossing blocks were established under greenhouse condition between October 2017 and February 2018. The 10 parents were stagger planted at a week interval for 3 cycles to synchronize flowering. The average temperature and humidity values in the greenhouse were 30/20^{o}C (day/night) and 65%, respectively. Crosses were generated by hand emasculation of florets and subsequent pollination following standard practices for wheat crossing (Curtis and Croy 1958). A total of 38 crosses were successfully generated (Table 5.2). Some of the planned cross combinations were not successful and hence a partial diallel mating design was adopted for this study.

Table 5.2 A 10×10 half-diallel mating scheme in bread wheat showing the successful crosses evaluated for combining ability

Parents	LM26	BW141	BW140	LM48	LM70	BW152	LM47	LM71	LM75	BW162
LM26	**X**				(1) LM26×LM70	(2) LM26×BW152	(3) LM26×LM47	(4) LM26×LM71	(5) LM26×LM75	(6) LM26×BW162
BW141		**X**	(7) BW141×BW140	(8) BW141×LM48	(9) BW141×LM70		(10) BW141×LM47	(11) BW141×LM71	(12) BW141×LM75	(13) BW141×BW162
BW140			**X**	(14) BW140×LM48		(15) BW140×BW152	(16) BW140×LM47		(17) BW140×LM75	(18) BW140×BW162
LM48				**X**	(19) LM48×LM70	(20) LM48×BW152	(21) LM48×LM47	(22) LM48×LM71		(23) LM48×BW162
LM70					**X**	(24) LM70×BW152	(25) LM70×LM47	(26) LM70×LM71	(27) LM70×LM75	(28) LM70×BW162
BW152						**X**	(29) BW152×LM47	(30) BW152×LM71	(31) BW152×LM75	(32) BW152×BW162
LM47							**X**	(33) LM47×LM71	(34) LM47×LM75	(35) LM47×BW162
LM71								**X**	(36) LM71×LM75	(37) LM71×BW162
LM75									**X**	(38) LM75×BW162
BW162										**X**

Numbers in parentheses (1 to 38) denote successful crosses which were evaluated together with the 10 parents (x) for combining ability

5.2.4 Trial management

A total of 48 genotypes (10 parents and 38 F_1 families) were established under greenhouse condition between May and September 2018. Five seeds were sown in 5-litre plastic pots containing 3 kilograms of soil. The number of plants was thinned to three plants per pot three weeks after emergence to achieve about 48 plants per square metre. Fertilizer was applied through an automated drip irrigation system at a rate of 300 kg N and 200 kg P_2O_5 ha^{-1}. During the experiment soil moisture content was monitored by a soil moisture sensor inserted in the center of the pot to a depth of 20 cm and monitored daily. The sensors use electrical capacitance to estimate the moisture content. Irrigation was based on the sensor readings and irrigation back to field capacity was initiated whenever the moisture content fell to 80 and 30% in the non-stressed and drought-stressed treatments, respectively. Two watering treatments (drought-stressed and non-stressed) were maintained until maturity (~120 days). In addition, pests and disease control were carried out as per standard practices.

5.2.5 Data collection

Days to heading (DTH) were recorded as the number of days from the date of planting to the date when 50% of plants in a plot had fully emerged spikes. The number of productive tillers (NPT) were counted per plant and plant height (PH) was measured at maturity stage as the average of three random major tillers per pot made from the soil surface to the tip of the spike, excluding awns. Days to maturity (DTM) were recorded as the number of days from planting to the day when 50% of the plants were dry. The above ground biomass was cut off at the soil surface to separate from below ground biomass. Roots were sampled from the entire soil volume per pot. All root biomass for all the experiments was separated from the soil in a two-step procedure adapted from Hirte et al. (2018). The grain yield (GY), shoot biomass (SB) and root (RB) biomass were weighed after the roots, shoots and grains were dried in an oven with forced air circulation at 60°C for 72 hours. The weight was converted to gram per square meter (gm^{-2}) accordingly using the plant population of 48 plants per square meter. Based on the dry weights of RB and SB, root: shoot (RS) ratios for each genotype were computed. Thousand-kernel weight (TKW) was determined by weighing a random sample of 250 seeds and multiplying by four to extrapolate to 1000 kernels.

5.2.6 Data analysis

The data was checked for normality using the Shapiro-Wilk test and subjected to analysis of variance in Genstat 18th edition (Payne et al. 2017). Means were separated using the Fischer's test procedure. Combining ability analysis was carried out following the partial diallel mating design II analysis using the following linear model presented by Kempthorne and Curnow (1961):

$$Y_{ij} = \mu + r_i + g_i + g_j + s_{ij} + \mathcal{E}_{ijk}$$

where Y_{ij} is the mean for the cross of the i^{th} parent with $_j{}^{th}$ parent, μ is the overall mean, r_i is the replicate effect, g_i and g_j are the main GCA effects of the i^{th} and j^{th} parents, respectively, s_{ij} is the SCA effects obtained from the cross between the i^{th} and $_j{}^{th}$ parents and Ɛijk is experimental error. The GCA estimates were calculated as follows:

$$GCA = X_{ij} - \mu$$

Where, X_{ij} is the mean of the i^{th} parent across j parents and μ is the overall mean.

The SCA estimates were calculated as follows:

$$SCA = X_{ij} - (GCA_i + GCA_j + \mu)$$

Where, Xij is the mean of the cross between i^{th} and j^{th} parents, GCA_i and GCA_j are the GCA estimates of the i^{th} and j^{th} parents in that order and μ is the overall mean. The significance of GCA effects were tested as follows: $t = \frac{GCA}{se}$ and for SCA effects: $t = \frac{SCA}{se}$ and se=standard error of the GCA or SCA estimates.

Variance components attributable to GCA and SCA were deduced from the analysis of variance of the traits according to Hallauer et al. (2010). The ratio of GCA and SCA variance ($2\sigma^2$gca/ $2\sigma^2$gca + σ^2sca) was used to test the relative importance of additive versus non-additive gene action (Baker 1978).

5.3 Results

5.3.1 Analysis of variance

Combined analysis of variance across water regimes revealed that genotype by water regime interaction effects were significant ($p<0.05$) for all traits except DTM (Table 5.3). There was also significant differences ($p<0.05$) among genotypes for all the traits. Water treatment had highly significant ($p<0.01$) effect on all the traits recorded.

Table 5.3 Mean squares and significance tests for grain yield, agronomic and biomass allocation traits of 10 wheat genotypes and their 38 F_1 families evaluated under drought-stressed and non-stressed conditions

SOV	DF	DTH	DTM	NPT	PH	RB	SB	RS	TKW	GY
Replicates	1	448.0	330.0	689.0	937.0	10233.0	25.0	10.0	144.0	564.0
Genotype (Gen)	47	51.9***	113.0	194.0***	401.0***	1446.0***	2173.0**	3.4*	3436.0***	2259.0***
Water treatment (WT)	1	405.1***	1261.1***	1569.5***	736.5***	23466.4***	25727.0***	26.4**	4501***	19264.2***
Gen*WT	47	95.0***	80.4	138.0***	108.0***	788.0***	2530.0*	3.4*	3340***	3083.0***
Residual	95	15.0	62.0	16.0	15.0	248.0	1560.0	2.0	8.0	187.0

SOV=source of variation; DF=degrees of freedom; DTH=number of days to 50% heading; DTM=number of days to maturity; NPT=number of productive tillers; PH=plant height cm; RB=root biomass dry weight gm^{-2}; SB=shoot biomass dry weight gm^{-2}; RS=root to shoot ratio; GY=grain weight gm^{-2} ; *, ** and *** denote significant differences at $p<0.05$, 0.01 and 0.001, respectively.

