Variation for Agronomic Traits, Biomass Allocation, and Carbon Storage in Sorghum (*Sorghum bicolor* [L.] Moench) Genotypes

By

Asande Satisfy Ngidi

BSc Agriculture (Plant Science, University of KwaZulu-Natal)

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College of Agriculture, Engineering and Science

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Dissertation Abstract

Sorghum (*Sorghum bicolor* [L.] Moench, $2n = 2x = 20$) is an ancient grain crop of Africa cultivated worldwide. The productivity of sorghum is low (< 1.5 t/ha) under smallholder farming systems in the region due to severe drought stress, poor soil health, diseases, insect pests, and noxious weeds. Besides its grain production for food, feed, and industrial raw materials, sorghum produces relatively high biomass for the biofuel and bioplastic industry. Sorghum's high biomass production can transfer atmospheric carbon (C) to the soil throughout its growth stages, thereby enhancing soil fertility and crop productivity through atmospheric C sequestration. There is a need to select sorghum genotypes with optimised agronomic traits, high biomass production and water and nutrient use efficiencies to enhance economic yield and carbon sequestration capacity. Therefore, the overall aim of this study was to screen and select sorghum genotypes with better agronomic traits, biomass allocation, and C storage. The specific objectives of the study were:

i. to quantify the extent of variation in biomass allocation and C storage between major crops, including sorghum for crop production, and C sequestration potential through a meta-analysis.
ii. to assess agronomic performance, biomass production and carbon accumulation in selected sorghum genotypes for production and breeding.
iii. to assess the extent of genetic variability for agronomic and carbon storage traits in selected sorghum genotypes to identify the best candidates for production or breeding.
iv. to assess the trend and magnitude of relationships between agronomic and carbon storage traits in sorghum to identify grain yield and carbon storage contributing traits and to guide future sorghum variety development and release.

A metanalysis was conducted from 40 global studies that reported on the allocation of plant biomass and C between roots and shoots of sorghum, maize, and wheat cultivars. Key statistics were calculated to determine the variability among the cultivars for total plant biomass (PB), shoot biomass (SB), root biomass (RB), root-to-shoot biomass ratio (RS), total plant carbon content (PCc), shoot carbon content (SCc), root carbon content (RCc), total plant carbon stock (PCs), shoot carbon stock (SCs), root carbon stock (RCs), and root-to-shoot carbon stock ratio (RCs/SCs). Maize exhibited the highest variability for PB (with a coefficient of variation [CV] of 31.2% and a mean of 4.2±1.3 Mg ha^{-1} yr^{-1}), followed by wheat (CV of 24.2% and mean of

1.5±0.4 Mg ha^{-1} yr^{-1}) and sorghum (16.8% and 2.0±0.8 Mg ha^{-1}), respectively. A similar trend was observed for PCs, with maize (CV of 40.1% and mean of 1.6±0.7 Mg ha^{-1}) showing the highest total plant C stock variability, followed by wheat (24.4% and 0.2±0.1 Mg ha^{-1}), and sorghum (16.3% and 0.9±0.3 Mg ha^{-1}), respectively. Maize exhibited the highest variability for RS (with a CV of 24.4% and mean of 0.1±0.03), while wheat exhibited the highest variability for RCs/SCs (30.92% and 0.2±0.05). The meta-analysis revealed that maize and sorghum have the highest variability for total plant biomass and plant carbon stocks, while wheat exhibits the highest variability for the below-ground biomass and carbon stocks.

In the first experiment, 50 sorghum genotypes were evaluated using a 5 x 10 alpha lattice design with two replications at three locations (Silverton, Ukulinga, and Bethlehem) in South Africa during the 2022/23 growing season. The following agronomic and carbon storage traits were assessed: days to 50% heading (DTH), days to 50% maturity (DTM), plant height (PH), PB, SB, RB, RS, GY, HI, GCc, SCc, RCc, PCs, SCs, RCs, RCs/SCs, and grain carbon stock (GCs). A combined analysis of variance revealed significant ($P < 0.05$) genotype x location interaction for DTH, DTM, PH, PB, SB, RB, RS, and GY. Genotypes AS115, AS251, and AS134 were the best performing with the highest GY of 5.08 g plant^{-1}, 21.83 g plant^{-1}, and 21.42 g plant^{-1}, respectively. Genotypes AS122 and AS27 ranked first and second, respectively, for all the carbon stock parameters except for RCs, whereas genotype AS108 had the highest RCs of 8.87 g plant^{-1}. The principal component analysis identified GY, DTH, PH, PB, SB, RB, RCs, RCs/SCs, PCs, SCs, and GCs as the most discriminated traits among the test genotypes. The cluster analysis using agronomic and carbon-related parameters delineated the test genotypes into three genetic groups. The selected sorghum genotypes are recommended for further breeding and variety release adapted to various agroecologies in South Africa.

Data from field experiments were computed to deduce variance components, heritability, and genetic advance to guide genotype selection. Higher phenotypic coefficient of variation (PCV) were recorded for PH (68.91%), followed by GY (51.8%), RB (50.51%), RS (41.96%), RCs/SCs (44.90%), and GCs (41.90%). In contrast, higher genotypic coefficient of variations (GCV) were recorded for GY (45.92%), followed by RB (39.24%), RCs/SCs (38.45), and RCs (34.62). The high PCV and GCV values suggest the availability of genetic variability among the test genotypes for the assessed traits. High to moderate broad-sense heritability and genetic advance were observed for HI (83.76 and 24.53%), GY (78.59 and 9.98%), PB (74.14 and 13.18%) and PCs (53.63 and 37.57%), respectively, suggesting a marked genetic contribution to the traits. High broad-sense heritability combined with increased genetic advance were computed for PB, RB, GY, HI, RS, GCs, RCs, and RCs/SCs, indicating that genetic effects primarily control these traits.

In the third experimental chapter, correlation and path coefficient analyses were computed to discern the trend and magnitude of associations of assembled traits to guide simultaneous selection for enhanced grain yield, its components and carbon storage. Significant ($P < 0.05$) positive phenotypic and genotypic correlations were observed between GY with HI at $r = 0.79$ and $r = 0.76$, DTH ($r = 0.31$ and $r = 0.13$), PH ($r = 0.27$ and $r = 0.1$), PB ($r = 0.02$ and $r = 0.01$), RB ($r = 0.06$ and $r = 0.05$), respectively. Further, the path analysis revealed significant positive direct effects of SB (0.61) and RB (0.46) on GY. The RS exerted a positive significant genotypic indirect effect (0.26) on GY through SB. The overall association analyses revealed that PB, SB, RB, RS, RCs, and RCs/SCs significantly influenced GY performance and are the principal traits when selecting sorghum genotypes with high carbon storage capacity.

The present study identified the following promising genotypes: AS251, SS27, AS134, AS203, and AS563 for their high biomass production, grain yield, and C sequestration potentials. The identified genotypes could be advanced for cultivar development and further evaluated for net carbon contribution to the soil.

Declaration

I, Asande Satisfy Ngidi declare that:

The research reported in this dissertation, except where otherwise indicated is my original research.

This dissertation has not been submitted for any degree or examination at any other university.

This dissertation does not contain other persons data, pictures, graphs, or other information, unless specifically acknowledged as being sourced from other persons.

This dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

> Their words have been re-written, but the general information attributed to them have been referenced.
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> Where their exact words have been used, then their writing have been placed in italics and inside quotation marks and referenced.

This dissertation does not contain text, graphs, or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the reference's sections.

Signed

[redacted]

Asande Satisfy Ngidi

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

[redacted]

Prof. Hussein Shimelis (Supervisor)

[redacted]

Dr. Sandiswa Figlan (Co-supervisor)

Dr. Vincent Chaplot (Co-supervisor)

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Dedication

I dedicate this dissertation to my parents, Nikiwe Simelane Ngidi and Scelo Ngidi, my grandmother, Balinganisile Zungu Ngidi, and my brothers and sisters; for their never-ending support, may god bless you abundantly.

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Abbreviations and Acronyms

ACCI	African Centre for Crop Improvement
ARC-SG	Agricultural Research Council – Small Grain
BD	Bulk density
C	Carbon
CV	Coefficient of variation
DF	Degrees of freedom
DTH	Days to heading
DTM	Days to maturity
GA	Genetic advance
GAM	Genetic advance as a percent of the mean
GCc	Grain carbon content
GCs	Grain carbon stock
GCV	Genotypic coefficient of variation
GWAS	Genome-wide association studies
GY	Grain yield
H^2	Broad sense heritability
HI	Harvest index
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
LSD	Least significant difference
MAP	Mean annual precipitation
MAT	Mean annual temperature
OC	Organic carbon
OM	Organic matter
PB	Total plant biomass
PCA	Principal component analysis
PCs	Total plant carbon stock
PCV	Phenotypic coefficient of variation
PH	Plant height
QTL	Quantitative trait locus

r	Correlation coefficient
R	Residual effect
RB	Root biomass
RCc	Root carbon content
RCs	Root carbon stock
RCs/SCs	Root-to-shoot carbon stock ratio
rg	Genotypic correlation coefficient
rph	Phenotypic correlation coefficient
RS	Root-to-shoot biomass ratio
SA	South Africa
SASRI	South African Sugarcane Research Institute
SB	Shoot biomass
SCc	Shoot carbon content
SCs	Shoot carbon stock
SD	Standard deviation
SE	Standard error
SOC	Soil organic carbon
UKZN	University of KwaZulu-Natal

Publications Pertaining to This Dissertation

Chapter 1

Ngidi, A., Shimelis, H., Chaplot, V., Shamuyarira, K.W., & Figlan, S. (2024). Biomass Allocation and Carbon Storage in the Major Cereal Crops: A Meta-analysis. Accepted for publication in Crop Science (15 May 2024).

Chapter 2

Ngidi, A., Shimelis, H., Abady, S., Figlan, S., & Chaplot, V. (2024). Response of *Sorghum bicolor* genotypes for yield and yield components and organic carbon storage in the shoot and root systems. Scientific Reports, 14(1), 9499. https://doi.org/10.1038/s41598-024-59956-x

Chapter 3

Ngidi, A., Shimelis, H., Abady, S., Figlan, S., & Chaplot, V. (2024). Genetic variation and association of yield, yield components, and carbon storage in sorghum (*Sorghum bicolor* [L.] Moench) genotypes. BMC Genomics Data. Under review.

Introduction to Dissertation

Background of study

Sorghum (*Sorghum bicolor* [L.] Moench, 2n = 2x = 20) is a climate resilient and food security crop in the semi-arid tropical regions of Asia, Africa, and Central America. It is the fifth most important carbohydrate-rich cereal crop after wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), maize (*Zea mays* L.), and barley (*Hordeum vulgare* L.) (Hossain *et al.,* 2022). Sorghum is cultivated across 86 countries globally in an area of 38 million hectares and with an annual grain production of about 58 million tons (Khalifa and Eltahir, 2023). In South Africa, sorghum production ranges from 100,000 to 180,000 tonnes per annum (Abah *et al.,* 2020). In sub-Saharan Africa (SSA), sorghum is mainly grown by smallholder farmers with limited access to essential production inputs such as improved varieties, crop protection chemicals, inorganic fertilizers, irrigation systems, and financial resources (Ahmad *et al.,* 2022). The average productivity in smallholder farming systems reaches up to 1.5 t/ha, significantly lower than the potential yields of 4 to 5 t/ha (Hungwe *et al.,* 2020). Additionally, a significant portion of sorghum is cultivated on marginal and impoverished soils, leading to low yields and poor crop quality.

With increasing global warming and climate change, sorghum presents a promising alternative crop for enhanced grain and biomass production, and carbon (C) sequestration. Sorghum is relatively tolerant to drought, heat stress, and waterlogging (Velmurugan *et al.,* 2020). Sorghum has a C4 photosynthetic pathway, which provides high photosynthesis efficiency and robust biomass production (Mullet *et al.,* 2014). Sorghum maintains its physiological activity and adequate moisture under drought stress conditions by extending its root length, density, and high water-use efficiency. Sorghum can reach heights of up to 3 meters, depending on the genotype and environmental conditions, and it can produce fresh biomass yields ranging from 45 to 112 t/ha (Rao *et al.,* 2015). Sorghum's high biomass production potential enhances carbon sequestration by effectively capturing and storing atmospheric carbon dioxide (CO_2) through photosynthesis, thus reducing and offsetting emissions (Meena *et al.,* 2020). Reports indicate that, on average, sorghum can sequester about 0.5 to 1.5 Mg C ha^{-1} annually (Srinivasarao *et al.,* 2012). Therefore, it is necessary to develop new sorghum varieties with desirable traits, such as high yield and biomass production, to enhance carbon sequestration, improve soil health, and mitigate climate change.

Increasing soil organic carbon (SOC) is one approach to reducing climate change (Powlson *et al.,* 2011). Agricultural soils could play a pivotal role in this effort, as they cover 34% of the land surface, and their management significantly impacts SOC storage by altering input and decomposition rates (Bhattacharya *et al.,* 2016). Root-derived organic carbon (OC) is a major contributor to SOC compared to shoot-derived OC and manure, comprising up to 90% of all carbon inputs to cultivated soils (Sokol and Bradford, 2019; Poeplau *et al.,* 2021). Root carbon has a longer residence time in soil because of its resistant chemical composition and tendency to be incorporated into more stable soil fractions (Hirte *et al.,* 2021). Studies have also reported that the mean residence time of OC entering the soil, computed from the ratio of total SOC stock to average annual C input or loss, can serve as a measure of the biogeochemical stabilization of OC in soils (Leifeld and Fuhrer, 2005; Poeplau *et al.,* 2021). Specifically, carbon inputs from roots into the deeper soil layers have been associated with prolonged carbon storage, attributed to the limited presence of decomposers and the increased storage capacity of deep unsaturated soil layers. Consequently, promoting more extensive and deeper root systems has been recommended as a strategy for enhanced drought tolerance, nutrient uptake, and atmospheric C sequestration, with an estimated potential to sequester approximately 1 Pg CO_2 annually (Lynch and Wojciechowski, 2015; Pierret et al., 2016; Zia et al., 2021).

Significant genetic variations have been reported for agronomic traits, biomass accumulation, and C allocation between roots and shoots of cereal crops, including sorghum (Gonzalez-Sanchez *et al.,* 2012; Luquet *et al.,* 2019; Satish *et al.,* 2019). Germplasm collection and characterization are crucial in developing a gene pool to enhance genetic variation for breeding (Kroc *et al.,* 2021). Genetic groupings can be established using data related to geographical origin, agronomic traits, pedigree, and molecular markers (Belaj *et al.,* 2012). Traditionally, the analysis of morphological traits has been used to assess the genetic diversity level within a given population. Phenotyping offers a relatively straightforward method for evaluating diversity; hence, it serves as a valuable tool for preliminary germplasm evaluation. Several researchers have used morphological traits to estimate the genetic diversity of sorghum (Yaqoob *et al.,* 2015; Motlhaodi *et al.,* 2017; Sejake *et al.,* 2020). Assessments of genetic variability crops is useful in selecting contrasting parents or generating segregating populations, thereby leveraging genetic diversity within a breeding program (Swarup *et al.,* 2021).