5.3.2 Mean performance of genotypes

The mean performance of the parents and their progenies showed differential response to drought-stressed and non-stressed conditions (Table 5.4). The mean root biomass was 127.0 g m^{-2} under non-stressed and 63.6 g m^{-2} under drought-stressed conditions. Mean shoot biomass was higher under non-stressed condition (209.0 g m-^{2}) compared with drought-stressed condition (130.0 g m-$^{2)}$. Root to shoot ratios declined by 18% under drought-stressed condition compared to non-stressed condition. Mean grain yield ranged between 80 and 221.0 g m^{-2} under drought-stressed condition, while the range was between 152.0 and 313.0 g m^{-2} under non-stressed conditions. The mean GY decreased by 28% from 216 g m^{-2} under non-stressed condition to 147.0 g m^{-2} under drought stressed condition. BW140 had the highest mean RB, while BW152 and BW162 exhibited consistently higher mean SB and GY under drought stress. Parental genotypes such as LM75 and BW152 and crosses such as LM47×LM75 and BW140×BW152 had the lowest DSI values and were associated with stable GY production across the two water treatments.

The crosses BW152×BW162, LM71×LM75 and LM47×LM75 accumulated higher root biomass under drought stressed compared to all the parental genotypes except LM75 (Table 5.4). There were eight crosses such as LM47×LM75 and LM70×LM47, which accumulated higher shoot biomass than all the parental genotypes under drought stress. Cross LM71×LM75 was among the top families with higher biomass allocation to roots (RS) exhibiting a mean of 0.6 under drought stress. There were 6 crosses: LM47×LM75, BW140×BW152, BW141×LM48, LM70×LM47, BW141×BW162 and LM26×LM70 that accumulated higher grain yield than all the parental genotypes under drought-stressed condition. The families of BW152×LM75, LM26×LM70, LM26×LM71 and BW152×LM71 attained higher grain than all the parental genotypes under non-stressed condition. Cross LM47×LM75 recorded the highest mean GY of 221.0 g m^{-2} under drought-stressed condition and was among the crosses with the lowest DSI.

Table 5.4 Mean values of grain yield, biomass and agronomic traits for 10 wheat genotypes and their 38 F_1 families evaluated under drought-stressed (DS) and non-stressed (NS) conditions

	DTH		DTM		NPT		PH		RB		SB		RS		TKW		GY		DSI
Genotypes	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	
Parents																			
BW140	56.8	69.1	99.2	116.7	16.7	64.1	73.9	85.2	87.4	146.7	95.8	151.3	0.5	1.0	28.1	142.3	184.5	224.9	0.2
BW141	53.8	69.4	110.5	116.5	15.4	43.9	75.7	93.3	44.0	104.8	135.0	247.7	0.4	0.4	34.8	43.8	155.7	210.1	0.3
BW152	63.2	69.3	105.9	119.9	19.5	37.9	89.1	78.6	50.4	108.5	152.3	192.1	0.4	0.6	38.6	44.5	171.0	179.0	0.0
BW162	65.9	79.0	100.1	134.3	26.6	20.3	83.2	89.2	61.1	99.6	163.5	174.6	0.3	0.6	44.8	42.3	177.1	247.3	0.3
LM26	61.0	71.7	106.9	123.1	7.1	-4.9	72.7	86.4	41.4	108.5	100.3	213.7	0.5	0.5	34.1	50.6	128.6	270.5	0.5
LM47	68.1	78.0	108.5	129.0	9.8	25.8	78.1	99.6	54.5	100.6	146.8	237.5	0.4	0.4	46.0	56.9	129.7	224.9	0.4
LM48	63.2	74.5	106.1	123.1	15.0	32.9	80.9	88.2	66.6	104.8	138.9	235.6	0.5	0.4	35.3	57.0	159.5	184.5	0.1
LM70	55.6	70.5	95.4	116.5	10.0	24.5	67.1	77.0	32.2	129.4	74.9	184.3	0.6	0.7	37.3	138.1	107.9	205.7	0.5
LM71	67.0	81.8	105.1	127.8	15.0	18.2	70.6	75.1	66.6	93.9	88.5	163.8	0.6	0.6	44.5	52.9	120.4	160.4	0.2
LM75	56.2	70.8	106.9	117.0	12.4	24.4	78.7	90.3	91.1	100.0	154.7	188.4	0.5	0.5	38.1	46.9	145.5	151.8	0.0
Families																			
BW140×BW152	64.6	78.0	108.4	119.4	23.3	28.8	78.6	86.3	66.1	107.0	153.2	259.1	0.4	0.4	38.7	48.5	200.0	212.2	0.1
BW140×BW162	62.7	77.3	109.3	131.3	16.7	18.4	71.2	87.1	37.0	94.4	98.0	157.4	0.5	0.6	40.2	45.7	146.8	161.7	0.1
BW140×LM47	68.7	70.5	100.1	116.5	18.3	35.5	83.1	94.7	59.6	121.6	156.4	224.7	0.4	0.5	38.3	50.6	183.8	246.0	0.3
BW140×LM48	64.6	75.1	106.1	116.3	4.1	32.2	81.8	79.5	70.9	145.7	84.6	215.4	0.6	0.7	39.2	45.7	103.2	220.8	0.5
BW140×LM75	59.4	79.0	99.2	122.6	13.1	29.3	84.9	108.0	58.9	138.3	98.0	212.2	0.6	0.7	35.5	54.5	125.8	249.8	0.5
BW141×BW140	76.7	62.5	106.0	116.8	4.6	23.1	54.3	65.7	25.8	120.3	150.2	171.8	0.3	0.7	47.6	43.0	167.7	201.6	0.2
BW141×BW162	61.0	72.6	106.1	116.3	19.5	31.8	84.6	95.7	63.9	132.3	148.1	230.6	0.4	0.6	30.8	45.4	189.6	220.3	0.1
BW141×LM47	65.3	73.9	109.0	122.6	11.2	28.8	84.3	94.7	62.2	99.2	129.8	205.3	0.5	0.5	24.8	41.4	146.4	173.4	0.2
BW141×LM48	61.5	75.1	104.3	130.0	17.4	40.9	79.3	80.9	46.7	153.2	150.7	197.9	0.4	0.8	31.6	46.2	195.6	233.5	0.2
BW141×LM70	65.3	79.6	97.6	90.6	15.2	65.4	73.3	102.0	74.5	123.3	113.8	173.8	0.5	0.7	44.2	135.9	127.1	203.5	0.4
BW141×LM71	67.9	60.9	107.6	110.9	5.3	23.7	55.8	69.5	61.0	118.5	121.1	191.7	0.5	0.6	36.3	42.0	148.4	204.4	0.3
BW141×LM75	58.9	77.3	94.6	135.8	21.6	21.0	77.6	105.3	82.9	146.4	98.2	182.6	0.3	0.8	36.0	116.5	122.1	181.5	0.3
BW152×BW162	58.3	74.5	110.5	130.6	18.1	30.2	75.1	85.7	87.9	131.9	133.4	187.7	0.6	0.7	49.1	51.3	158.0	170.8	0.1
BW152×LM47	58.9	77.3	112.6	125.2	10.0	28.8	76.5	99.6	63.2	129.0	121.0	219.5	0.5	0.6	38.8	60.8	142.4	196.0	0.3
BW152×LM71	65.3	78.0	115.4	130.0	4.5	34.8	72.2	108.8	15.6	103.7	71.9	252.5	0.5	0.4	51.0	48.1	84.7	273.5	0.7
BW152×LM75	56.2	70.5	96.1	117.4	10.0	34.8	71.7	97.5	63.9	144.3	112.8	245.7	0.5	0.6	48.6	50.9	111.2	312.5	0.6
LM26×BW152	62.1	72.6	111.4	120.8	15.0	38.6	77.0	83.6	63.9	146.8	142.2	147.4	0.4	1.0	34.8	152.7	157.9	248.8	0.4
LM26×BW162	65.3	75.5	109.7	125.8	13.9	36.9	67.4	82.3	58.4	169.8	113.0	226.5	0.5	0.7	40.6	45.4	139.4	269.8	0.5
LM26×LM47	66.2	72.2	112.1	121.9	16.7	30.2	80.9	86.3	72.0	135.8	149.8	176.6	0.4	0.8	39.0	49.9	158.0	169.9	0.1
LM26×LM70	65.9	75.5	98.5	119.0	21.2	34.8	96.6	91.5	83.2	141.7	169.1	296.6	0.4	0.5	33.7	45.4	185.7	291.0	0.4
LM26×LM71	67.0	78.0	105.9	125.6	13.9	36.9	65.8	99.6	78.2	131.7	129.4	271.9	0.5	0.5	50.1	52.7	117.0	278.2	0.6
LM26×LM75	70.5	58.4	105.9	115.3	-0.8	22.6	53.9	61.9	58.1	119.9	154.8	177.4	0.4	0.7	44.4	43.7	127.8	210.3	0.4
LM47×BW162	65.3	75.1	111.8	125.6	11.6	38.6	68.9	82.8	84.2	155.5	131.3	214.7	0.6	0.7	35.0	50.5	140.8	233.7	0.4
LM47×LM71	63.2	79.0	111.8	132.3	24.2	45.8	68.5	72.1	77.7	166.9	170.2	236.2	0.4	0.7	32.5	45.1	124.0	166.8	0.3
LM47×LM75	57.7	76.1	103.4	121.5	30.2	27.1	93.9	102.1	90.6	164.3	182.0	200.2	0.4	0.8	39.7	56.4	221.0	233.1	0.1
LM48×BW152	63.8	72.0	113.6	117.4	16.3	23.7	72.1	97.5	65.0	123.4	114.9	210.5	0.5	0.6	35.5	115.1	80.4	177.8	0.5
LM48×BW162	59.4	73.9	105.2	125.2	11.6	41.3	78.1	81.5	53.6	101.6	125.0	184.1	0.5	0.6	36.3	40.6	148.2	173.3	0.1
LM48×LM47	60.4	79.6	101.8	118.5	13.3	27.9	76.4	86.6	82.9	114.5	116.0	192.3	0.6	0.6	41.4	47.1	146.2	213.6	0.3
LM48×LM70	65.3	71.2	110.6	116.3	13.1	48.1	80.5	93.3	85.2	139.4	136.3	237.5	0.5	0.6	37.4	246.3	121.3	156.3	0.2
LM48×LM71	65.9	81.7	109.0	125.8	24.9	28.4	87.1	86.4	56.1	102.6	166.2	194.9	0.3	0.5	36.6	50.5	182.1	202.9	0.1
LM70×BW152	61.5	74.5	105.2	130.0	14.6	33.6	88.0	87.1	39.1	98.2	138.3	232.4	0.4	0.4	46.0	161.0	167.2	262.2	0.4
LM70×BW162	69.4	74.5	101.8	121.9	23.3	24.5	94.7	85.0	68.7	155.5	171.4	212.5	0.3	0.7	38.7	45.4	177.6	231.4	0.2