Grain yield, biomass production and carbon storage remain the target traits of interest in sorghum breeding. High grain yield response is a must-have trait to satisfy the needs of the farmers, breeders, and value chain (Kaminski *et al.,* 2013). However, grain yield is controlled by various inter-related agronomic and biomass traits that contribute differently during genotype selection. Studies have indicated that indirect selection for grain yield through yield components is the most effective approach to enhance productivity, drought tolerance, and carbon storage (Abdolshahi *et al.,* 2021; Reynolds *et al.,* 2016). Evaluating yield components helps pinpoint the crucial traits influencing yield, simplifying the selection process for sorghum genotypes with high biomass production and carbon sequestration potential (Gurmu *et al.,* 2018).

Yield components influence grain yield both directly and indirectly. Hence, identifying the direct and indirect effects of traits improves selection efficiency in sorghum breeding programs (Shukla *et al.,* 2015). Correlation and path coefficient analyses are statistical techniques commonly used to determine the association between traits, aiding in the and used in selection for optimum biomass production and increased carbon storage. Selecting correlated traits with high heritability also increases genetic gains in plant breeding programs (Silva *et al*., 2016). Reports show that significant yield increases worldwide have resulted from improving components that were associated with grain yield (Gebreyohannes *et al.,* 2018; Enyew *et al.,* 2021). Therefore, employing diverse selection strategies and techniques to develop varieties with enhanced biomass production and carbon storage provides the most effective method for enhancing sorghum yields in South Africa's agroecologies.

Rationale of study

Sorghum is among South Africa's indigenous cereal crops cultivated across several provinces. Its production in dryland regions of the country is mostly under low agricultural input systems, which are highly constrained by poor soil fertility and recurrent droughts associated with climate change, resulting in a decline in sorghum production over the years. Breeding for sorghum genotypes with desirable agronomic traits and high biomass production can improve C sequestration capability and yield gains through enhanced water and nutrient use efficiencies and help mitigate climate change. Currently, limited studies compared the major cereal crops for C storage for regenerative agriculture and to optimize C sequestration strategies. Understanding the C input between roots and shoots of major cereal crops allows for assessing options for enhancing soil C storage. Therefore, there is a need to differentiate potential powerhouse crops with high biomass production that involve high C sequestration to guide plant breeding and crop production programs. Additionally, limited studies documented the genetic diversity of sorghum integrating agronomic traits, balanced biomass allocation, and C sequestration under South African production conditions. Previous studies have reported differences in plant C stocks between crop types with limited emphasis on intra-specific variations to guide breeding (Ordonez *et al.,* 2020; Zhao et al., 2022), especially for C sequestration potential. Genetically diverse sorghum accessions were collected from major producing countries, including Ethiopia, Tanzania, and South Africa, for selection under South African growing conditions. From this gene pool, 50 accessions were sampled based on their high grain yield, biomass, and ethanol production (Mangena *et al.,* 2018)

Research aim

The aim of the study was to screen and select, sorghum genotypes with better agronomic traits, biomass allocation, and C storage.

Research objectives

The specific objectives of the study were:

i. to quantify the extent of variation in biomass allocation and C storage between major crops, including sorghum for crop production, and C sequestration potential through a meta-analysis.
ii. to assess agronomic performance, biomass production and carbon accumulation in selected sorghum genotypes for production and breeding.
iii. to assess the extent of genetic variability for agronomic and carbon storage traits in selected sorghum genotypes to identify the best candidates for production or breeding.
iv. to assess the trend and magnitude of relationships between agronomic and carbon storage traits in sorghum to identify grain yield and carbon storage contributing traits and to guide future sorghum variety development and release.

Outline of dissertation

Table 0.1 shows the outline of the dissertation. The dissertation is written in the form of discrete research chapters, each following the format of a stand-alone research paper followed by a general overview and implications of findings from the study. This is the dominant dissertation format adopted by the University of KwaZulu-Natal. As such, there is unavoidable repetition of some references and some introductory information between chapters. Chapter 1 has been accepted for publication in Crop Science. Chapter 2 was published in Scientific Reports, Doi: 10.1038/s41598-024-59956-x. Chapter 3 and 4 have been submitted to BMC Genomics Data and currently under review.

Table 0.1 Outline of the dissertation

Chapter	Title
-	Introduction to dissertation
1	Biomass allocation and carbon storage in the major cereal crops: A meta-analysis
2	Response of sorghum (*Sorghum bicolor* [L.] Moench) genotypes for yield and yield components and organic carbon storage in the shoot and root systems
3	Genetic variation of yield, yield components, and carbon storage in sorghum (*Sorghum bicolor* [L.] Moench) genotypes
4	Association of agronomic traits and carbon storage on grain yield response in sorghum
-	Overview and implications of the study

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CHAPTER 1: Biomass Allocation and Carbon Storage in the Major Cereal Crops: A Meta-analysis

Abstract

Crop biomass is the reservoir of carbon (C), a valuable input to the soil, thus supporting the soil fauna and enhancing soil health. There are limited studies that compared the major cereal crops for C storage for regenerative agriculture and optimize C sequestration strategies. The objective of this study was to quantify the extent of variation in biomass allocation and C storage between maize, sorghum, and wheat for crop production, and C sequestration potential. The study used metadata from 40 global studies that reported the allocation of plant biomass and C between roots and shoots of the major cereal crops. Key statistics were computed to determine the variability between genotypes for total plant biomass (PB), shoot biomass (SB), root biomass (RB), root-to-shoot biomass ratio (RS), total plant carbon content (PCc), shoot carbon content (SCc), root carbon content (RCc), total plant carbon stock (PCs), shoot carbon stock (SCs), root carbon stock (RCs), and root-to-shoot carbon stock ratio (RCs/SCs). Maize exhibited the highest variability for PB (with a coefficient of variation [CV] of 31.2% and a mean of 4.2±1.3 Mg ha^{-1} yr^{-1}), followed by wheat (CV of 24.2% and mean of 1.5±0.4 Mg ha^{-1} yr^{-1}) and sorghum (16.8% and 2.0±0.8 Mg ha^{-1}), respectively. A similar trend was observed for PCs, with maize (CV of 40.1% and mean of 1.6±0.7 Mg ha^{-1}) showing the highest total plant C stock variability, followed by wheat (24.4% and 0.2±0.1 Mg ha^{-1}), and sorghum (16.3% and 0.9±0.3 Mg ha^{-1}), respectively. Maize (with a CV of 24.4% and mean of 0.1±0.03) exhibited the highest variability for RS, while wheat (30.92% and 0.2±0.05) exhibited the highest variability for RCs/SCs. Correlation analysis revealed the following significant associations: PB and mean annual temperature (MAT) (r = -0.47), and SB and MAT (r = -0.43), and PB and mean annual precipitation (MAP) (r = -0.34), and SB and MAP (r = -0.30). RB had a strong, significant positive correlation with MAT (r = 0.72) and MAP (r = 0.85). The meta-analysis revealed that maize and sorghum have the highest variability for total plant biomass and plant carbon stocks, while wheat exhibited the highest variability for the below-ground biomass and carbon stocks. The data aided in crop selection and suggested that the best cultivars could be developed and identified for production and C sequestration potential for cultivation by farmers, land rehabilitation, and climate change mitigation.

Keywords: Carbon stocks; biomass allocation; C sequestration; major cereals; coefficient of variability

1.1 Introduction

Crop biomass is the major reservoir of organic carbon (OC), which is incorporated into the soil, enhancing soil fauna and quality. Soils constitute the greatest terrestrial pool of carbon (C) and store two to three times the C found in the atmosphere (Minasny *et al.,* 2017). Soil carbon, which is found as part of organic matter (OM) within the soil, provides energy and nutrients for soil micro-organisms and is vital for ecosystem functioning, such as food production and climate regulation. However, due to the conversion of natural ecosystems to agricultural production, most of the original soil C has been lost to the atmosphere. Harvested crops export enormous amounts of nutrients from soils, thus leading to organic matter depletion (Chaplot and Smith, 2023). Abbas *et al.,* (2020) estimated the total amount of C lost to the atmosphere during the five and 50 years in the tropical to amount to 20 Mg C ha^{-1} and 50 Mg C ha^{-1} for tropical and temperate regions, respectively. Therefore, C replacement and soil enrichment appear to be a credible strategy for rehabilitating denuded croplands and reduce the carbon buildup in the atmosphere (Daba and Dejene, 2018).

The atmosphere-plant-soil system is the most crucial part of the global C cycle, with about 17% of the 720 Gt atmospheric C stock flowing through it yearly (Jaradat, 2013). The assumption is that increasing soil C stocks would only require a slight increase in the flux of C from the atmosphere to plants and from plants to soils (Mathew *et al.,* 2017). Plants capture atmospheric C through photosynthesis for assimilation and release OC into the soil through rhizodeposition and decomposition of plant residues such as leaves, stems and roots (Abbas *et al.,* 2020). Balesdent and Balabane (1996) indicated that most of the C released into the soil by plants comes from roots rather than shoots. Evidence from other studies (Cardinael *et al.,* 2018; Hirte *et al.,* 2021; Katterer *et al.,* 2011) show that crops with higher root-to-shoot ratios (RS) have up to 20% higher capacity to sequester carbon in soils than crops with low RS. Furthermore, Lorenz and Lal (2014) observed that root-derived soil organic carbon (SOC) is 1.5 to 3.0 times higher than shoot-derived carbon. The reason for higher soil C sequestration by crops with high RS is that roots are physically embedded in the soil, providing them with a more stable and secure environment while providing soil fauna with a high variety of sugars that feed soil micro-fauna whole cells ultimately turning into soil organic matter (Kramer *et al.*, 2012). In addition, soil provides a buffer against environmental factors such as temperature fluctuations, moisture changes, and exposure to light, which enhances the decomposition of fresh above-ground organic matter (Buytaert *et al.,* 2011).

Variation in total biomass and C and their allocation between roots and shoots have been observed among crop species in several studies (Gonzalez-Sanchez *et al.*, 2012; Mathew et al, 2017). In a global meta-analysis, Mathew *et al.*, (2017) reported that maize had 11 and 32% higher shoot biomass and shoot C stocks than sorghum. Conversely, in Canada, Thivierge *et al.* (2016) reported higher shoot biomass for sorghum (19 kg ha^{-1}) followed by maize (17.6 kg ha^{-1}) and pearl millet (13.40 kg ha^{-1}). However, crop production environments as affected by soil type, climate, and management practices also significantly influence biomass and C allocation. For instance, Kukal and Benbi (2009) reported that wheat allocated 55% C to shoots in manure-fertilized soils but allocated 45% C to the shoots in soils applied with inorganic fertilisers. Similarly, Amujoyegbe *et al.* (2007) reported an increase in root biomass and root carbon stock allocation in maize by 35% and sorghum by 18.2 % in Nigeria when N application rate was increased. This information can be used to match crop types to land and inform on the best management practices to adopt to increase biomass allocation to the roots for land rehabilitation and climate change mitigation (Kellogg and Schware, 2019). Carbon allocation into crops differs not only between crop types and between land management systems but also between cultivars of a crop. Aquino *et al.* (2017) pointed out that the accumulation of carbon in maize shoots was 46% higher in a newly developed genotype “USM Var 10” than in “Crystal”, which is the local variety.

Maize (*Zea mays*), sorghum (*Sorghum bicolor*), and wheat (*Triticum aestivum*) are the major cereal crops in terms of grain production, food security and marketing. Over two-thirds of the global cereal outputs are used for food for an estimated 35% of the world’s population, and one-fifth is used to feed livestock (Grote *et al.*, 2021). The global production of maize and wheat is approximately 1127 million tons and 750 million tons annually, respectively (OECD, 2022). The global sorghum production amounts to an annual average of 58.87 million tons (OECD, 2022) . The crops can be grown in a wide range of agroecologies, including diverse temperatures and latitudes, and land and soil types. Furthermore, sorghum can withstand high temperatures and extended periods of drought due to its deep root system to access soil moisture (Chen *et al.*, 2020). Crop production in Africa has been challenged by drought stress, poor soil health, diseases, insect pests, and parasitic plants (Macauley and Ramadjita, 2015). Crops have high biomass production and can transfer atmospheric C to the soil over their life cycle which can enhance the potential for carbon sequestration.

There are limited studies that compared the major cereal crops for C storage for regenerative agriculture and to optimize C sequestration strategies. Understanding the C input between roots and shoots allows for assessing options for enhancing soil C storage. There is a need to differentiate potential powerhouse crops with high biomass production that involve high C sequestration to guide plant breeding and crop production programs (Wegener *et al.*, 2015). By integrating data collected from global studies, it would be possible to assess the variability in carbon allocation to shoots and roots among different crop cultivars. Hence, the objective of this paper was to integrate results from different studies worldwide to assess the variation in plant biomass production and C allocation of maize, sorghum, and wheat cultivars. Understanding the differences in biomass and carbon allocation between roots and shoots, can help assess the capacity of the major cereal crops to sequester atmospheric C and screen crop types for carbon efficiency to enhance soil health and productivity and subsequently mitigate climate change.

1.2 Materials and methods

1.2.1 Study setup

Research articles published between 1980 and 2022, and reporting on plant biomass and carbon variables for shoots and roots were identified using Google Scholar, Scopus, and Web of Science. Keywords used to identify relevant articles were “carbon partition”, “carbon allocation”, “plant carbon sequestration”, “root: shoot biomass carbon”, “rhizodeposition”, “plant/soil organic C stocks”, “root and shoot carbon”, “cereal”, “maize”, “sorghum” and “wheat”. All relevant articles were entered into a Microsoft Excel database. Articles included in the database had to meet the following criteria: i) they had to report on plant (both root and shoot) biomass, C stocks and C content variables, ii) they had to report on data for either maize, sorghum or wheat cultivars, and iii) they had to report on experiments conducted in the field rather than in pots or controlled environments. For articles reporting on multi-year experiments, each year was treated as a separate and independent experiment, while in the case of replicated values, a mean was calculated for each treatment to avoid duplication and bias. The final database (summarized in Table 1.1) consisted of 509 data points from 40 research articles, reporting on 133 variable genotypes of maize, sorghum, and wheat. Nine main variables, namely total plant biomass (PB), shoot biomass (SB), root biomass (RB), total plant carbon content (PCc), shoot carbon content (SCc), root carbon (RCc) content, total plant carbon stocks (PCs), shoot carbon stocks (SCs), and root carbon stocks (RCs) were included in the final database. The observations in the final database were stratified using long-term climate variables (mean annual precipitation (MAP) and mean annual temperature (MAT)) and soil parameters (pH and texture). When climatic variables were not explicitly described in each article, data was retrieved from climate-data.org (2021) for the location where the experiment was conducted. The soil texture was cited from journal articles or determined using a soil texture triangle according to Mutema *et al.* (2015) when proportions of sand, silt and clay were reported. The soil pH derived from the research articles was converted using the $CaCl_2$ scale and averaged across the soil profile to allow comparison using standardized values between research articles.

Table 1.1 References included in databases with locations, crops, and climatic zones under which the studies were conducted.

Paper ID	**Author and year**	**Crop**	**No. of tested genotypes**	**Country**	**Climate**	**Tillage**
1	Amujoyegbe *et al.*, 2007	Maize, sorghum	2	Nigeria	Sub-tropical	No tillage
2	Anderson, 1988	Maize	1	USA	Temperate	Conventional, minimum tillage, no tillage
3	Aquino *et al.*, 2017	Maize	2	Philippines	Tropical	Conventional
4	Bolinder *et al.*, 1997	Wheat	8	Canada	Temperate	Conventional
5	Christiansen-Weniger *et al.*, 1992	Wheat	3	Netherlands	Tropical	Conventional
6	Comin *et al.*, 1999	Maize	2	Brazil	Tropical	Conventional
7	Das *et al.*, 2016	Maize, Sorghum	2	USA	Temperate	No tillage
8	Figueroa-Bustos *et al.*, 2018	Wheat	5	Australia	Tropical	Conventional
9	Gan et al. 2009	Wheat	1	Canada	Temperate	Conventional
10	Geng *et al.*, 2006	Wheat	2	China	Sub-tropical	Conventional
11	Hebert *et al.*, 2001	Maize	7	France	Temperate	Conventional
12	Hussein and Alva, 2014	Sorghum	1	Egypt	Tropical	Conventional
13	Mathew *et al.*, 2019	Wheat	15	South Africa	Temperate	Conventional
14	Kanchikerimath and Singh, 2001	Maize	1	India	Sub-tropical	Conventional
15	Kaushik *et al.*, 2005	Wheat	3	India	Tropical	Conventional
16	Khorramdel *et al.*, 2013	Maize	1	Iran	sub-tropical	Conventional
17	Kundu *et al.*, 2007	Wheat	1	India	Sub-tropical	Conventional
18	Liang *et al.*, 2020	Maize	2	China	Temperate	Conventional
19	Liu *et al.*, 2014	Maize	4	China	Temperate	No tillage
20	Martin and Kemp, 1980	Wheat	12	Australia	Temperate	Conventional

Table 1.1 Continued

Paper ID	**Author and year**	**Crop**	**No. of tested genotypes**	**Country**	**Climate**	**Tillage**
21	Meki *et al.*, 2013	Sorghum	1	USA	Tropical	Conventional, minimum tillage, no tillage
22	Meskelu *et al.*, 2014	Maize	1	Ethiopia	Sub-tropical	Conventional
23	Montanez *et al.*, 2012	Maize	2	Uruguay	Temperate	Conventional
24	Msongaleli *et al.*, 2017	Sorghum	3	Tanzania	Tropical	Minimum tillage
25	Nguyen *et al.*, 2019	Wheat	2	Australia	Tropical	Conventional
26	Promkhambut *et al.*, 2010	Sorghum	4	United Kingdom	Tropical	Conventional
27	Sainju *et al.*, 2005	Sorghum	1	USA	Temperate	No tillage
28	Schortemeyer *et al.*, 1997	Maize	4	USA	Tropical	Conventional, minimum tillage
29	Shaheen and Hood-Nowotny, 2005	Wheat	4	Austria	Sub-tropical	Conventional
30	Shen *et al.*, 2007	Wheat	1	China	Temperate	No tillage
31	Srinivasarao *et al.*, 2012	Sorghum	1	India	Sub-tropical	Conventional
32	Teravest *et al.*, 2015	Maize	1	Malawi	Tropical	No tillage
33	Thivierge *et al.*, 2016	Maize, sorghum	2	Canada	Temperate	Minimum tillage
34	Van de Broek *et al.*, 2020	Wheat	4	Switzerland	Tropical	Conventional
35	Wang *et al.*, 2007	Wheat	3	China	Sub-tropical	Conventional
36	Wang *et al.*, 2018	Maize	5	China	Temperate	No tillage
37	Xia *et al.*, 2021	Maize	2	China	Temperate	Conventional
38	Xu *et al.*, 2019	Maize	10	Belgium	Temperate	Conventional
39	Xu *et al.*, 2020	Maize	10	Belgium	Temperate	Conventional
40	Zan *et al.*, 2001	Maize	1	Canada	Temperate	Conventional

1.2.2 Biomass and C allocation variables

Definitions for PB, RB, SB, RS, PCc, SCc, RCc, PCs, SCs, RCs, and RCs/SCs are summarized 1.2. Soil properties (clay content, bulk density, and pH) are also described in Table 1.3. All the definitions used in the current study were exclusively for the purposes of the current analysis and are not intended to be used in other contexts. These definitions matched most of the studies, except for a few studies where the authors did not separate roots from shoots. For the purposes of this study, all biomass was considered as shoot biomass when no distinction was made between roots and shoots. In articles where plant biomass and plant carbon variables were not provided, they were derived from adding shoot and root variables for biomass and carbon respectively. In instances were plant biomass, C stocks and C content variables were not reported directly, estimates were obtained using harvest indices and root-to-shoot ratios reported in the experiment. Where the biomass and carbon variables were not explicitly stated in the paper, they were estimated using ratios according to the following formulae:

$$RB = RS \times SB$$

$$SB = RS \times RB$$

$$PB = SB + RB$$

$$PCc = \frac{PCs}{PB} \times 100$$

$$SCc = \frac{SCs}{SB} \times 100$$

$$RCc = \frac{RCs}{SB} \times 100$$

Also, where the carbon variables were not stated, they were estimated according to Bar-On *et al.* (2018) using the following formulae:

$$SCs = SB \times SCc$$

$$RCs = RB \times RCc$$

$$PCs = SCs + RCs$$

Where RB is the root biomass (Mg ha^{-1}), SB the shoot biomass (Mg ha^{-1}), PB the total plant biomass (Mg ha^{-1}), RS the root-to-shoot biomass ratio, RCc the root carbon content (g C kg^{-1}), SCc the shoot carbon content (g C kg^{-1}), PCc the total plant carbon content (g C kg^{-1}), SCs the shoot carbon stock (Mg C ha^{-1}), RCs the root carbon stock (Mg C ha^{-1}), and PCs the total plant carbon stock (Mg C ha^{-1}).

Table 1.2 Descriptions of biomass and carbon variables used in this study.

Variable	Symbol	Unit	Definition
Total plant biomass	PB	Mg ha^{-1}	The total mass of root and shoot biomass of the crop.
Root biomass	SB	Mg ha^{-1}	The mass of above-ground biomass (stems and leaves) of the crop.
Shoot biomass	RB	Mg ha^{-1}	The mass of below ground biomass of the crop, excluding harvestable components.
Total plant carbon content	PCc	g C kg^{-1}	The total concentration of carbon in the roots and shoots.
Shoot carbon content	SCc	g C kg^{-1}	Concentration of carbon in the shoots.
Root carbon content	RCc	g C kg^{-1}	Concentration of carbon in the roots.
Total plant carbon stock	PCs	Mg C ha^{-1}	The total quantity of carbon contained in the entire plant, as stated by the authors, or as the sum of root and shoot carbon stocks.
Shoot carbon stock	SCs	Mg C ha^{-1}	The total quantity of carbon in the shoot biomass as stated by the authors or calculated as shoot biomass multiplied by shoot carbon concentration.
Root carbon stock	RCs	Mg C ha^{-1}	The total quantity of carbon in the root biomass stated by authors or calculated as root biomass multiplied by root carbon concentration.
Root-to-shoot ratio of biomass	RS		An expression of root biomass as a fraction of shoot biomass.
Root-to-shoot ratio of carbon stock	RCs/SCs		An expression of root carbon stocks as a fraction of shoot carbon stocks.

Table 1.3 Environmental factors and their categories

Factor	Remarks	Categories	Symbol	Class
Soil pH	Soil pH as reported in the article	< 5.5 6.5–7.5 > 7.5	pH	Acidic Neutral Alkaline
Soil bulk density ($g\ cm^{-3}$)	Average bulk density (BD) of soil profile	< 1.5 > 1.5	BD	Low High
Fertilizer application	Amount of fertilizer applied on the soil, as cited on the paper	N (kg/ha) P as P_2O_5 (kg/ha) K as K_2O (kg/ha)	NPK	Applied Nitrogen Applied Phosphate fertilizer. Potassium applied.
Climatic region	Based on the study site's average annual temperature and precipitation	Precipitation > 1000 mm Temperature > 20 °C Precipitation 300–1000 mm Temperature 10–20 °C Precipitation < 800 mm Temperature < 10 °C	Hot and warm Warm and arid humid Cool and arid to moist	Tropical Sub-tropical Temperate
Soil texture	Soil texture based as cited on the paper or based on soil texture triangle	% Clay % Silt % Sand	Texture	Clay, Sand, Loam, Sandy clay, Sandy clay loam, loamy sand, clay loam, silt loam, etc.
Tillage	The mechanical manipulation of the soil for the goal of crop production.	No ploughing at all Targeted ploughing Deep ploughing	Tillage	No-tillage. Minimum Conventional
Mulching	Covering of soil between plants with a layer of material (plastic)	Soil mulch Plastic mulch Organic mulch	Mulch	No mulch Half mulch Full mulch

1.2.3 Variability of biomass and carbon variables

Key statistics were computed to determine genotype variability based on plant and soil parameters. Standard deviations were calculated as a measure of variability between maize, sorghum, and wheat cultivars in PB, SB, RB, RS, PCc, SCc, RCc, PCs, SCs, RCs, and RCs/SCs. Variability was also expressed using the coefficient of variation (CV) as the ratio of the standard deviation and the mean for each biomass, C content, and C stock variables.

1.2.4 Data analyses

Standard deviations were calculated using Genstat 18th edition (Payne et al., 2011) for eachpaper to measure the variability of cultivars in that location. Summary statistics were generated for standard deviations of biomass allocation, C content, and C stocks using Genstat 18th edition (Payne *et al.,* 2011), which were outlined by mean, median, minimum, maximum, first quartile (Q_1) and third quartile (Q_3), standard deviation (SD), coefficient of variation (CV), skewness, and kurtosis. Box plots were used to depict the variability of datasets based on standard deviations obtained per individual site for the three crop types. Each boxplot recorded the outliers, minimum, maximum, median, mean, Q_1 and Q_3 values. Bar graphs showing the variability between crop cultivars expressed in percent of mean total biomass, C content, and C stocks were generated using Microsoft Excel 2016. Correlation coefficients (*r*), based on Spearman Rank correlations, were carried out using IBM SPSS statistics (Wagner III, 2019) to determine the magnitude of associations between variables. A biplot principal component analysis (PCA) was conducted using R statistical software (Core, 2019) to show the multiple relationships of the variation for biomass allocation, C allocation and C content with environmental factors.

1.3 Results

1.3.1 Variation of plant biomass, carbon content, and C stocks of cereal cultivars

The variabilities for biomass, carbon content, and C stocks recorded at individual sites of maize, sorghum, and wheat are summarized in Tables 1.4 to 1.6. Maize with a mean plant biomass of 4.18 Mg ha^{-1} accumulated the highest value followed by sorghum (2.02 Mg ha^{-1}) and wheat (1.10 Mg ha^{-1}) (Table 1.4). All the crops showed a similar trend for biomass and C allocation variability in shoots and roots, with shoots showing higher variability than roots across crop types (Figures 1.1a and c). Wheat had the lowest variability in shoot biomass (SB) than sorghum and maize but higher variability in root biomass (RB) compared to sorghum, with mean variability in SB and RB of 1.11 Mg ha^{-1} and 0.51 Mg ha^{-1}, respectively. Maize showed great variability across plant and shoot variables, whereas sorghum showed more variability when compared to wheat. Wheat had the highest mean (0.13) variability in root-to-shoot biomass ratio (RS), followed by maize (0.07) and sorghum (0.04).

Maize had the highest variability for total plant carbon content (PCc) with the maximum variability of 37.42 g C kg^{-1} followed by wheat (6.63 g C kg^{-1}) and sorghum (2.24 g C kg^{-1}) (Table 1.5 and Figure 1.1b). Wheat had the highest variability in shoot carbon content (SCc) and sorghum had the highest variability in root carbon content (RCc) with mean variability values of 0.58 g C kg^{-1} and 0.64 g C kg^{-1}, respectively. There is very low variation for carbon content between cultivars with constant variables recorded as coefficient of variation and standard deviation. Maize had the highest variability in total plant carbon stocks (PCs), ranging from 0.02 Mg C ha^{-1} to 14.36 Mg C ha^{-1} with a mean variability value of 1.55 Mg C ha^{-1} followed by sorghum (0.83 Mg C ha^{-1}) (Table 1.6 and Figure 1.1d). The variability in root biomass and carbon stocks was low across all crop types compared to variability in shoot parts. Wheat had the highest mean (0.18) variability in root-to-shoot carbon stock ratio (RCs/SCs), followed by maize (0.06) and sorghum (0.05).

Table 1.4 Summary statistics of biomass variables for maize, sorghum, and wheat

Statistics	**PB**			**SB**			**RB**			**RS**		
	Mg ha^{-1}											
	Maize	Sorghum	Wheat	Maize	Sorghum	Wheat	Maize	Sorghum	Wheat	Maize	Sorghum	Wheat
No.	19	8	13	19	8	13	19	8	13	19	8	13
Mean	4.18	2.02	1.49	3.31	1.76	1.11	1.27	0.32	0.51	0.07	0.04	0.13
Median	1.51	0.85	1.22	1.43	0.68	0.87	0.38	0.13	0.40	0.02	0.04	0.10
Min.	0.06	0.04	0.11	0.04	0.03	0.06	0.02	0.01	0.04	0.00	0.001	0.03
Max.	20.48	6.73	6.48	14.47	5.34	3.85	11.03	1.39	1.86	0.57	0.25	0.44
Q1	0.91	0.49	0.49	0.67	0.25	0.35	0.14	0.04	0.26	0.001	0.02	0.04
Q3	4.56	3.08	1.84	4.40	3.05	1.68	0.85	0.30	0.60	0.08	0.10	0.18
SD	5.80	2.23	1.56	4.22	1.94	1.05	2.55	0.45	0.46	0.13	0.08	0.12
SEM	1.33	0.79	0.42	0.97	0.69	0.28	0.58	0.16	0.12	0.03	0.03	0.03
Variance	33.66	4.96	2.43	17.82	3.75	1.10	6.50	0.20	0.21	0.02	0.01	0.01
%CV	138.79	110.03	105.01	127.51	110.16	94.35	200.08	140.35	90.01	186.60	179.42	94.80
Skewness	2.03	1.08	2.19	1.81	0.82	1.23	3.06	1.63	1.75	3.22	1.90	1.13
Kurtosis	4.16	0.98	7.51	3.23	-0.64	1.73	11.71	3.99	4.79	13.66	5.81	1.63

No = number of values, Min = minimum, Max = maximum, Q_1 = first quartile, Q_3 = third quartile, SD = standard deviation, SEM = standard error of mean, CV = coefficient of variation, PB = total plant biomass (Mg ha^{-1}), SB = shoot biomass (Mg ha^{-1}), RB = root biomass (Mg ha^{-1}), RS = root-to-shoot biomass ratio.