Table 5.4 continued

	DTH		**DTM**		**NPT**		**PH**		**RB**		**SB**		**RS**		**TKW**		**GY**		**DSI**
Genotypes	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	
LM70×LM47	64.6	80.6	100.8	131.3	23.7	20.7	75.5	77.1	84.7	112.3	180.3	195.3	0.4	0.6	49.5	48.2	190.0	205.1	0.1
LM70×LM71	70.2	71.2	113.6	117.0	13.9	22.8	71.1	87.1	74.0	116.8	79.8	207.5	0.6	0.6	38.8	42.0	113.6	228.9	0.5
LM70×LM75	63.8	81.8	105.1	121.9	17.8	28.8	82.7	99.6	56.0	139.6	118.6	216.2	0.5	0.6	30.8	48.6	134.4	188.1	0.3
LM71×BW162	68.4	72.6	114.3	127.1	16.7	35.5	79.8	87.1	62.3	181.9	129.3	239.2	0.5	0.8	32.0	54.5	156.6	253.0	0.4
LM71×LM75	66.7	69.1	105.9	117.4	7.1	29.3	72.7	89.1	90.3	118.9	128.1	211.3	0.6	0.6	44.3	43.8	105.6	223.4	0.5
LM75×BW162	57.0	70.8	92.5	123.1	8.3	29.3	71.5	105.5	32.8	140.3	118.7	215.3	0.4	0.7	37.4	53.2	108.0	249.8	0.6
MEAN	63.5	74.0	105.8	122.0	14.8	31.2	76.6	88.5	63.6	126.7	130.3	208.6	0.5	0.6	38.9	65.4	146.6	216.4	0.3
LSD (5%)	7.55		19.8		6.01		7.76		7.36		31.2		0.06		5.73		11.6		0.05
CV (%)	5.56		7.59		18.2		4.84		16.8		16.5		33.9		6.62		6.93		8.45
SE	3.81		13.67		4.04		3.92		2.22		5.76		0.05		2.89		7.86		0.02

DS=drought stressed condition; NS=non-stressed condition; DTH=number of days to 50% heading; DTM=number of days to maturity; NPT=number of productive tillers; PH=plant height cm; RB=root biomass dry weight gm^{-2}; SB=shoot biomass dry weight gm^{-2}; RS=root to shoot ratio; SL=spike length; TKW=thousand kernel weight g1000^{-1} seeds; GY=grain weight gm^{-2}; DSI=drought sensitivity index; SE=standard error; LSD=least significant difference at the 0.05 probability level; CV=coefficient of variation

5.3.3 Combining ability effects

The analysis of variance for yield, biomass and agronomic traits of wheat genotypes revealed that both GCA and SCA effects were significant ($p<0.05$) for all traits under drought stress (Table 5.5). Under non-stressed condition, GCA effects were significant ($p<0.05$) for DTH, PH, RB, TKW and GY. All traits, except TKW, exhibited significant SCA mean squares. GY under drought stressed exhibited the highest proportion of variance due to GCA, while it was the least for RS under non-stressed condition. The σ^2GCA/σ^2SCA ratios were below unity for all traits. The highest σ^2GCA/σ^2SCA ratio was calculated for RB (0.4) under non-stressed condition followed by SB (0.3) under drought-stressed condition indicating that non-additive genetic effect is important controlling these traits.