Table 1.5 Summary statistics of carbon content variables for maize, sorghum, and wheat

Statistics	**PCc**			**SCc**			**RCc**		
				g C kg^{-1}					
	Maize	Sorghum	Wheat	Maize	Sorghum	Wheat	Maize	Sorghum	Wheat
No.	19	8	13	19	8	13	19	8	13
Mean	2.30	0.52	0.95	0.10	0.30	0.58	0.12	1.19	0.64
Median	0.03	0.03	0.40	0.61	0.00	0.00	0.00	0.00	0.00
Min.	0.001	0.001	0.00	0.001	0.00	0.00	0.001	0.00	0.00
Max.	37.42	2.24	6.63	1.30	2.42	4.51	2.03	8.03	6.85
Q1	0.001	0.00	0.06	0.001	0.00	0.00	0.00	0.00	0.00
Q3	0.38	0.56	0.72	0.99	0.00	0.00	0.18	0.36	0.00
SD	8.32	0.84	1.68	0.32	0.80	1.43	0.45	2.63	1.81
SEM	1.91	0.30	0.45	0.07	0.28	0.38	0.30	0.93	0.48
Variance	69.24	0.71	2.83	0.10	0.64	2.04	0.21	6.93	3.27
%CV	361.86	161.96	177.88	307.62	264.57	246.73	388.74	222.00	281.94
Skewness	3.94	1.24	2.74	3.03	2.27	2.10	3.96	2.13	2.90
Kurtosis	18.54	0.79	10.06	10.86	8.00	4.33	18.63	7.20	10.97

No = number of values, Min = minimum, Max = maximum, Q_1 = first quartile, Q_3 = third quartile, SD = standard deviation, SEM = standard error of mean, CV = coefficient of variation, PCc = total plant carbon content (g C kg^{-1}), SCc = shoot carbon content (g C kg^{-1}), RCc = root carbon content (g C kg^{-1}).

Table 1.6 Summary statistics of carbon stock variables for maize, sorghum, and wheat

Statistics	**PCs**			**SCs**			**RCs**			**RCs/SCs**		
	Mg C ha^{-1}											
	Maize	Sorghum	Wheat	Maize	Sorghum	Wheat	Maize	Sorghum	Wheat	Maize	Sorghum	Wheat
No.	19	8	13	19	8	13	19	8	13	19	8	13
Mean	1.55	0.85	0.21	0.82	0.73	0.12	0.29	0.16	0.11	0.06	0.05	0.18
Median	0.46	0.46	0.16	0.38	0.30	0.09	0.09	0.06	0.07	0.01	0.02	0.13
Min.	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.003	0.001	0.001	0.001	0.01
Max.	14.36	2.94	0.83	3.56	2.35	0.48	2.52	0.59	0.56	0.53	0.24	0.69
Q1	0.22	0.21	0.05	0.16	0.11	0.03	0.05	0.04	0.03	0.0002	0.00	0.01
Q3	1.22	1.25	0.27	1.08	1.24	0.12	0.19	0.27	0.12	0.07	0.05	0.29
SD	3.20	0.92	0.23	1.04	0.80	0.13	0.58	0.19	0.14	0.12	0.08	0.19
SEM	0.73	0.33	0.06	0.24	0.28	0.03	0.13	0.07	0.04	0.03	0.03	0.05
Variance	10.23	0.85	0.05	1.08	0.64	0.02	0.34	0.04	0.02	0.01	0.01	0.04
%CV	206.66	107.96	109.46	127.16	109.94	107.32	197.46	115.51	126.97	206.13	155.54	106.22
Skewness	3.39	1.26	1.55	1.80	0.94	1.60	3.07	1.24	2.48	3.26	1.83	1.24
Kurtosis	14.46	2.41	2.84	3.21	0.29	3.53	11.75	2.14	8.86	13.82	5.58	2.21

No = number of values, Min = minimum, Max = maximum, Q_1 = first quartile, Q_3 = third quartile, SD = standard deviation, SEM = standard error of mean, CV = coefficient of variation, PCs = total plant carbon stock (Mg C ha^{-1}), SCs = shoot carbon stock (Mg C ha^{-1}), RCs = root carbon stock (Mg C ha^{-1}), RCs/Scs = root-to-shoot carbon stock ratio.

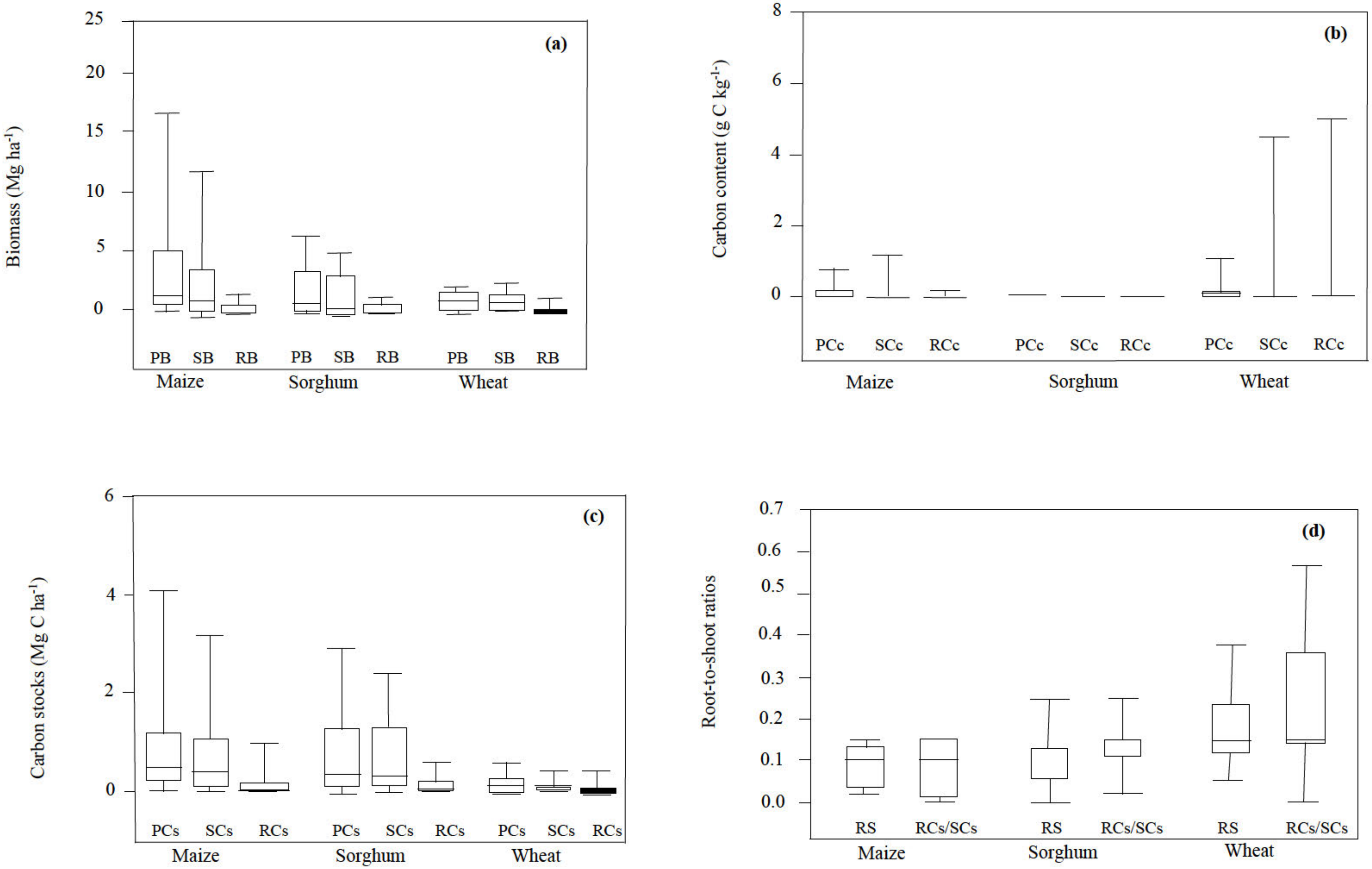

Figure 1.1 Variability between crop cultivars in total plant biomass (PB), shoot biomass (SB), and root biomass (RB) (a); total plant carbon content (PCc), shoot carbon content (SCc), and root carbon content (RCc) (b); total plant carbon stock (PCs), shoot carbon stock (SCs), and root carbon stock (RCs) (c); root-to-shoot biomass ratio (RS) and root-to-shoot carbon stocks ratio (RCs/SCs) (d) of maize, sorghum, and wheat. Each box plot presents the minimum, maximum, median, quartile 1 (25%), and quartile 3 (75%). See Table 1.2 for trait descriptions and units.

1.3.2 Variability expressed in percent of mean biomass, C content, and C stocks

The variability between cultivars expressed in percent of mean plant biomass, carbon content, and C stocks are presented on Table 1.7 and Figure 1.2. Maize and wheat had higher variability expressed in the percent of mean PB, SB, and RB (Figure 1.2a). Sorghum showed the lowest variability expressed in percent of mean PB (16.82%), SB (18.13%), and (36.96%), respectively, compared to maize and wheat. The variability expressed in the percent of mean SB in maize and wheat was greater than 27.97% and was twice the variability expressed in percent of mean SB in sorghum. Maize had variability expressed in percent of mean PB exceeding 31.89% compared to 24.15% of wheat.

Similar trends were observed for carbon content variables with maize excelling higher than the other crops. Maize (8.39%) had the highest variability expressed in percent of mean PCc, followed by wheat (6.15%), and sorghum (1.19%). Wheat amassed the highest variability expressed in percent of mean SCc and RCc (4.32% and 3.15%, respectively), followed sorghum (0.69% and 2.82%, respectively) and maize (0.42% and 0.51%, respectively) (Figure 1.2b).

Maize exhibited higher variability expressed in percent of mean PCs, SCs, and RCs compared to sorghum and wheat with the values of 40.13%, 30.76%, and 50.38%, respectively. Sorghum had the lowest variability expressed in percent of mean carbon stocks for all variables measured (Figure 1.2c). Maize had the highest variability expressed in percent of mean RS and wheat displayed the highest variability expressed in percent of mean RCs/SCs with values of 24.38% and 30.92%, respectively (Figure 1.2d).

Table 1.7 Mean variability, the standard deviation of variability, and percent of mean variability for plant biomass and carbon stocks for maize, sorghum, and wheat.

Parameters and crop	**PB**	**SB**	**RB**	**RS**	**PCc**	**SCc**	**RCc**	**PCs**	**SCs**	**RCs**	**RCs/SCs**
Mean variability		Mg ha^{-1}				g C kg^{-1}			Mg C ha^{-1}		
Maize	4.18	3.31	1.27	0.07	2.3	0.1	0.42	1.55	0.82	0.29	0.06
Sorghum	2.02	1.76	0.32	0.04	0.52	0.30	1.19	0.85	0.73	0.16	0.05
Wheat	1.49	1.11	0.51	0.13	0.95	0.58	0.64	0.21	0.12	0.11	0.18
SD of variability											
Maize	5.80	4.22	2.55	0.13	8.32	0.32	0.45	3.20	1.04	0.58	0.12
Sorghum	2.23	1.94	0.45	0.08	0.84	0.80	2.63	0.92	0.80	0.19	0.08
Wheat	1.56	1.05	0.46	0.12	1.68	1.43	1.81	0.23	0.13	0.14	0.19
Coefficient of variation						%					
Maize	31.89	31.78	51.97	24.38	8.39	0.42	0.51	40.13	30.76	50.38	22.02
Sorghum	16.82	18.13	13.64	16.79	1.19	0.69	2.82	16.30	17.03	16.76	16.76
Wheat	24.15	27.97	29.94	21.09	6.15	4.32	3.15	24.35	23.14	30.92	30.92

See Table 1.2 for trait descriptions and units.

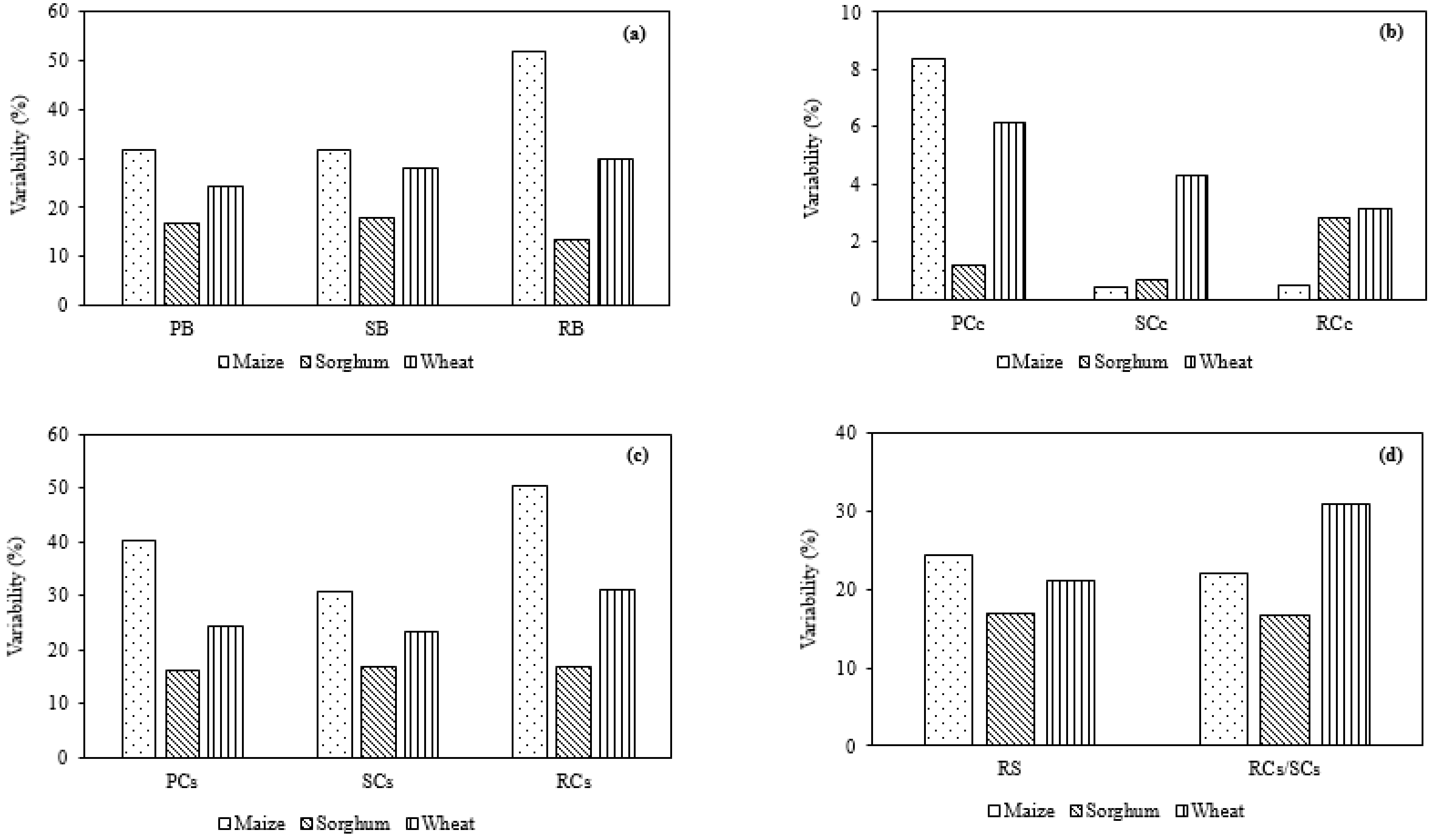

Figure 1.2 Variability between crop cultivars expressed in percent of mean total plant biomass (PB), shoot biomass (SB), and root biomass (RB) (a); total plant carbon content (PCc), shoot carbon content (SCc), and root carbon content (RCc) (b); total plant carbon stock (PCs), shoot carbon stock (SCs), and root carbon stock (RCs) (c); root-to-shoot biomass ratio (RS) and root-to-shoot carbon stocks ratio (RCs/SCs) (d) of maize, sorghum, and wheat. See Table 1.2 for trait descriptions and units.