5.3.4 General combining ability effects of parents

The GCA estimates showed that LM47, LM75 and BW140 had positive and significant GCA effects for RB under drought-stressed condition (Table 5.6). LM47 had the highest GCA effects for RB (8.0), while BW141 exhibited the least (-12.9) for RB under drought-stressed condition. Under non-stressed condition, LM70 had the highest GCA effects (11.6) for RB followed by BW162 (4.5). Parents LM47, LM75 and BW140 also exhibited significant and positive GCA effects for SB under drought stressed condition, while LM71 and LM48 exhibited significant but undesirable GCA effects for SB under drought stressed condition. The highest GCA estimates for SB under non-stressed condition were recorded in LM26 followed by BW152 and LM70. BW162 and BW140 had significant but negative GCA effects for SB under non-stressed condition. Only LM48 and LM71 had positive and significant GCA effects for RS under drought-stressed condition. Conversely, BW141, BW162 and LM47 exhibited negative and significant GCA values for RS under drought stress. Under non-stressed condition, only five parents exhibited significant GCA effects for RS. The parents BW162 and LM70 had positive GCA effects, while BW141, BW152 and LM26 exhibited negative and significant GCA effects for RS. The parents LM75 and BW152 exhibited desirable negative GCA effects for DTH, while LM75 also exhibited negative GCA effects for DTM under drought stress. BW141 and LM26 had significant and negative GCA effects for DTH under non-stressed condition. However, theses genotypes had undesirable GCA effects for DTM, NPT and TKW. Parental genotypes BW162 and LM47 exhibited the highest positive GCA effects for NPT under drought-stressed condition. The highest GCA effects for

TKW were realized in the parent BW152 and LM71. Parents LM26, LM71 and BW162 exhibited significant positive GCA for GY under non-stressed condition, while BW162, BW140, LM47 and BW141 were good combiners for GY under drought.

Table 5.5 Mean squares and significance tests for general and specific combining ability effects, genetic variance and their ratios for agronomic traits, biomass allocation and grain yield from 10 parental wheat genotypes and their 38 F_1 families evaluated under drought-stressed and non-stressed conditions

					Drought stressed					
Parameter	**DF**	**DTH**	**DTM**	**NPT**	**PH**	**RB**	**SB**	**RS**	**TKW**	**GY**
GCA	9	56.1*	64.8*	37.6*	98.3**	447.0*	637.0**	0.005**	31.9*	1565.0**
SCA	38	25.7**	26.3***	53.5*	110.6***	393.0***	828.0**	0.009***	36.6**	1412.0***
Residual	48	11.9	9.3	4.7	9.2	127.5	208.6	0.002	6.1	157.2
δ^2GCA		2.5	3.2	5.5	13.5	59.1	174.6	0.001	4.4	297.7
δ^2SCA		13.8	17.1	48.8	101.5	265.4	619.6	0.007	30.5	1254.9
δ^2GCA/ δ^2SCA		0.2	0.2	0.1	0.1	0.2	0.3	0.121	0.1	0.2
					Non-stressed					
	DF	**DTH**	**DTM**	**NPT**	**PH**	**RB**	**SB**	**RS**	**TKW**	**GY**
GCA	9	53.2*	58.9	85.9	90.0*	342.0*	1127	0.01	25.41*	1272.0*
SCA	38	40.6***	38.1***	121.0***	157.0***	790.0***	1123.0***	0.01***	41.5	1165.0***
Residual	48	6.5	6.8	17.7	14.5	211.7	38.6	0.00	8.7	251.9
δ^2GCA		4.2	7.5	12.5	33.1	60.5	151.8	0.01	3.8	169.2
δ^2SCA		34.1	31.3	102.8	142.2	166.5	1083.9	0.05	23.1	913.2
δ^2GCA/ δ^2SCA		0.1	0.2	0.1	0.2	0.4	0.1	0.30	0.2	0.2

SOV=source of variation; DF=degrees of freedom; GCA=general combining ability effects; SCA=specific combining ability effects; δ^2GCA=variance of general combing ability; δ^2SCA=variance of specific combining ability; DTH=number of days to 50% heading; DTM=number of days to maturity; NPT=number of productive tillers; PH=plant height cm; RB=root biomass dry weight gm^{-2}; SB=shoot biomass dry weight gm^{-2}; RS=root to shoot ratio; GY=grain weight gm^{-2}; *, ** and ***=significance at $p<0.05$, 0.01 and 0.001, respectively.

Table 5.6 Estimates of general combining ability effects for agronomic traits, biomass allocation and grain yield of 10 wheat genotypes evaluated under drought-stressed (DS) and non-stressed (NS) conditions

	DTH		**DTM**		**NPT**		**PH**		**RB**		**SB**		**RS**		**TKW**		**GY**	
Parents	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS
BW140	1.6*	-1.4*	-0.90	-3.8***	-1.10	3.2**	-0.90	-3.1**	3.4*	-3.2*	4.7*	-10.2**	0.00	-0.01	-0.47	-0.8*	8.2**	0.20
BW141	-0.04	-4.0***	-1.10	-0.40	-1.4*	2.7*	-4.6***	-1.8*	-12.9***	-1.90	3.00	-5.5*	-0.04**	-0.02*	-2.2***	-2.7***	7.7**	-8.3**
BW152	-2.5**	-0.50	1.4*	1.00	0.40	1.3*	1.6*	1.40	-5.00*	-7.6**	-4.5*	11.3***	0.00	-0.05**	2.8***	1.2**	-4.7*	-5.2*
BW162	-0.70	2.0**	-0.30	3.9***	3.5**	-0.80	2.6**	1.5*	-1.60	4.5*	2.00	-11.3***	-0.02*	0.06**	-0.06	-1.1**	17.0***	8.1**
LM26	2.6**	-1.4*	2.6**	0.20	-1.70	-4.8***	-1.8*	-1.6*	-0.80	0.40	-4.20*	17.9***	0.01	-0.02*	-1.0*	-0.52	-2.30	25.0***
LM47	0.02	3.3**	1.3*	2.7**	2.3**	-0.20	3.2**	3.5**	8.0**	-0.70	13.3*	0.20	-0.02*	0.01	0.05	2.8***	13.1***	-0.50
LM48	-0.80	1.10	1.10	-1.5*	-0.80	2.6*	1.8*	-0.80	2.70	0.60	-5.6*	1.91	0.02*	0.01	-2.3**	-0.44	-7.16**	-14.1***
LM70	-0.10	0.60	-2.9**	-0.09	1.1*	1.1*	2.6**	-1.20	1.80	11.6***	-2.80	7.5**	0.01	0.02*	1.0*	1.3**	-0.90	-1.30
LM71	3.5**	1.5*	3.0**	0.03	-0.90	-1.6*	-4.3***	-2.9**	-2.50	-5.2*	-12.2*	-4.8*	0.02**	0.00	1.8**	-0.21	-19.3***	4.1*
LM75	-3.6***	-1.06	-4.0***	-2.2**	-1.4*	-3.4**	-0.10	5.0***	6.8**	1.40	6.3*	-7.0*	0.01	0.01	0.6	0.7*	-11.7***	-3.2*
SE	0.68	0.57	0.68	0.58	0.49	0.85	0.68	0.87	1.93	1.69	2.3	1.39	0.01	0.01	0.12	0.14	1.14	1.45

DTH=number of days to 50% heading; DTM=number of days to maturity; NPT=number of productive tillers; PH=plant height cm; RB=root biomass dry weight gm^{-2}; SB=shoot biomass dry weight gm^{-2}; RS=root to shoot ratio; GY=grain weight gm^{-2}; SE=standard error; *, ** and ***=significance at $p<0.05$, 0.01 and 0.001, respectively.