1.3.3 Global variability expressed in percent of mean plant biomass, C content, and C stocks

The variability between cultivars expressed in percent of mean plant biomass, C content, and C stocks for different continents are presented in Figure 1.3. Europe (PB = 40.02%, SB = 41.39%, and RB = 70.74%) had the highest variability for all the biomass variables, followed by Africa (PB = 35.46%, SB = 35.81%, and RB = 37.18%) and Asia (PB = 31.18%, SB = 33.9%, and RB = 38.13%). The continents with the lowest variability expressed were South America, North America, and Oceania (Figure 1.3a) in descending order. Similar trends were observed for C content, with Europe continuing to excel for variability expressed in percent of mean C content for all the C content variables (Figure 1.3b).

Europe (PCs = 65.13%, SCs = 38.55%, and RCs = 66.22%) had the highest variability expressed in percent of mean C stocks for all the carbon variables, followed by Asia (PCs = 33.00%, SCs = 38.19%, and RCs = 38.31%), and Africa (PCs = 32.46%, SCs = 31.34%, and RCs = 37.82%) (Figure 1.3c). Europe exhibited the highest variability expressed in percent of mean RS and RCs/SCs (36.73% and 24.84%, respectively) (Figure 1.3d).

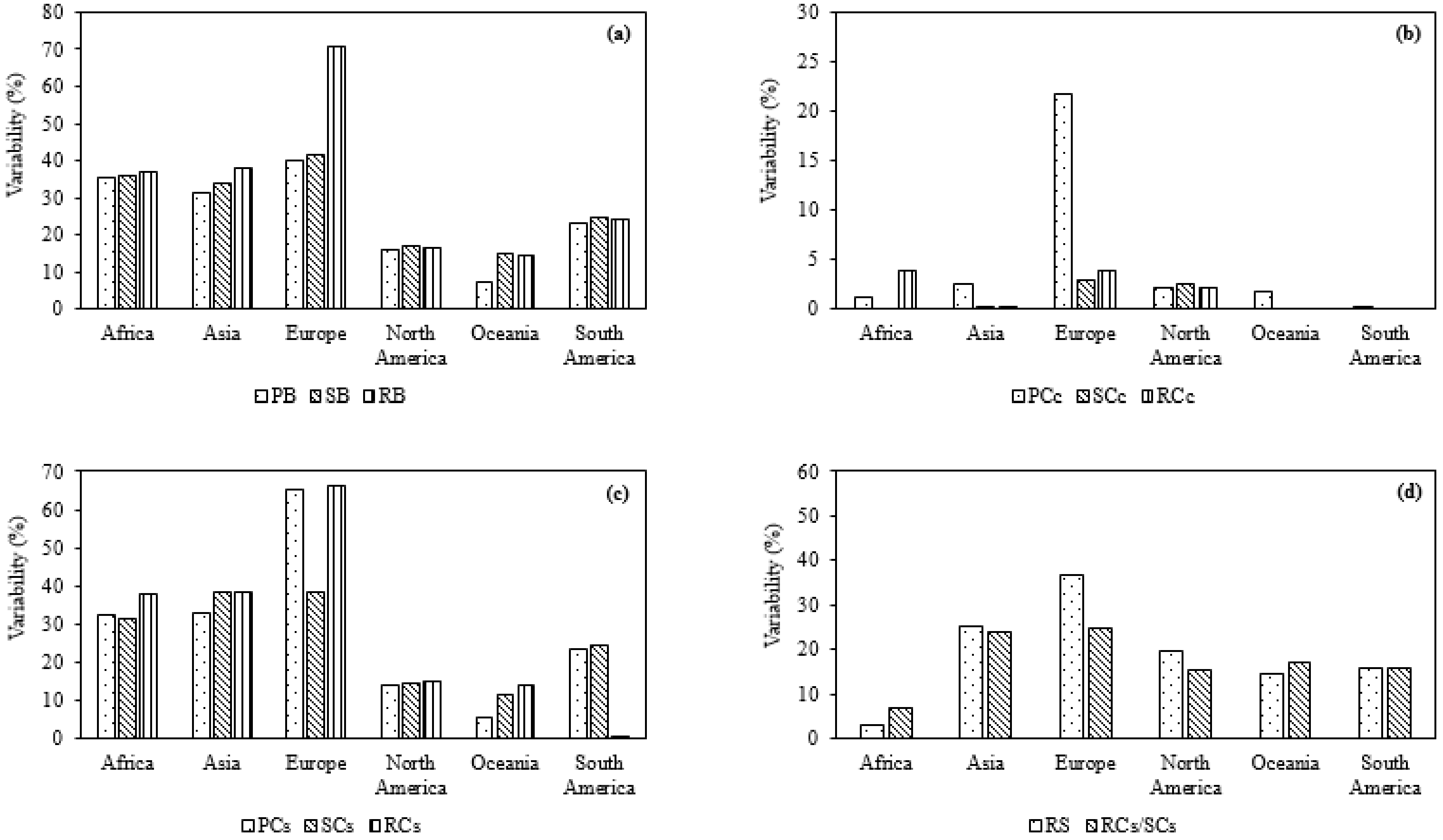

Figure 1.3 Variability between crop cultivars expressed in percent of mean total plant biomass (PB), shoot biomass (SB), and root biomass (RB) (a); total plant carbon content (PCc), shoot carbon content (SCc), and root carbon content (RCc) (b); total plant carbon stock (PCs), shoot carbon stock (SCs), and root carbon stock (RCs) (c); root-to-shoot biomass ratio (RS) and root-to-shoot carbon stocks ratio (RCs/SCs) (d) of maize, sorghum, and wheat for different continents. See Table 1.2 for trait descriptions and units.

1.3.4 Associations between environmental factors and variabilities for biomass, C stocks, and carbon content

Mean annual precipitation and variability in RB displayed the strongest significant positive correlation (*r* = 0.85, $p < 0.05$), suggesting a direct link between the two (Table 1.8). The variability in PB and SB significantly negatively correlated with both MAP and MAT. The variability in PCs and SCs followed the same trend but showed an insignificant correlation. Mean annual temperature had the strongest significant positive correlation (*r* = 0.72, $p < 0.05$) with variability in RB. Mean annual precipitation exhibited the strongest correlations with variability in RS and RCs/SCs (*r* = 0.80 and *r* = 0.80, $p < 0.05$, respectively) compared to MAT. This trend was the same with variability in RCs. The variability in PCc, SCc, and RCc exhibited non-significant correlations with all the environmental factors.

Table 1.8 Correlations showing relationship between variability in biomass, C variables, and environmental factors.

Plant variables	MAT	MAP
PB	-0.47*	-0.34*
SB	-0.43*	-0.30*
RB	0.72*	0.85*
RS	0.67	0.81*
PCc	0.58	0.73
SCc	0.39	0.57
RCc	0.49	0.66
PCs	-0.45	-0.31
SCs	-0.70	-0.63
RCs	0.60	0.76
RCs/SCs	0.65	0.80*

* Significance at $P \leq 0.05$. See Table 1.2 for trait descriptions and units.

1.3.5 Principal component biplot for variability of biomass, C content, C stocks, and environmental factors

A biplot based on the principal component analysis (PCA) of variables relationship between the variation of biomass, carbon stock, carbon content, and environmental factors of different cereals is shown in Figure 1.4. The first and second principal components (PC1 and PC2) accounted for a total variation of 90%, with PC1 accounting for 60.9% of the variation while PC2 accounted for only 29.1%. The variability between cultivars in PB, RB, SB, PCc, and RCs, were strongly associated with PC1. On the other hand, PC2 was positively correlated with the variability in RS, SCc, and RCs/SCs. The variability in PCs was associated with SCs and could thus be interpreted as an axis of carbon enrichment. Maize varieties were associated to PC1, while wheat varieties contributed more to PC2. Sorghum had a negative association with both PC1 and PC2. The first PC correlated with MAP, and PC2 was closely correlated with MAT on the negative coordinates. Several of the studied variables, including variability in RS and RCs/SCs showed negative coordinates on Axis 2. Conversely, variability in PB, SB, and carbon stocks between cultivars increased as MAT decreased, and variability in SCc increased with increasing MAP.

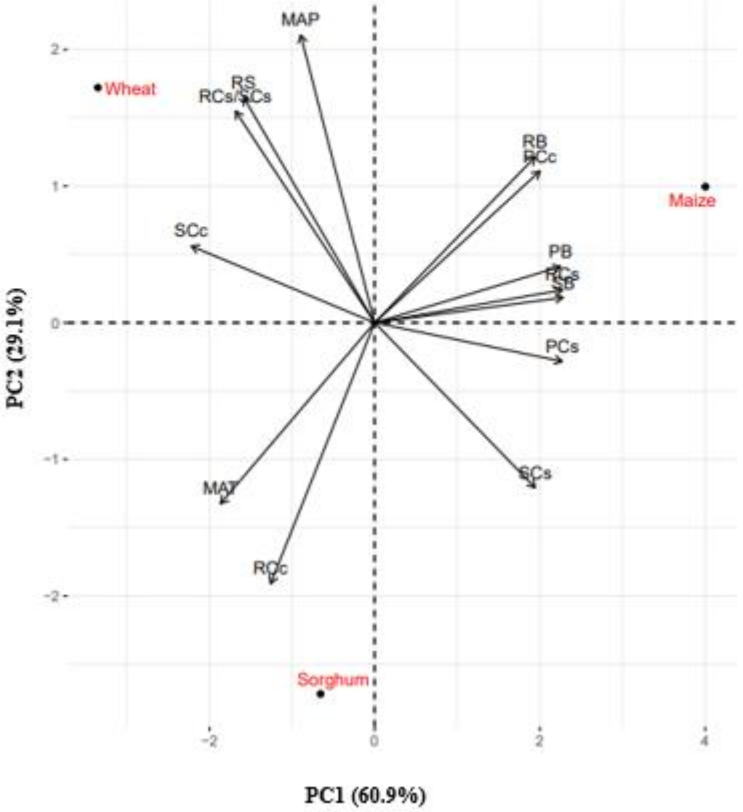

Figure 1.4 Principal component biplot displaying the relationship among the variability in plant biomass, C content, C stocks, and environmental factors in cultivars of maize, sorghum, and wheat. See Table 1.2 for trait descriptions and units.

1.4 Discussion

1.4.1 Causes of variation in biomass allocation amongst crop types

The present study shows that different crop types exhibited significant variations in biomass allocation, agreeing with Monti *et al.* (2008) (Table 1.5). A higher amount of biomass was measured in maize compared to sorghum and wheat for all the plant variables, consistent with Ritchie *et al.* (1998) and Guzman and Al-kaisi (2010). Compared to other cereals, maize produces more biomass because it maximizes light absorption for synthesising carbon assimilates that are used to drive biomass production (Stewart, 2013). Additionally, the C4 photosynthetic pathway in crops such as maize and sorghum is more efficient at utilizing carbon dioxide than the C3 crops such as wheat. This enables C4 species to photosynthesize more effectively, producing higher biomass (Sales *et al.,* 2021). Furthermore, C4 species are better acclimated to high temperatures and droughts, typical in many regions where maize and sorghum are cultivated (Brown, 1999). These factors collectively contribute to the higher biomass accumulation observed in maize and sorghum than wheat. Global maize improvement programs have achieved yield gains using traditional maize landraces and improved open-pollinated varieties (OPV) or hybrid varieties. The result of maize improvement has thus been increased yield. Maize has higher hybrid vigour compared to sorghum or wheat, and new cultivars have been developed that generate more biomass than sorghum or wheat (Hiremath *et al.,* 2013). Studies in maize conducted by Ibraheem and El-Ghareeb (2019) and Li *et al.,* (2018) reported that F_1 hybrid of maize showed strong heterosis for agronomic traits and increased biomass compared to parents. These results are consistent with the ones reported by Singh *et al.* (2014), who indicated that crosses involving complementary inbred lines with different genetic compositions result in hybrid vigor, which produces superior phenotypes with higher yield, accelerated growth rate and development, improved biomass, better quality, and improved resistance to biotic and abiotic stress. This also explains the high variability in biomass variables in maize compared to other cereals. With landraces, OPVs, and commercial cultivars grown worldwide, wide variation in biomass production is expected in maize.

Wheat accumulated less biomass and C compared to maize and sorghum for all the biomass and C variables, and this may be due to the low plant stature (Figure 1.1a). The crop size of wheat is generally smaller than that of maize and sorghum. In addition, Theocharis *et al.* (2012) reported that one of the main environmental stressors that restricts wheat growth and photosynthetic output and lowers grain yield is low temperature. Wheat is mainly grown in temperate regions, and cold stress often extends the period of crop growth and lowers net photosynthetic rate and biomass accumulation (Li *et al*., 2015; Whaley *et al.,* 2004; Yamori *et al.,* 2014). Interestingly, wheat had higher root-to-shoot biomass ratios (RS). However, the size of the wheat root systems remains lower than that of maize and sorghum, and as such, the latter two crops will contribute more to carbon sequestration than wheat.

Open-pollinated and landrace sorghum genotypes could produce comparable or higher biomass than maize. However, improved sorghum cultivars, such as hybrids, have shorter plant stature and reduced biomass than maize. Maize has undergone extensive genetic improvement efforts over the years, leading to the development of high-yielding varieties optimized for biomass production than sorghum (Gedil and Menkir, 2019). In contrast, sorghum has not been widely researched and bred, aiming to increase biomass yields (Hao *et al.,* 2021). Therefore, sorghum possesses great untapped potential in breeding for biomass production. Sorghum breeding for ethanol production led to substantial genetic gains for biomass production (Pfeiffer *et al.,* 2019). However, this has been limited to sweet stem sorghums, and the adoption of such varieties will not be beneficial to resource-poor farmers in drier areas where they depend on sorghum grain for food.

1.4.2 Causes of variation in C accumulation and allocation amongst crop type

Sorghum had higher total plant carbon content and plant carbon stock than maize and wheat, making it a more efficient crop in increasing carbon fluxes from the atmosphere to the soil (Figure 1.1c and d). Its big and fibrous root system will ensure deeper C deposition in the soil which will be crucial for the long-term stability of SOC (Zuazo and Pleguezuelo, 2009). The large and deep root system of sorghum distinguishes its root architecture (Kell, 2011). According to Xiong *et al.,* (2020), sorghum roots can reach soil depths of up to 2 m, whereas maize and wheat roots typically reach 1 m or less. Sorghum has deeper root system and deposits organic matter, including carbon, in deeper soil horizons. Because of its notable drought tolerance, sorghum develops deep roots as an adaptation strategy. Wheat and maize, on the other hand, have shallower root systems despite being sensitive to drought stress. Drought-stressed conditions can promote deeper root growth in sorghum (Chadalavada *et al.,* 2021). The authors observed sorghum roots extending deeper during dry spells. Gautam *et al.* (2020) reported that sorghum produced 20% more aboveground biomass, which enhanced carbon inputs into the soil profile.

1.4.3 Variations of plant biomass and carbon variables between crop type cultivars

The variation between cultivars in root and shoot biomass could result from different specific allocation patterns caused by genetic variation between major cereal species. These patterns could be high shoot biomass production in maize, deep root systems, and balanced allocation of shoot and root biomass in sorghum and tillering in wheat (Irving, 2015). Maize had higher variability in PB, SB, and RB between cultivars compared to sorghum and wheat (Figure 1.1a). Pittelkow *et al.*, (2015) reported that the increase in biomass production from subtropical to tropical regions corresponds to increases in temperature and precipitation. These results are consistent with the ones reported in the current study as variability in RB had the strongest, highest positive correlation with MAP and MAT (Table 1.8). Hence, lower precipitation limits root biomass production in temperate and subtropical climates, whereas low temperatures further limit biomass production in temperate climates.

Sorghum also exhibited high variability in PB, SB, and RB between cultivars than wheat. This is due to sorghum's genetic diversity. There is a wide range of stem biochemical compositions suitable for different end uses, such as bioenergy or fodder (Perrier *et al.,* 2017). Due to its drought tolerance, sorghum can sustain biomass production under water-stressed conditions. The wide differences in shoot biomass compared to root biomass for maize, sorghum, and wheat are due to their adaptations. Performance variations between genotypes represent genetic diversity which is influenced by genetic compositions, the production environment and their interaction (Hughes *et al.*, 2008).

There was an increase in variation between genotypes of sorghum for PCs (Figure 1.1c). Cultivars may respond differently to growing conditions (Anderson-Teixeira *et al.,* 2013). This is shown by the decrease in variation between cultivars of maize for total PCs by the different factors such as tillage system, fertilizers, and environmental conditions used across the studies. Wheat remains to be the crop with the lowest PCs, SCs, and RCs. There was no marked variation between genotypes for the carbon content of all the tested crops, as revealed by the constant performance (Figure 1.1b). The trend could be attributed to higher proportions of proteins and lipids, which are crucial components of plant tissues in maize, sorghum, and wheat. In turn, this contributed to a higher carbon content as a major constituent. Biomass and C allocation varied between roots and shoots as RS ratios varied significantly amongst the wheat cultivars across the studies (Figure 1.1d). In a study conducted by Toscano *et al.* (2019) it was reported that heat-tolerant wheat genotypes exhibit a high RS, which indicates their capacity to sustain productivity even under conditions of simultaneous drought and heat stress.

This allowed the heat-tolerant genotypes to allocate more biomass to root development than the heat susceptible genotypes. Such genotypes with high biomass accumulation and heat endurance are more appropriate for SSA, where heat stress and drought are frequently co-occurring conditions.

1.4.4 Associations between plant biomass and carbon variables

The strong correlations of variability in SCs and RCs and variability in RB with PC1 show that the three traits were the most important in explaining variation among the cereal crops. Therefore, identifying shoot and root carbon stocks in varieties could be important in cultivar selection for carbon sequestration. Carbon content may be less effective in differentiating varieties. The carbon content in varieties is relatively constant and varies slightly between varieties and, in most cases, even between crop types (Ma *et al.*, 2018). Maize was correlated with PC1, which showed that maize varieties exhibited most of the variation in this panel of cultivars while wheat varieties had the least variation. In new sorghum cultivars, biomass production can be harnessed through hybrid breeding and new genomic technologies that will accelerate sorghum improvement (Hao *et al.*, 2021). There is high biomass production and carbon accumulation between maize cultivars but a low variability in carbon enrichment in plant parts. In contrast, wheat showed a high variableness in carbon enrichment but a low variableness in biomass production and C accumulation. Sorghum genotypes only marginally varied for plant C content and exhibited low variability in biomass and C accumulation.

1.5 Conclusions

Maize, sorghum, and wheat showed significant variation in biomass production, carbon accumulation, and allocation to roots and shoots, demonstrating the importance of these genetic resources for selecting and developing varieties with improved C sequestration potential. However, sorghum presents the greatest potential for breeding to increase biomass production due to limited breeding in the crop. Using sorghum as a model crop for increasing carbon sequestration can go a long way in mitigating the effects of climate change. Maize and wheat will also remain important crops that can be used to support climate-smart agriculture. These findings improve our understanding of how C is allocated within roots and shoots and possibly to soils. This is especially needed when best practices such as zero tillage or cover cropping do not store carbon into soils as much as has been claimed (Baker *et al.*, 2007; Chaplot and Smith, 2023). The meta-analysis revealed that maize and sorghum have the highest variability for total plant biomass and plant carbon stocks, while wheat exhibited the highest variability for the below-ground biomass and carbon stocks. The data aided in crop selection and suggested that the best cultivars could be developed and identified for production and C sequestration potential for cultivation by farmers, land rehabilitation, and climate change mitigation. The present study used data based on 40 global studies that reported on allocating plant biomass and C between roots and shoots of the major cereal crops. The independent studies used varied experimental setups, data collection and reporting, which may introduce inconsistencies and biases in the integrated analysis and conclusions. Standardization of the study protocols and data reporting may improve the reliability of the findings and recommendations. Further, the study recommends integrating multiple traits related to biomass production and carbon allocation from ongoing and diverse studies. Leaf area, root architecture, and photosynthetic efficiency should be included in future studies due to their influence on crop performance and biomass and C allocation between roots and shoots.

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CHAPTER 2: Response of Sorghum (*Sorghum bicolor* [L.] Moench) Genotypes for Yield and Yield Components and Organic Carbon Storage in the Shoot and Root systems

Abstract

Sorghum is a vital food and feed crop in the world's dry regions. Developing sorghum cultivars with high biomass production and carbon sequestration can contribute to soil health and crop productivity. The objective of this study was to assess agronomic performance, biomass production and carbon accumulation in selected sorghum genotypes for production and breeding. Fifty sorghum genotypes were evaluated at three locations (Silverton, Ukulinga, and Bethlehem) in South Africa during 2022 and 2023 growing seasons. Significant genotype x location ($p < 0.05$) interactions were detected for days to 50% heading (DTH), days to 50% maturity (DTM), plant height (PH), total plant biomass (PB), shoot biomass (SB), root biomass (RB), root-to-shoot biomass ratio (RS), and grain yield (GY). The highest GY was recorded for genotypes AS115 (25.08 g plant^{-1}), AS251 (21.83 g plant^{-1}), and AS134 (21.42 g plant^{-1}). Genotypes AS122 and AS27 ranked first and second, respectively, for all the carbon stock parameters except for root carbon stock (RCs), whereas genotype AS108 had the highest RCs of 8.87 g plant^{-1}. The principal component analysis identified GY, DTH, PH, PB, SB, RB, RCs, RCs/SCs, total plant carbon stock (PCs), shoot carbon stock (SCs), and grain carbon stock (GCs) as the most discriminated traits among the test genotypes. The cluster analysis using agronomic and carbon-related parameters delineated the test genotypes into three genetic groups, indicating marked genetic diversity for cultivar development and enhanced C storage and sustainable sorghum production. The selected sorghum genotypes are recommended for further breeding and variety release adapted to various agroecologies in South Africa.

Keywords: carbon sequestration; genetic diversity analysis; sorghum; yield components

2.1 Introduction

Sorghum (*Sorghum bicolor* [L.] Moench, $2n = 2x = 20$) is the fifth most important cereal crop after maize, wheat, rice, and barley cultivated globally. It is the primary food source for approximately 750 million people living in the semi-arid tropics of Africa, Asia, and Latin America, with an annual global production of 61.62 million tonnes (Khoddami *et al.,* 2023). It serves as a raw material for feed, bioenergy, and industrial applications. It has unique nutritional value and adaptation to dryland conditions. In South Africa, sorghum is one of the most widely grown crops with an annual production of 215,000 tons (Tonapi *et al.,* 2020). In the country, the largest sorghum production is found in Mpumalanga (41%) followed by Limpopo (34%), Free State (31%), and North West (25%) Provinces. In sub-Saharan Africa, sorghum is predominantly produced by smallholder farmers who have limited access to production inputs such as improved seeds, crop protection chemicals, inorganic fertilizers, irrigation facilities, and finance (Muimba-Kankolongo, 2018). Furthermore, the major proportion of sorghum is cultivated under marginal and poor soil conditions, resulting in low crop yields and quality.

Reportedly, sorghum is one of the major crops with high biomass production with a substantial capacity for carbon (C) storage in agricultural soils (Xiang *et al.,* 2017). Improved sorghum cultivars with high biomass production and carbon sequestration can contribute to soil health and crop productivity. Agricultural soils cover up to 34% of the global land surface (Ritchie and Roser, 2021). Hence, adequate management of agricultural lands is vital to maintaining soil organic C storage by incorporating crop biomasses and residues with enhanced decomposition (Janzen, 2015; Paustian *et al.,* 2016)

There is a high carbon loss in cultivated croplands in Africa due to the removal of most plant residues after harvest for various household uses (e.g. fuel wood, livestock feed, and construction material). Also, high biomass decomposition rates and nutrient loss due to erosion causes poor soil fertility and low yield gains (Poeplau and Don, 2015). Approximately 17% of all atmospheric CO_2 flows through the plant-soil-atmosphere interaction each year, making soil and plant C critical to the global C cycle (Bruggemann *et al.,* 2011). Root C is a major contributor to soil organic C, accounting for up to 90% of all C inputs to arable soils (Katterer *et al.,* 2011). Due to its unique and stable chemical composition (Rasse *et al.,* 2005) and partitioning into more stable components (Ghafoor *et al.,* 2017), root C has more extended residence in the soil bank compared to C-derived from above-ground crop residue (Katterer *et al.,* 2011; Menichetti *et al.,* 2015; Zhang *et al.,* 2015). Plant roots have relatively low decomposer association and high C storage capacity in deep soil layers (Rumpel *et al.,* 2012; Sanaullah *et al.,* 2016), serving as a long-term C reservoir (Russell *et al.,* 2009; Fan *et al.,* 2019). Therefore, crop ideotypes such as sorghum genotypes with fibrous and deep root systems have been identified as promising contributors to enhanced carbon in soils, with an estimated potential to sequester 1 Pg yr^{-1} of atmospheric CO_2 (Lynch and Wojciechowski, 2015; Pierret *et al.,* 2016; Paustian *et al.,* 2016).

There is a marked difference among crops and genotypes within a crop in C storage and deposition of plant C into soils. For instance, Mathew *et al.,* (2020) reported that barley transferred 29% C into the soil, followed by maize (20%), and wheat (18%). Similarly, Bolinder *et al.,* (1997) reported higher (50%) annual C transferred to the soil by barley compared to oats (48%) and winter wheat (32%). Manna *et al.,* (2005) also reported a 45% C transfer into the soil by sorghum into as compared to 33% by rice under different soil types. Significant differences for C storage by wheat cultivars have also been reported Chaplot *et al.,* (2023), where the authors found that plant C stocks under 25% field capacity were the highest for genotypes BW152 (1059 g C m^{-2}) and BW141 (1004 g C m^{-2}), while genotype BW140 and LM26 had values below 850 g C m^{-2}, and genotype BW141 had the highest plant C stock of 2260 g C m^{-2} under 75% field capacity. Ahmed *et al.,* (2020) reported that Wary sorghum genotype exhibited the highest root C allocation at 386 Mg C ha^{-1}, while UNL-hybrid-5 demonstrated the lowest root C allocation at 140 Mg C ha^{-1}. Moreover, the authors reported that genotype BATAEM-4 exhibited the highest shoot C allocation at 3334 Mg C ha^{-1}, whereas UNL-hybrid-5 exhibited the lowest shoot C allocation, measured at 1007 Mg C ha^{-1} at different locations. Xiang *et al.,* (2017) reported that growing sorghum varieties with high biomass could significantly increase C sequestration in soils. In another study by Liang *et al.,* (2020), maize varieties had high yield potential and greater capacity to store C in plant biomass and soil. Amujoyegbe *et al.,* (2007) reported increased root biomass and root C stocks allocation in maize by 35% and sorghum by 18.2 % when the soil nitrogen content increased. From the above literature, it is necessary to evaluate specific crop types and genotypes in the target production environments for the targeted recommendation and devise the best management practices that can be adopted to increase biomass allocation to the roots for land rehabilitation, soil C storage and crop productivity.

Mangena *et al.,* (2018) reported significant differences for agronomic traits and biomass production between sorghum genotypes evaluated, where the use of 190 diverse sweet sorghum genotypes played a crucial role in shaping the observed variations. The authors found that biomass yield varied from 6.67 to 111.20 t ha^{-1}, with genotype AS203 producing 20% higher yield than all other genotypes. Abraha *et al.,* (2015) reported that under drought conditions, sweet sorghum genotypes EG 469 and Hamelmalo had the highest biomass production of 16.70 t ha^{-1} and 18.10 t ha^{-1}and produced the highest grain yields of 2.70 t ha^{-1} and 2.6 t ha^{-1}, respectively. Complementary and contrasting genotypes can be used to create new breeding populations to develop a sorghum ideotype characterized by high root biomass and improved yield gains.

There is limited knowledge that documented the genetic diversity of sorghum integrating agronomic traits, balanced biomass allocation, and C sequestration under South African production conditions. Previous studies have reported differences in plant C stocks between crop types with limited emphasis on intra-specific variations to guide breeding, especially for C sequestration potential. Screening genetically diverse sorghum lines will enable the selection of best-performing genotypes for crop breeding and selection programs. Sorghum genotypes with desirable agronomic traits and high biomass production can improve C sequestration capability and yield gains through enhanced water and nutrient use efficiencies. Therefore, the objective of this study was to assess agronomic performance, biomass production and carbon accumulation in genetically diverse sorghum lines across three different locations to select unique genotypes for production and breeding. The findings may be beneficial for crop breeders to assess the variations in biomass allocation and agronomic performances. This is crucial for developing sorghum genotypes with increased grain yield, drought tolerance, water use efficiency, and the capacity for C sequestration into soils.

2.2 Materials and methods

2.2.1 Plant materials

Fifty sorghum genotypes consisting of landraces, pure lines and commercial hybrids were used in this study (Table 2.1). The test germplasms were obtained from different sources, including Zimbabwe, South Africa, Ethiopia, and Tanzania. South African genotypes were collected from KwaZulu-Natal, Eastern Cape, and Limpopo Provinces and mainlined at the African Centre for Crop Improvement (ACCI) of the University of KwaZulu-Natal (UKZN) in South Africa. The genotypes were selected for their high grain yield, biomass, and ethanol production (Mangena *et al.,* 2018).

Table 2.1 Detailed description of sorghum genotypes used in this study.