5.3.5 Specific combining ability effects of crosses

Fourteen families had positive and significant SCA values for RB ranging between 8.8 and 31.8 under drought stress, which is in a desirable direction (Table 5.7). The family BW141×BW162 had the highest SCA value (31.8), while LM26×LM75 had the lowest SCA value (-47.9) for RB under drought-stressed condition. Under non-stress condition, there were 16 crosses that recorded positive and significant SCA values for RB ranging between 12.0 (recorded in the family LM47×LM71) and 97.3 (family BW141×LM70). The family LM26XLM47 exhibited the highest positive and significant SCA effects of 50.3 for SB under drought stress (Table 5.7). Parental genotype BW162 was involved in the crosses BW152×BW162 (exhibiting SCA value of 35.3) and LM70×BW162 (30.9) for SB under drought-stressed condition. BW141 was the progenitor of the families BW141×LM48 (with SCA value of 28.7) and BW141×LM71 (25.4), which were among the families with the highest positive SCA effects for SB under drought-stressed condition. Under non-stress condition, BW140 and BW152 each had families showing significant but negative SCA values for SB in undesirable direction. However, BW152 combined well with LM26, LM48 and BW162 resulting in positive SCA for SB under non-stress condition. The significant SCA values for RS ranged between -0.2 and 0.1 under drought stress. The least combiners for RS under drought stress were LM26×LM47 although LM26 was involved in other families such as LM26×BW152, LM26×LM70 and LM26×LM71, which exhibited positive SCA for RS under drought-stressed condition. Under non-stress condition, LM70×LM47 exhibited the lowest SCA for RS (-0.13), while BW141×LM70 recorded the highest (0.3).

There were 13 families that exhibited desirable negative and significant SCA effects for DTH, while there were 7 and 18 families with desirable effects for DTM and PH, respectively, under drought-stressed condition (Table 5.7). For NPT and TKW, there were 11 and 15 families, respectively, that exhibited desirable positive SCA effects. The following four families: LM70×LM71, LM70×LM75, LM26×BW162 and BW140×BW152 exhibited desirable negative SCA effects for DTH and DTM. For reducing PH under drought-stressed conditions, BW162 could be a good parent as it was a progenitor of three families: BW140×BW162, LM48×BW162 and LM70×BW162, which exhibited the highest negative and significant SCA values for PH. The parent LM48 was involved in LM48×BW152, LM48×LM47 and BW141×LM48 that exhibited the most positive SCA effects on NPT under drought stress (Table 5.7).

Table 5.7 Estimates of specific combining ability effects for agronomic traits, biomass allocation and yield for 38 F_1 families of wheat evaluated under drought-stressed (DS) and non-stressed (NS) conditions