Name	Pedigree	Source	Seed colour	Country	Name	Pedigree	Source	Seed colour	Country
05-POTCH-138	50-POTCH-138	ARC-GCI	White	SA	**AS143**	Red Swazi	ACCI	Brown	SA
16MZ	-	-	Brown	-	**AS145**	AWN98	ACCI	Brown	SA
AS106	Landrace	ACCI	Cream	SA	**AS147**	MRS94	ACCI	Red	SA
AS108	P9504B	ACCI	Cream	SA	**AS148**	SDS 3472	ACCI	Brown	SA
AS109	P9511B	ACCI	Cream	SA	**AS152**	01MN1589	ACCI	Brown	SA
AS111	P9539B	ACCI	Cream	SA	**AS194**	Mtentu Imphe	D Vatcha	Brown	-
AS113	TX2737/91BE7414	ACCI	Cream	SA	**AS203**	SA landrace LP 49	J M Donaldson	Brown	SA
AS114	BTx3197	ACCI	Cream	SA	**AS205**	SA landrace LP 51	J M Donaldson	Brown	SA
AS115	BTx631	ACCI	Cream	SA	**AS251**	AS97 OPV	ACCI	Red	SA
AS116	01Aphid207	ACCI	Cream	SA	**AS391**	SS27 OPV	Mtentu	Brown	SA
AS117	01Aphid148	ACCI	Cream	SA	**AS449**	#12 235926 OC	Ethiopia	Red	Ethiopia
AS121	Kat 369 x EX-1 Chira	ACCI	Brown	SA	**AS560**	IESV 92028 DL	ICRISAT	Brown	-
AS122	KSV 12	ACCI	Cream	SA	**AS563**	IS 2331	ICRISAT	Brown	-
AS129	KARI Mtama X ICS 3-1	ACCI	Cream	SA	**AS72**	KAT-487	UK-SGVT 07-49	Cream	-
AS130	Gambella 1107	ACCI	Cream	SA	**AS74**	ICSV 111	UK-SGVT 07-51	Brown	-
AS131	WK#1025 Sudan	ACCI	Cream	SA	**G50**	TZA 5557	Tanzania	Brown	Tanzania
AS132	Parc 1260793	ACCI	Cream	SA	**ICS634**	-	ICRISAT	Brown	-
AS133	Marimanti Co 1110	ACCI	Cream	SA	**ICSV92001**	-	ICRISAT	Brown	-
AS134	P6 NQ#23 Sudan	ACCI	Brown	SA	**LP4403**	LP4403	ARC-GCI	Brown	SA
AS135	Dinkmash	ACCI	Cream	SA	**MAMOLOKWANE**	Mamolokwane	ARC-GCI	White	SA
AS136	FLO (107) x GS 3541	ACCI	Cream	SA	**NW5393**	-	ARC-GCI	Brown	SA
AS137	IESV 92022 DL	ACCI	Grey	SA	**NW5430**	-	ARC-GCI	Brown	SA
AS138	Mugeta	ACCI	White	SA	**PAN8816**	PAN8816	Pannar	Red	SA
AS140	Kaguru	ACCI	Red	SA	**SS27**	SS27	ARC	Brown	SA
AS141	Kiboko loca	ACCI	Red	SA	**SV07002**	-	ICRISAT	Brown	-

ARC-SG = Agricultural Research Council – Small Grain, ACCI = African Centre for Crop Improvement, UKZN = University of KwaZulu-Natal, ICRISAT = International Crops Research Institute for the Semi-Arid Tropics, SA= South Africa, - = unknown

2.2.2 Study sites

Field experiments were conducted during the 2022 and 2023 growing seasons at three South African locations (Silverton, Ukulinga, and Bethlehem). The Silverton location is the main research station of the Agricultural Research Council – Agricultural Engineering, located on the outskirts of Pretoria (latitude: 25°44' S, longitude: 28°14' E). The mean annual temperature and rainfall for the location was 18.4 ℃ and 661 mm, respectively. The Ukulinga Research location is located at Farm of the University of KwaZulu-Natal in Pietermaritzburg (latitude: 30°24' S, longitude: 29°24 E'). The long-term average temperature and rainfall for Ukulinga are 16.7 ℃ and 966 mm, respectively. The soil at Ukulinga farm is loam, fertile and friable, with good drainage and a pH of 4.5. However, it is susceptible to cracking and crusting under flooding. The Bethlehem location is situated at the Agricultural Research Council – Small Grain (latitude: 28°09' S, longitude: 28°18' E). The mean annual temperature and rainfall for Bethlehem was 14.4 ℃ and 702 mm, respectively. The weather data of the locations during the study periods are presented in Table 2.2.

Table 2.2 Monthly weather data during the field trials at Silverton, Ukulinga and Bethlehem, South Africa, during 2022/2023 growing seasons.

Location	**Month**	**Year**	**Rainfall (mm)**	**Tmax (°C)**	**Tmin (°C)**	**RH (%)**
Silverton	February	2022	34	29	17	62
	March	2022	19	27	16	62
	April	2022	174	25	12	61
	May	2022	16	23	8	56
	June	2022	16	20	5	54
	July	2022	0.76	20	4	53
	August	2022	2.2	23	7	46
Ukulinga	November	2022	121	23	14	79
	December	2022	137	25	15	81
	January	2023	140	25	16	83
	February	2023	118	26	17	82
	March	2023	106	25	16	80
	April	2023	62	23	13	77
	May	2023	32	22	9	70
Bethlehem	November	2022	109	25	11	56
	December	2022	150	25	13	63
	January	2023	148	25	14	68
	February	2023	102	25	14	67
	March	2023	97	24	12	65
	April	2023	55	21	9	62
	May	2023	27	18	5	56

Tmax = average maximum temperature, Tmin = average minimum temperature, RH = relative humidity.

2.2.3 Experimental design and field trial establishment

The 50 sorghum genotypes were field evaluated using a 5 x 10 alpha lattice design with two replications. Each genotype was planted on a two-meter-long row with inter-row spacing of 90 cm and intra-row spacing of 25 cm. Two seeds were planted and later thinned to one plant. Standard agronomic practices were kept constant in all three locations according to sorghum production guidelines in South Africa (DAFF, 2010). Supplementary irrigation was used to maintain optimum soil moisture conditions throughout the cropping season.

2.2.4 Data collection

2.2.4.1 Agronomic traits

Data were collected on the following agronomic parameters: days to 50% heading (DTH) recorded as the number of days from planting to when 50% of the genotypes in each plot had fully exerted panicles; days to 50% maturity (DTM) recorded as the number of days from planting to when 50% of the genotypes in each plot had dried panicles; biomass production (root and shoot); and grain yield. Shoot biomass (SB) was recorded as the total mass of the above-ground biomass cut from the base of the plant, excluding the grain. The shoots were oven-dried at 70 °C for 48 hours, weighed and expressed in g plant^{-1}. Root biomass (RB) was recorded as the total root dry matter harvested per genotype per plot. Root samples for each plot were harvested to a depth of 50 cm. The roots were separated from the soil by hand and washed under running water to remove all soil particles. The remaining soil was mixed with water and the suspension was sieved through a 2 mm sieve. Fine roots were collected from the sieve residue and added to the large roots. The roots were oven-dried at 60 °C for 72 hours. The dried roots were weighed on a balance to get the RB which was adjusted to g plant^{-1}. Total plant biomass (PB) was the sum of all dry plant material for each genotype including RB and SB harvested from the test plots and recorded in g plant^{-1}. Root to shoot biomass ratio (RS) was the ratio of the root to shoot biomass as recorded above. Grain yield (GY) was the weight of harvested grain at 12.5% moisture content per genotype per plot and expressed in g plant^{-1}. Harvest index (HI) was also calculated using the following formula and expressed in percent:

$$HI = \frac{GY}{SB+GY}\, x\, 100$$

Where HI is the harvest index (%), GY the grain yield (g plant^{-1}), and SB is the shoot biomass (g plant-1).

2.2.4.2 Carbon stocks determination

Due to the high cost of carbon analysis, the 50 genotypes were sub-sampled, and a select number were retained. Twenty-five genotypes were selected from the Silverton trials based on their grain yield performance and subjected to carbon analysis using two replications. Among the 25 selections, 10 were the top, and 10 were the bottom performing, while five genotypes were random samples.

The carbon analysis involved collecting shoot samples to determine shoot carbon content (SCc), root samples for root carbon content (RCc), and grain samples for grain carbon content (GCc). These samples were oven-dried at 70°C for 48 hours and transformed into fine powder, weighing five grams each. The shoots were pulverized into fine powder using a blender, while the roots and grains were processed into fine powder using a ZM 200 ultra centrifugal mill. The total carbon content of shoot, root, and grain samples was determined by combustion at the South African Sugarcane Research Institute (SASRI) using a LECO TruMac CNS Analyzer (Rayment and Lyons, 2011).

The shoot (SCs), root (RCs), and grain (GCs) carbon stocks were defined as the total amount of C measured in the respective plant parts according to Grewer *et al.*, (2018). These C stocks in the two parts (SCs and RCs) were summed up to derive total plant carbon stocks (PCs). The carbon stocks were calculated based on the carbon content and corresponding biomasses using the following formulas:

$$SCs = \frac{SCc}{100} * SB$$

$$RCs = \frac{RCc}{100} * RB$$

$$GCs = \frac{GCc}{100} * GY$$

Where SCs is the shoot carbon stock (g plant^{-1}), RCs is the root carbon stock (g plant^{-1}), GCs is the grain carbon stock (g plant^{-1}), SCc is the shoot carbon content (%), RCc is the root carbon content (%), GCc is the grain carbon content (%), SB is the shoot biomass (g plant^{-1}), RB is the root biomass (g plant^{-1}), and GY is the grain yield (g plant^{-1}).

2.2.5 Data analysis

The data collected from the 50 genotypes for the agronomic traits and 25 selected genotypes for carbon storage traits were analyzed separately. A combined analysis of variance was performed after homogeneity of variance test procedure (Levene, 1960) using the Statistical Analysis System (SAS) software version 9.4 program using the PROC general linear model (GLM) procedure (Guide, 2014). The mean values of the test genotypes for the measured traits were compared using Fisher's least significant difference (LSD) procedure at 5% probability level. Data were subjected to parametric and non-parametric analyses using IBM SPSS statistics 29.0 program (Pallant, 2020). The rotated component matrix and principal component analysis (PCA) biplots were generated for agronomic and carbon storage traits using the R software version 4.2.3 (Team, 2013). A hierarchical cluster analysis based on the agglomerative clustering method was also performed using R software to establish genetic relationships among genotypes.

2.3 Results

2.3.1 Analysis of variance for agronomic traits

Combined analysis of variance revealed significant ($p < 0.05$) differences among the sorghum genotypes for all the assessed agronomic traits except DTM (Table 2.3). Significant genotype by location ($p < 0.05$) interactions were recorded for DTH, DTM, PH, PB, SB, RB, RS, and GY.

Table 2.3 Combined analysis of variance and significance tests for agronomic traits of 50 sorghum genotypes across three locations in South Africa.

Souce of variation	**DF**	**DTH**	**DTM**	**PH**	**PB**	**SB**	**RB**	**RS**	**GY**	**HI**
Location	2	91.83	349.41	20546.17***	20.14	18.49	22.83**	0.004	13.70**	50.34
Replication	3	479.85	519.62**	28833.24***	9.61	7.93	0.37	0.01	7.43*	18.37
Block	24	167.77	111.44	2757.55	25.17	11.53	3.31	0.005	4.42*	21.91
Genotype	49	307.42*	130.78	3194.9**	349.57***	151.72***	129.22***	0.26***	181.77***	1031.47***
Genotype x Location	98	207.06**	111.63*	2257.43**	18.36***	14.44**	4.5*	0.004*	2.57***	15.34
Error	123	195.51	135.71	1826.3	15.99	14.52	5.06	0.004	2.73	17.83
CV (%)		17.58	8.44	27.39	13.25	23.36	9.35	12.46	13.88	15.53
LSD (5%)		15.97	13.22	48.55	5.09	4.24	2.4	0.28	1.98	5.94

*, ** and *** denote significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, SOV = source of variation, DF = degrees of freedom, CV = coefficient of variation, LSD = least significant difference at 5% probability level, DTH = days to 50% heading, DTM = Days to 50% maturity, PH = plant height (cm), PB = total plant biomass (g plant^{-1}) , SB = shoot biomass (g plant^{-1}), RB = root biomass (g plant^{-1}), RS = root to shoot biomass ratio, GY = grain yield (g plant^{-1}), HI = harvest index (%).

2.3.2 Analysis of variance for carbon storage

The analysis of variance test genotypes for carbon parameters revealed significant differences ($p < 0.001$) among test genotypes for GCc, PCs, SCs, RCs, RCs/SCs, and GCs (Table 2.4).

Table 2.4 Analysis of variance and significance tests for carbon storage of the 25 selected sorghum genotypes at Silverton during the 2022 growing season.

Source of variation	DF	SCc	RCc	GCc	PCs	SCs	RCs	RCs/SCs	GCs
Replication	1	1.24*	5.21	0.03	1.34	2.85	0.29	0.004	0.86
Block	8	0.28**	7.45**	0.01**	10.88	13.41	0.38	0.05*	0.21
Genotype	24	0.65	12.77	0.49***	29.02**	20.13*	5.47***	0.13***	8.44**
Error	16	0.32	8.03	0.01	8.76	7.61	0.42	0.02	0.34
CV		1.28	6.9	0.28	23.14	33.64	14.05	20.72	11.65
LSD (5%)		1.11	5.13	0.23	6.28	6.04	1.34	0.29	0.91

*, ** and *** denote significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, SOV = source of variation, DF = degrees of freedom, CV = coefficient of variation, LSD = least significant difference at 5% probability level, SCc = shoot carbon content (%), RCc = root carbon content (%), GCc = grain carbon content (%), PCs = total plant carbon stocks (g plant^{-1}), SCs = shoot carbon stock (g plant^{-1}), RCs = root carbon stock (g plant^{-1}), RCs/SCs = root to shoot carbon stock ratio, GCs = grain carbon stock (g plant^{-1}).

2.3.3 Performance of sorghum genotypes for agronomic traits and carbon allocation

2.3.3.1 Agronomic performance

The mean performance of sorghum genotypes for nine agronomic traits across three locations is summarized in Table 2.5. The top ten genotypes, distinguished by their superior grain yield production, are highlighted in bold fonts. The mean DTH and DTM were 80 and 138 days, respectively. Genotype G50 was the earliest to reach 50% heading and maturity at 61 and 114 days, respectively, followed by AS72 (69 and 133 days), AS122 (69 and 139 days), AS141 (69 and 137 days), and AS117 (70 and 139 days). Extended flowering and maturity periods were recorded for genotypes AS136 and AS135 with mean values of 92 and 71 days, and 149 and 151 days in that order. Plant height varied from 107.06 to 223.28 cm. The mean plant height for the evaluated genotypes was 156.01 cm. The tallest genotypes with mean plant height greater than 180 cm were AS205, AS391, AS109, AS113, and AS111. The shortest genotype across the testing locations was PAN8816. The mean total plant biomass of the evaluated genotypes was 27.94 g plant^{-1}. The total plant biomass of the genotypes ranged from 14.07 to 43.75 g plant^{-1}, with genotypes AS122, AS391, SS27, AS203, AS74 having the highest total plant biomass of 43.75, 41.31, 38.65, 38.19, 37.83 g plant^{-1}, respectively. The mean shoot biomass varied from 6.49 to 24.87 g plant^{-1}, with a grand mean of 16.31 g plant^{-1}. The most productive genotypes with the highest shoot biomass were SS27, AS122, AS203, and AS391, with 24.87, 23.90, 23.45, and 23.27 g plant^{-1}, respectively. The grand mean root biomass for the evaluated genotypes was 11.62 g plant^{-1}, ranging from 5.92 to 21.02 g plant^{-1}. The highest shoot biomass was 21.02, 20.31, 19.87, 19.85, 19.46, and 18.04 g plant^{-1} observed on genotypes AS106, AS74, AS72, AS122, and AS152. The root-to-shoot biomass ratio ranged from 0.32 to 3.00. The genotypes that allocated more biomass to their roots than their shoots were AS152, AS106, and 05-POTCH-138, with the highest root-to-shoot biomass ratio of 3.00, 2.50, and 1.95, respectively. The wide genetic variation in grain yield spanned from 2.53 to 25.08 g plant^{-1}, averaging 11.90 g plant^{-1}. Genotypes AS115, AS251, AS134, AS145, and AS130 were the five best-performing genotypes with mean yields of 25.08, 21.83, 21.42, 19.43, 18.50 g plant^{-1}, respectively. The harvest index ranged from 15.34 to 66.66%. Genotypes AS115, AS130, and AS251 exhibited the highest harvest index ≥ 60%.