	DTH		**DTM**		**NPT**		**PH**		**RB**		**SB**		**RS**		**TKW**		**GY**	
Family	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS
BW140XBW152	-3.0***	2.4*	-6.5***	-3.3*	-2.0	-5.1*	-3.6*	-2.7	9.4**	-13.5***	-25.4***	-21.9***	0.1***	0.0	4.6***	-3.4***	-10.6	20.9**
BW140XBW162	7.2***	-11.9***	1.5	-5.6***	-11.5***	0.2	-19.3***	-28.2***	-10.5***	-10.0**	19.0***	-50.4***	-0.1***	0.1***	5.7***	-4.6***	-9.1	-18.8**
BW140XLM47	1.1	1.9*	2.9**	-0.9	-3.5**	-4.4**	10.7***	6.2**	4.4	-26.2***	-20.2***	3.2	0.1***	-0.1***	-12.1***	-6.8***	-25.3***	-25.2***
BW140XLM48	2.9***	-1.4	5.3***	-2.7*	2.9*	-10.8***	-6.4***	10.3***	4.5	2.3	-8.6	-9.7	0.0	0.0	-4.0***	-3.8***	-58.6***	-31.7***
BW140XLM75	-2.9***	1.7	4.***	0.2	-6.5***	-3.0	-3.4*	8.0***	-2.5	9.3	-21.5***	-3.2	0.1***	0.0	-3.1**	8.7***	-16.9**	-5.6
BW141XBW140	10.9***	-4.9***	2.3*	-1.5	-6.7***	-13.5***	-15.3***	-16.1***	-27.3***	-2.6	8.7	-31.5***	-0.1***	0.2***	11.1***	-1.5	1.0	2.3
BW141XBW162	-2.8*	0.2	3.5***	1.9	0.5	-0.9	-4.3*	-3.9*	31.8***	7.0	2.2	-26.2***	0.1***	0.1***	7.3***	3.1***	-1.5	-34.4***
BW141XLM47	3.3***	9.4***	6.3***	3.2*	4.3***	0.4	5.1**	9.1***	-15.4***	-1.5	-18.5***	6.7	0.0	0.0	-9.9***	-1.5	-3.8	-14.9*
BW141XLM48	-2.9***	1.0	0.3	-0.1	5.6***	0.0	15.7***	6.9***	13.1***	35.5***	28.7***	-1.9	0.0	0.2***	-0.0	4.8***	80.8***	27.2***
BW141XLM70	2.0**	-3.3***	6.6***	-3.5**	-1.1	13.8***	1.0	8.7***	17.9***	97.3***	14.2**	50.9***	0.0	0.3***	-0.3	-2.7**	13.6*	-33.2***
BW141XLM71	9.9***	-8.8***	-1.0	-17.0***	-12.5***	-7.2***	-9.7***	-11.8***	9.4**	-2.7	25.4***	-24.5***	0.0	0.1***	0.5	-4.2***	-71.1***	-13.6*
BW141XLM75	1.4	-3.7***	5.7***	1.0	0.4	7.2***	6.3***	1.9	3.6	54.6***	5.7	38.0***	0.0	0.1***	-8.9***	7.6***	8.0	38.5***
BW152XBW162	-4.5***	1.5	1.6	5.2***	7.2***	16.9***	-5.5**	-15.1***	9.4**	44.7***	35.3***	23.8***	-0.1***	0.1***	-8.4***	-5.6***	44.8***	-44.1***
BW152XLM47	-0.7	-2.5**	2.3*	-1.9	2.4*	4.5**	4.4**	-2.3	2.2	8.1	7.0	-58.7***	0.0	0.2***	0.8	-0.6	8.3	-65.7***
BW152XLM71	15.3***	-13.7***	1.2	-1.9	-6.7***	-5.3***	-14.8***	-17.3***	-21.7***	-9.9**	-25.6***	-51.9***	0.1***	0.1***	3.0**	-2.6**	-16.0**	-25.2***
BW152XLM75	-2.4**	3.9***	4.7***	7.2***	0.6	-14.6***	-5.6**	2.1	-27.6***	-35.0***	-42.5***	-43.1***	0.1***	0.0	1.6	-0.5	-29.2***	-48.9***
LM26XBW152	-0.4	2.6**	0.1	0.1	-7.9***	-4.2**	5.8***	-3.2	2.1	20.2***	-48.3***	14.8**	0.1***	0.1***	3.0*	-1.2	-48.6***	27.4***
LM26XBW162	-3.3***	5.2***	-5.6***	3.7**	2.9*	12.6***	-3.2	17.5***	18.9***	8.5	12.1**	40.0***	0.0	-0.1***	10.2***	5.2***	-12.2*	41.9***
LM26XLM47	-1.0	6.4***	-1.0	5.9***	2.8*	-3.3*	14.6***	3.4	-6.8*	-20.8***	50.3***	-13.0*	-0.2***	-0.1***	-1.9	3.0***	78.5***	-15.2*
LM26XLM70	2.7***	-3.6***	7.8***	-3.5**	0.0	-7.4***	-2.3	4.5*	-11.9***	-17.6***	-38.9***	-2.4	0.1***	-0.1***	-3.1**	-7.2***	-17.0**	18.7**
LM26XLM71	2.7***	-4.1***	1.1	-1.0	-4.3***	3.6*	2.0	0.2	23.3***	-5.4	0.3	17.0**	0.1***	-0.1***	2.8**	-4.9***	-14.3*	15.2*
LM26XLM75	0.3	-9.3***	-0.1	-10.8***	-6.1***	-8.1***	-10.3***	-12.6***	-47.9***	-2.4	-3.5	-8.8	-0.1***	0.0	-2.4*	-3.2***	9.2	1.3
LM47XBW162	-0.9	2.1*	1.5	0.3	-1.6	11.9***	-8.6***	-4.3*	-1.8	36.9***	-18.5***	6.8	0.1***	0.1***	2.6*	-1.1	-26.2***	34.5***
LM47XLM71	-2.9***	8.6***	-1.6	7.3***	1.8	-1.2	10.8***	19.4***	-13.9***	12.**	-46.8***	21.1***	0.1***	0.0	-3.8***	6.5***	-21.5***	45.4***
LM47XLM75	-2.6***	1.8*	1.6	-8.6***	3.6**	-0.7	11.5***	9.2***	15.7***	1.7	9.3*	33.9***	0.0	-0.1***	-6.0***	1.1	14.0*	18.1**
LM48XBW152	1.3	7.1***	2.2*	1.0	8.3***	-6.4***	2.9	1.4	5.0	-10.2**	19.2***	33.9***	-0.1***	-0.1***	-2.7**	0.0	57.9***	6.5
LM48XBW162	0.7	-9.2***	-3.9***	-15.4***	-9.2***	-4.3**	-18.1***	-26.6***	-16.9***	-16.2***	6.7	-16.6**	-0.1***	0.0	7.1***	-6.8***	-31.8***	-7.0
LM48XLM47	-1.8*	5.2***	-1.5	8.8***	5.7***	5.0**	7.1***	-3.2	-5.8	26.4***	19.6***	-8.4	-0.1***	0.2***	-2.9**	1.2	44.2***	48.6***
LM48XLM70	2.4**	0.3	6.3***	-1.3	-1.8	5.6***	-3.5*	12.6***	8.8**	-15.1***	-36.2***	-6.1	0.1***	-0.1***	-6.9***	0.3	-20.1***	2.2
LM48XLM71	2.9***	-4.2***	-6.0***	-3.6**	3.4**	1.8	5.8***	7.7***	-14.5***	-2.6	4.6	22.8***	-0.1***	-0.1***	-0.4	0.4	11.7*	38.9***
LM70XBW152	-3.3***	-2.0*	-1.5	1.5	-4.8***	9.0***	-1.4	-5.8**	-10.2**	-31.6***	-5.1	-21.3***	0.0	-0.1***	-0.4	-6.0***	-12.5*	-23.0***
LM70XBW162	1.9**	-14.0***	2.5*	-6.3***	-11.5***	-12.3***	-15.8***	-20.3***	14.2***	-23.6***	30.9***	-33.9***	0.0	0.0	9.0***	-7.6***	6.6	-7.3
LM70XLM47	-0.1	1.7	1.0	5.9***	-0.8	0.5	8.6***	0.3	-20.4***	-33.8***	11.9*	-1.5	-0.1***	-0.1***	3.1**	5.4***	22.***	11.0
LM70XLM71	-2.9***	-2.9**	-9.0***	0.2	-7.5***	2.9	-6.1***	12.3***	-35.1***	6.3	-23.3***	25.2***	0.0	0.0	-2.3*	5.5***	-48.2***	42.5***
LM70XLM75	0.1	4.3***	5.2***	5.8***	-8.7***	4.4**	-0.2	23.6***	-39.7***	-11.4**	-45.1***	22.6***	0.0	-0.1***	7.3***	-1.1	-42.1***	67.3***
LM71XBW162	1.7*	-3.0**	4.9***	-2.7*	-7.9***	9.0***	-12.0***	-8.9***	15.0***	23.6***	-17.8***	18.8***	0.1***	0.0	-4.1***	0.6	-40.2***	23.8***
LM71XLM75	-1.9**	-0.7	-7.0***	-2.0	-2.8*	6.3***	-4.9**	4.4	-0.5	22.5***	-22.7***	20.6***	0.0	0.0	6.1***	0.9	-23.3***	38.2***
LM75XBW162	1.2	10.2***	-4.1***	12.9***	1.7	31.0***	0.2	18.2***	22.9***	36.1***	-10.6*	13.9**	0.1***	-0.1***	6.3***	9.3***	-30.5***	-27.3***
Standard error	0.7	0.8	0.9	1.3	1.1	1.4	1.6	1.8	2.9	3.5	4.4	5.0	0.01	0.02	1.0	0.8	5.4	6.3

DTH=number of days to 50% heading; DTM=number of days to maturity; NPT=number of productive tillers; PH=plant height cm; RB=root biomass dry weight gm^{-2}; SB=shoot biomass dry weight gm^{-2}; RS=root to shoot ratio; TKW=thousand kernel weight; GY=grain weight gm^{-2}; *, ** and ***=significance at $p<0.05$, 0.01 and 0.001, respectively.

5.4 Discussion

5.4.1 Genetic variance and environmental impact

The significant interactions between genotypes and water regime revealed by analysis of variance (Table 5.3) is associated with quantitative traits. Quantitative traits are highly amenable to changes in environment (Houle 1992) which cause crossover ranking among the genotypes in different environments. Crossover ranking complicates the breeding process as environmental variance confounds trait expression. In some cases it provides opportunities to deduce the variation attributable to genetic effects and also to select genotypes adapted for specific environments (Yan and Tinker 2006). Genotypic variation in RB, SB, RS and GY have been reported previously in wheat under contrasting environments (Hendriks et al. 2015; Chen et al. 2018), providing opportunities for improving biomass allocation. The variation in trait means among the genotypes can be attributable to genotypic inheritance and differential response to water availability (Rebetzke et al. 2003). This indicated that the germplasm was diverse enough to conduct detailed gene action analysis.

The higher mean performance by some of the crosses compared to their parents (Table 5.4) indicates the possibility of genetic advancement in biomass accumulation. In addition, the high relative performance in crosses compared to their parents is an indication of dominance gene action and provides a basis for transgressive selection (Langridge 2017). Crosses such as BW152×BW162, LM71×LM75 and LM47×LM75 showed higher RB and SB under drought stressed condition than their mid-parents and can be used for transgressive selection under drought stress, while crosses BW141×LM70 and LM70×LM75 can be selected under non-stress to achieve higher genetic gains for RB, SB and GY, respectively. Crosses such as LM47×LM75 and BW152×BW162 should be selected for drought tolerance and C sequestration potential as they exhibited higher mean values for RB, RS and GY.

5.4.2 Combining ability effects

Quantifying the additive and non-additive components is fundamental for devising appropriate breeding strategy for a trait under consideration (Dhanda et al. 2002). The analysis of variance for yield and agronomic traits revealed that GCA and SCA effects were significant for biomass and other agronomic traits (Table 5.5) showing that they were under the control of both additive and non-additive gene action, in that order. The presence of GCA effects for more traits under drought compared to non-stressed condition agrees with other studies which revealed that GCA effects were more important under stress condition (Subhani and Chowdhry 2000; Betran et al. 2003). Furthermore, the GCA variances were higher for all traits except NPT and SB under drought stress, which agreed with reports by Khahani et al. (2017) that additive effects were more important for the inheritance of traits under drought stress compared to non-stressed condition. Proportionally, the $2\sigma^2GCA/2\sigma^2GCA + \sigma^2SCA$ ratios were below 0.50 showing that non-additive gene effects were more important than additive gene effects for all traits under both water conditions. Rad et al (2013) also found high preponderance of non-additive gene effects in the inheritance of yield related traits in wheat. Very low σ^2GCA/σ^2SCA ratios point to possible existence of epistasis (Longin et al. 2013). The preponderance of non-additive gene effects for traits in this population would favor family selection followed by pure line selection in advanced generations as suggested by Edwards et al. (1976). The inconsistent GCA variance of traits across the water regimes shows that there is need to select families for specific environmental adaptation (Makumbi et al. 2011), while higher GCA compared to SCA mean squares suggests that genetic advancement will be achieved by selection in advanced segregating generations (Gravina et al. 2004).

5.4.3 General combining ability effects of parents

Parental genotypes with suitable GCA effects for biomass allocation, agronomic traits and GY can be used for developing breeding populations (Maich et al. 2000). Genotypes such as BW140 and LM47, which exhibited positive GCA effects for RB, SB or GY and other agronomic traits under drought stress (Table 5.6) make them ideal selections for developing improved cultivars or for use in recurrent selection for biomass allocation and drought tolerance. Negative GCA effects indicate that alleles for low mean traits were largely inherited from parents with low means for the respective traits (Rebetzke et al. 2003). For instance, parent LM70, which had the lowest mean RB, SB and GY, was involved in crosses such as LM70×LM75, LM70×LM71 and LM26×LM70, which consistently exhibited negative SCA for the traits. The presence of additive gene effects indicate that selection for parents with higher mean values for RB and GY could be effective for improving drought tolerance. Parental genotypes that exhibit low DSI and have favorable combining ability under drought-stressed condition can be selected for genetic advancement of drought tolerant genotypes. Genotypes including LM75 and BW152 can be selected due to their negative GCA effects for DTH under drought stress. Such negative GCA effects indicate that these parents will contribute genes to reduce the time to flowering and contribute to drought avoidance. Parents with desirable GCA effects under specific condition transfer additive genes to their offspring although those parents that exhibit the desirable GCA effects under diverse conditions will be more useful for breeding (Dholariya et al. 2014). In addition to biomass allocation to roots, shoots and grains, plant height should also be considered in adaptation to drought. Genotypes with semi-dwarfing genet *Rht1* and *Rht2* have been reported to be drought tolerant by increasing rooting capacity in the top soil layers (Miralles et al. 1997). Parents such as BW141, LM71 and LM26 with negative GCA for PH under both conditions must be selected for breeding to reduce PH.

5.4.4 Specific combining ability effects of crosses

There existed a preponderance of non-additive gene effects for RB, SB, RS, GY and other agronomic traits which could result in transgressive selection. The presence of families such as BW152×BW162, LM71×LM75 and LM47×LM75 that had higher mean values for RB and SB than their mid-parent values points to the possible existence of oligo- or polygenic control of the traits (Rebetzke et al. 2003). Improvement of traits under non-additive gene effects can be achieved by selection of transgressive segregants at advanced generations after successive inbreeding followed by pure line selection (Hallauer and Miranda 1988; Susanto 2018). For drought tolerance and C sequestration potential, families such as BW141×LM48 and LM47×LM75 which attained high mean GY and exhibited positive and significant SCA effects for RB, SB, RS and GY under drought stressed condition (Table 5.7) should be selected for further advancement. Families that include BW141×LM75, LM26×BW152, LM47×LM71 LM71×BW162 and LM71×LM75 can be considered for non-drought environments because of their positive SCA effects for RB, SB and GY under non-stress condition. Crosses such as BW141×LM48, BW141×LM70 and BW152×BW162 exhibited positive SCA effects for RB, SB and GY under drought-stressed and non-stressed conditions despite their parents having negative GCA effects for biomass traits. Such crosses that exhibit high SCA, even though they are derived from low GCA parents, show that there may be dominance × dominance gene action involved in the control of the traits (Wassimi et al. 1986). Parent LM70 could be a source of genes for early flowering and maturity as exhibited by its involvement in crosses LM70×LM71, LM70×LM75 and LM70×BW152, which exhibited desirable SCA effects for DTH and DTM. For reduction in plant height to enhance drought tolerance (Monneveux et al. 2012), parent BW162 can be selected as it was a progenitor of families such as BW140×BW162, LM48×BW162 and LM70×BW162 that exhibited the highest negative SCA effects for PH under drought stress.

5.5 Conclusions

Families such as BW152×BW162, LM71×LM75 (which accumulated higher root biomass), and LM47×LM75 (with higher shoot biomass) and LM47×LM75, BW140×BW152 and BW141×LM48 (which had higher GY) compared to their mid-parent values under drought stress condition are recommended for transgressive selection. Parental genotypes LM47 and LM75 had positive and significant GCA effects for root biomass under drought stressed condition, while BW162 had positive and significant GCA for GY under both drought-stressed and non-stressed conditions. These genotypes can be used in recurrent selection to improve the respective traits for adaptation to specific conditions. The families BW141×LM48 and LM47×LM75 were good specific combiners for RB, SB and GY under drought-stress, while BW141×LM75, LM26×BW152, LM47×LM71 LM71×BW162 and LM71×LM75 were good combiners for RB, SB and GY under non-stress condition. The families such as BW140×BW162, LM48×BW162 and LM70×BW162 that exhibited negative SCA effects for PH indicated that BW162 is a possible source of plant height reducing genes, which are important for drought tolerance. Non-additive gene effects were significant in the inheritance of biomass allocation traits suggesting that recurrent selection will be the appropriate method for improving the traits under consideration. Further research under multiple field conditions and advanced populations are required to substantiate the present findings and for targeted breeding and cultivar release.

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Overview of the Research Findings

Introduction and objectives of the study

In sub-Sahara Africa, including in South Africa, wheat is mainly cultivated under dry-land and low input farming systems that are dependent on residual soil moisture and nutrients. Therefore, wheat production and productivity is challenged by recurrent drought stress and poor soil health. Modern wheat cultivars with improved root systems and well-balanced biomass allocation will have better water- and nutrient-use efficiency and, hence, increased productivity under dry-land farming systems. The success of developing wheat cultivars with well-balanced biomass allocation for drought tolerance and carbon (C) sequestration depends on effective screening procedures to identify the requisite phenotypic and genetic variation in the key traits for breeding. Hence, this study aimed to improve biomass allocation in wheat for drought tolerance and enhanced C sequestration capacity under water-limited conditions. This overview highlights the study objectives, the summary of research findings and finally, the implications of the findings for wheat breeding with enhanced biomass allocation and drought tolerance. The specific objectives of the study were:

- To evaluate agronomic performance and quantify biomass production and allocation between roots and shoots in selected wheat genotypes in response to different soil water levels to select promising genotypes for breeding for drought tolerance and C sequestration.
- To determine variance components and heritability of biomass allocation and grain yield related traits among 99 genotypes of bread wheat and triticale (*Triticosecale* Wittmack) to optimize biomass partitioning for drought tolerance.
- To deduce the population structure and genome-wide marker-trait association of yield and biomass allocation traits in wheat to facilitate marker-assisted selection for drought tolerance and C sequestration.
- To estimate the combining ability of selected wheat genotypes and their progenies for agronomic traits, biomass allocation and yield under drought-stressed and non-stressed conditions for future breeding and genetic advancement for drought tolerance and C sequestration.

Research findings in brief

Selection of wheat genotypes for biomass allocation to improve drought tolerance and biomass allocation

Ninety five bread wheat genotypes obtained from the International Maize and Wheat Improvement Center (CIMMYT), two local checks and two commercial cultivars, and a triticale accession were evaluated under drought-stressed and non-stressed conditions. The genotypes were evaluated under greenhouse and field conditions using a 10×10 alpha lattice design with two replications. Phenotypic traits such as number of days to heading (DTH), number of productive tillers per plant (NPT), plant height (PH), days to maturity (DTM), spike length (SL), thousand kernel weight (TKW), root biomass (RB), shoot biomass (SB), root to shoot ratio (RS), grain yield (GY) and carbon content in plant biomass were measured and subjected to multivariate analysis. The main findings of the study were as follows:

- Significant ($p<0.05$) variation in agronomic performance, biomass allocation and grain yield production were found among the genotypes and across the treatments, indicating differential response of genotypes.
- Drought stress reduced total biomass production by 35% and root to shoot ratio by 14%. The decline in biomass production and RS indicate that C sequestration will be low under low moisture availability.
- Significant ($p<0.05$) and stronger phenotypic correlations between GY and RB ($r=0.46$) and SB ($r=0.59$) under drought-stressed compared to non-stressed conditions suggested that biomass allocation to RB can be achieved without compromising GY.
- Ten genotypes BW140, BW141, BW152, BW162, LM26, LM47, LM48, LM70, LM71 and LM75 with low drought sensitivity indices were selected for their drought adaptability from different clusters to capture as much diversity as possible. BW162 accumulated the highest root carbon stocks. These genotypes were selected for population development and genetic advancement.

Variance components and heritability of traits related to root: shoot biomass allocation and drought tolerance in wheat

Phenotypic data from the field and greenhouse experiments were subjected to analysis of variance and regressions and correlation analyses. Analysis of variance was based on the general linear model (GLM) in which environment and water regimes were treated as fixed factors, while genotype effects were treated as random factor. The main outcomes were:

- The genetic variance for RS and GY under non-stressed conditions were 45.50 and 24.60%, respectively. Under drought stress, the genetic variances were significantly reduced to 30.50 and 3.95%, respectively.
- RS and GY were found to have low heritability, below 30%, under drought stressed condition, which complicates selection under drought stress condition.
- The low heritability and low genetic variance for RS and GY suggests the need to include diverse germplasm pool to widen the genetic diversity.
- GY and RS showed significant and negative genetic correlations ($r<-0.33$, $p<0.05$) under drought-stressed and non-stressed conditions showing that they cannot be improved simultaneously via direct selection. Alternatively, RS and GY can be improved via secondary traits such DTH, PH, RB and TKW, which exhibited favorable genetic correlations with RB, SB and GY.

Genome wide association study of drought tolerance and biomass allocation in wheat

The study analyzed the genetic structure and marker-trait associations in a sample of 99 bread wheat (*Triticum aestivum* L.) genotypes and one triticale (*Triticosecale* Wittmack) accession using 28,356 DArTseq derived SNP markers. After imputation, 16,362 SNPs were used to deduce the population structure and marker-trait associations using STRUCTURE and GAPIT softwares, respectively. The main findings were:

- The population was grouped into seven clusters and the average polymorphic information content of 0.42, showing considerable degree of diversity.
- A total of 54 significant marker-trait associations (MTAs) were identified. Twenty-one of the MTAs were detected under drought stress and 89% of the significant MTAs were identified loci that have not been previously reported, thus they provide new information for biomass allocation.

- The four markers, 1B|040.630655570|3570152|3570152, 1B|149.556316800|1003008|1003008, 2B|078.880456780|1863039|1863039 and 3B|097.589047780|1025605|1025605, which exhibited pleiotropy for RB and SB could be targeted for simultaneous selection for root and shoot biomass.

Combining ability of selected wheat genotypes for drought tolerance and biomass allocation

The following 10 wheat genotypes: BW140, BW141, BW152, BW162, LM26, LM47, LM48, LM70, LM71 and LM75 were crossed using a half diallel mating design. The parents and their crosses were evaluated using a completely randomized design in the greenhouse between February and October 2018. Agronomic, biomass and grain yield traits were measured and subjected to statistical analysis in Genstat 18th edition. The core findings were:

- Non-additive gene effects were more significant for the inheritance of biomass and grain-yield related traits.
- Parental genotypes LM75, BW162, LM71 and BW140, which exhibited good general combining ability for RB, SB, RS and GY across drought-stressed and non-stressed conditions should be considered for recurrent selection.
- Family selection method among the crosses involving of BW141/LM48 and LM47/LM75 under drought stress and LM26/LM70 and LM26/LM71 under non-stress conditions will improve biomass allocation and GY because they showed favorable SCA effects under the specific conditions.

Implications of findings for breeding for biomass allocation to improve drought tolerance and carbon sequestration

- The wheat genotypes LM26, LM47, BW140, LM70, LM48, BW152, LM75, BW162, LM71 and BW14110 exhibited low drought sensitivity and genetic diversity for biomass, yield and yield components. These are vital genetic resources for improving biomass allocation for drought tolerance and C sequestration in wheat.
- The low heritability of root to shoot ratios and grain yield imply that drought tolerance breeding and C sequestration depends on identifying proxy traits such as root and shoot

biomass, days to heading, plant height and thousand kernel weight with favorable correlations rather than selecting for root to shoot ratios and grain yield *per se*.

- 54 significant marker-trait associations (MTAs) identified in this study can be used for marker-assisted breeding to increase selection efficiency for shoot and root biomass, root to shoot ratios and grain yield to eliminate environmental variance confounding phenotypic selection.
- The pleiotropic markers for root and shoot biomass indicate that they share some common genomic loci implying that drought tolerance and C sequestration are tightly linked and hence, can be selected simultaneously.
- The predominance of specific combining ability effects found in this study shows that improvement of drought tolerance and C sequestration in the population will be achieved via family selection followed by pure line selection in advanced generations.
- The families BW141×LM48 and LM47×LM75 were good specific combiners for RB, SB and GY under drought-stress, while BW141×LM75, LM26×BW152, LM47×LM71 LM71×BW162 and LM71×LM75 were good combiners for RB, SB and GY under non-stress condition. The families BW140×BW162, LM48×BW162 and LM70×BW162 indicated that BW162 is a possible source of plant height reducing genes.
- This is the first study that evaluated biomass allocation in wheat as a strategy to improve drought tolerance and carbon sequestration.
- Further research under multiple field conditions and advanced populations are required to substantiate the present findings and for targeted breeding and cultivar release.
- There is also a need to assess the carbon dynamics in wheat and other food security crops using more precise techniques such as isotopic carbon tracing to quantify carbon input during and after crop growth.