Table 2.5 Mean values for the agronomic traits among the ten best and five bottom genotypes after evaluating 50 sorghum genotypes across three locations.

Genotype	DTH	DTM	PH	PB	SB	RB	RS	GY	HI
Top ten genotypes									
AS115	88	130	182.50	21.39	12.54	8.85	0.71	25.08	66.66
AS251	85	142	131.39	27.60	14.63	12.97	0.89	21.83	59.88
AS134	78	135	162.72	35.52	20.37	15.16	0.74	21.42	51.26
AS145	86	141	157.67	32.20	21.95	10.25	0.47	19.43	46.95
AS130	77	145	163.33	25.56	11.29	14.27	1.26	18.50	62.09
SS27	79	142	135.92	38.65	24.87	13.77	0.55	17.58	41.41
AS138	78	140	146.17	28.54	15.03	13.51	0.90	17.45	53.73
AS132	94	133	171.06	25.90	15.61	10.29	0.66	16.89	51.98
AS563	84	135	173.94	33.02	21.31	11.71	0.55	16.83	44.12
AS203	82	131	174.50	38.19	23.45	14.74	0.63	16.63	41.49
Bottom five genotypes									
AS147	80	138	145.22	16.20	7.51	8.69	1.16	4.63	38.13
AS116	77	138	142.11	21.52	14.89	6.63	0.45	4.35	22.62
PAN8816	75	140	107.06	33.58	17.47	16.12	0.92	3.75	17.68
AS129	84	143	132.28	26.89	17.41	9.49	0.54	3.28	15.87
AS111	84	144	185.67	23.22	13.97	9.25	0.66	2.53	15.34
Mean	79.52	138.01	156.01	27.94	16.31	11.62	0.8	11.9	41.47
SD	6.82	5	22.74	6.56	4.74	4.26	0.52	5.03	12.09
SE	0.97	0.71	3.22	0.93	0.67	0.6	0.07	0.71	1.71
Skewness	0.5	0.38	0.47	0.23	-0.26	0.6	2.48	0.25	-0.34
kurtosis	0.22	0.09	0.4	-0.25	-0.73	-0.55	7.41	-0.13	-0.3

SD = standard deviation, SE = standard error, DTH = days to 50% heading, DTM = days to 50% maturity, PH = plant height (cm), PB = total plant biomass (g plant^{-1}), SB = shoot biomass (g plant^{-1}), RB = root biomass (g plant^{-1}), RS = root to shoot biomass ratio, GY = grain yield (g plant^{-1}), HI = harvest index (%).

2.3.3.2 Carbon allocation to roots and shoots

The mean performance of the 25 selected sorghum genotypes for carbon storage (SCc, RCc, GCc, PCs, SCs, RCs, RCs/SCs, and GCc) is summarized in Table 2.6. The top ten genotypes, based on their high root carbon stock, are highlighted in bold fonts. All the carbon content variables ranged from 40 to 45%. The total plant carbon stocks ranged from 7.52 to 24.64 g plant^{-1}, with a mean of 12.65 g plant^{-1}. The genotypes that sequestered more carbon with the highest total plant carbon stock were SS27, AS122, AS134, AS203, and AS563, with values of 24.64, 18.00, 16.48, 15.55, and 14.99 g plant^{-1}, respectively. The lowest carbon sequestration with the lowest total plant carbon stock of 7.96, 7.69, and 7.52 g plant^{-1} was recorded in genotypes NW5393, AS116, and AS115, respectively. The shoot carbon stock of the selected genotypes had a mean of 7.98 g plant^{-1}, spanning from 3.25 to 19.04 g plant^{-1}. The genotypes that allocated more carbon to the shoots with the highest shoot carbon stock were SS27, AS122, ICSV92001, and AS563 with 19.04, 10.42, 10.34, and 10.33 g plant^{-1}, respectively. The root carbon stocks varied from 1.38 to 8.37 g plant^{-1}. The mean root carbon stock for the selected genotypes was 4.67 g plant^{-1}. The genotypes that had the highest root carbon stock were AS108 with 8.87 g plant^{-1}, followed by AS122 (7.58 g plant^{-1}), AS134 (7.13 g plant^{-1}), AS251 (6.49 g plant^{-1}), and AS203 (6.40 g plant^{-1}). Genotypes AS145 and AS116 were among the genotypes with the lowest root carbon stock with 1.74 and 1.37 g plant^{-1}, respectively. The root-to-shoot carbon stock ratio ranged from 0.18 to 1.56. The genotypes allocated more carbon to their shoots than their roots. The genotypes that allocated more carbon to their roots than their shoots were AS108 and AS115, with the highest root-to-shoot carbon stock ratio of 1.56 and 1.31, respectively. The grain carbon stocks ranged from 1.04 to 12.92 g plant^{-1}, with a grand mean of 5.84 g plant^{-1}. The genotypes AS115 and AS134 were the highest grain carbon stock, with mean grain carbon stock of 12.92 and 11.38 g plant^{-1}, respectively.

Table 2.6 Mean values for carbon storage traits of the 25 selected sorghum genotypes.

Genotype	SCc	RCc	GCc	PCs	SCs	RCs	RCs/SCs	GCs
16MZ	**43.20**	**44.41**	**43.24**	**14.92**	**9.83**	**5.09**	**0.52**	**5.24**
AS108	**43.74**	**44.79**	**43.96**	**14.55**	**5.68**	**8.87**	**1.56**	**3.92**
AS109	43.95	42.53	43.06	11.40	6.47	4.93	0.76	9.40
AS111	**43.91**	**45.40**	**43.64**	**12.86**	**7.40**	**5.46**	**0.74**	**1.04**
AS115	43.44	45.34	43.56	7.52	3.25	4.27	1.31	12.92
AS116	43.78	40.12	43.34	7.69	6.33	1.37	0.22	1.78
AS117	43.51	38.33	43.51	12.23	9.13	3.09	0.34	4.91
AS122	**43.62**	**40.39**	**43.43**	**18.00**	**10.42**	**7.58**	**0.73**	**3.69**
AS130	43.73	40.55	43.40	8.65	4.33	4.32	1.00	9.26
AS131	43.51	41.76	44.06	13.15	8.76	4.39	0.50	3.38
AS132	44.48	38.55	43.16	13.77	9.20	4.57	0.50	9.31
AS134	**44.41**	**39.34**	**43.25**	**16.48**	**9.34**	**7.13**	**0.76**	**11.38**
AS136	44.17	39.15	43.58	9.00	4.97	4.03	0.81	6.57
AS138	44.87	40.72	43.36	8.52	5.35	3.17	0.59	8.04
AS143	44.68	43.67	44.24	8.16	5.41	2.76	0.51	3.87
AS145	45.16	33.69	43.64	11.64	9.90	1.74	0.18	6.68
AS203	**44.56**	**43.41**	**43.47**	**15.55**	**9.15**	**6.40**	**0.70**	**7.23**
AS251	**43.51**	**43.26**	**42.89**	**14.97**	**8.48**	**6.49**	**0.76**	**4.57**
AS563	44.70	40.26	44.49	14.99	10.33	4.66	0.45	5.91
ICSV92001	44.55	39.94	44.65	14.33	10.34	3.99	0.39	3.35
LP4403	**43.73**	**43.76**	**45.31**	**13.89**	**8.38**	**5.51**	**0.66**	**3.35**
MAMOLOKWAN	44.35	41.17	43.44	8.94	5.47	3.47	0.64	5.33
NW5393	45.09	39.40	44.07	7.96	5.20	2.75	0.53	5.73
PAN8816	**44.44**	**37.71**	**43.24**	**12.54**	**7.38**	**5.16**	**0.70**	**1.62**
SS27	**45.48**	**39.92**	**43.27**	**24.64**	**19.04**	**5.61**	**0.29**	**7.61**
Mean	44.18	41.10	43.65	12.65	7.98	4.67	0.65	5.84
SD	0.61	2.76	0.56	3.96	3.14	1.77	0.31	3.02
SE	0.12	0.55	0.11	0.79	0.63	0.35	0.06	0.60
Skewness	0.35	-0.44	1.42	0.96	1.66	0.34	1.25	0.56
Kurtosis	-0.81	0.64	2.10	2.01	5.41	0.24	2.50	-0.07

SD = standard deviation, SE = standard error, SCc = shoot carbon content (%), RCc = root carbon content (%), GCc = grain carbon content (%), PCs = total plant carbon stocks (g plant^{-1}), SCs = shoot carbon stock (g plant^{-1}), RCs = root carbon stock (g plant^{-1}), RCs/SCs = root to shoot carbon stock ratio, GCs = grain carbon stock (g plant^{-1}). The top ten performing genotypes based on RCs are highlighted in bold fonts.

2.3.4 Principal component and biplot analyses for agronomic traits

Table 2.7 displays the rotated component matrix, illustrating the percentage variance associated with various principal components (PCs) and the corresponding loadings for recorded agronomic traits. Four principal components (PC1 to PC4) attributed to 86.21% of the total genotypic variation for agronomic- and biomass-related traits. Total plant biomass and SB made the highest contributions to PC1, followed by RB and RS, with positive contributions to PC2. The highest positive loadings for PC3 were for PH and DTM, and for PC4 were for GY and HI, respectively.

Table 2.7 Principal components showing variation and contribution by nine agronomic traits among 50 sorghum genotypes evaluated during the 2022 and 2023 growing seasons at three locations in South Africa.

Trait	**PC1**	**PC2**	**PC3**	**PC4**
DTH	0.03	-0.21	**0.66**	0.13
DTM	-0.01	-0.28	-0.74	0.003
PH	0.09	-0.05	**0.88**	0.04
PB	**0.94**	0.34	0.08	0.02
SB	**0.93**	-0.32	0.09	-0.05
RB	**0.41**	**0.89**	0.02	0.09
RS	-0.34	**0.92**	-0.05	0.05
GY	0.23	-0.06	0.15	**0.95**
HI	-0.36	0.25	0.08	**0.89**
Eigenvalue	2.37	2.27	1.84	1.29
Variance (%)	26.29	25.17	20.39	14.36
Cumulative variance (%)	26.29	51.46	71.85	86.21

PC = principal component, DTH = days to 50% heading, DTM = days to 50% maturity, PH = plant height, PB = total plant biomass, SB = shoot biomass, RB = root biomass, RS = root to shoot biomass ratio, GY = grain yield, HI = harvest index. The highest loading scores for each PC are highlighted in bold.

Biplots generated through principal component analysis for agronomic traits is illustrated in Figure 2.1. The first principal component (PC1) was positively correlated with DTH, PH, PB, SB, RB, and GY. On the contrary, PC2 was negatively correlated with DTM, RS, and HI. Agronomic traits like DTH, PH, PB, SB, RB, and GY were positively associated with each other, evident in their vectors aligning in the same direction and forming acute angles between them (Figure 2.1). Similarly, DTM, RS, and HI were positively correlated to each other. High-yielding genotypes such as AS134 and SS27 had high PB and SB, while AS115 and AS251 were associated with RB, HI, and RS (Figure 2.1).

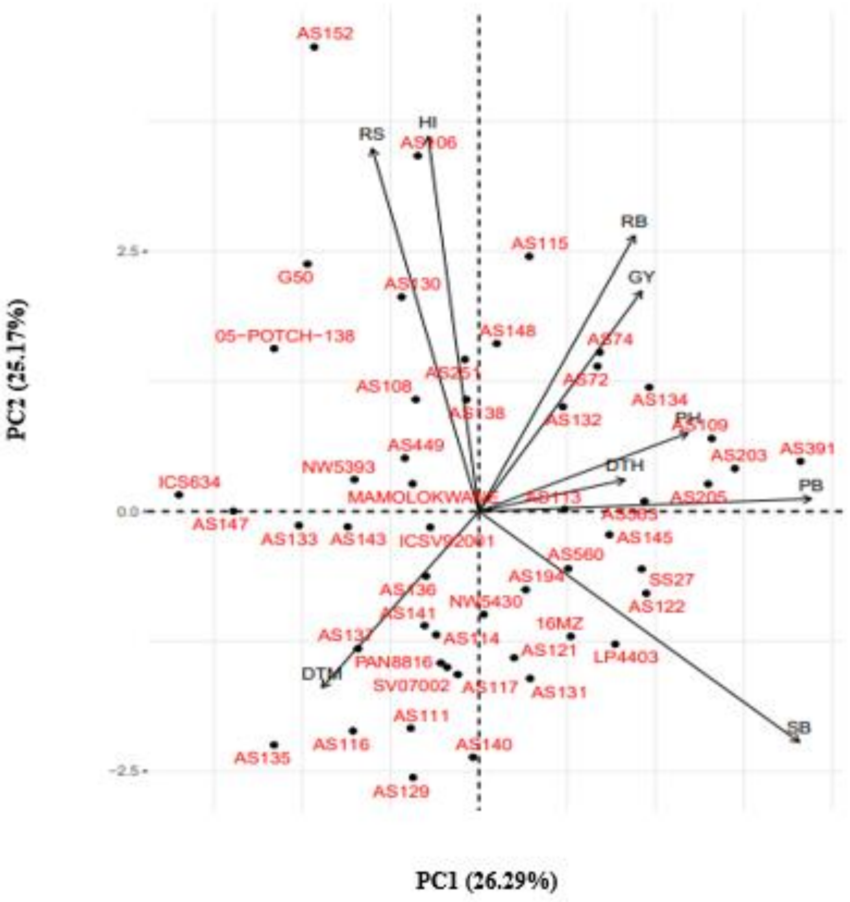

Figure 2.1 Principal component biplot displaying the relationship among nine agronomic traits of 50 sorghum genotypes evaluated in three locations in South Africa. PC1 = first principal component, PC2 = second principal component, DTH = days to 50% heading, DTM = days to 50% maturity, PH = plant height, PB = total plant biomass, SB = shoot biomass, RB = root biomass, RS = root to shoot biomass ratio, GY = grain yield, HI = Harvest index.

2.3.5 Principal component and biplot analyses for carbon storage

The PCA showed that three PCs accounted for 78.18% of the total variation. The first component had the major contribution of 32.68% to the variation (Table 2.8). Variation in PC1 was mainly from the positive loadings of RCs/SCs, RCc, and RCs and negative loadings of SCc. The second principal component (PC2) contributed 27.68% to the variation, mainly from the positive contributions of PCs, SCs, and RCs. The variation in PC3 (17.83%) was due to positive GCs and RCs/SCs loadings, and the negative loadings were due to GCc and RCc.

Table 2.8 Principal components showing variation and contribution by carbon storage among 25 selected sorghum genotypes.

Trait	**PC1**	**PC2**	**PC3**
SCc	-0.72	0.15	0.12
RCc	**0.83**	0.01	-0.18
GCc	0.03	-0.07	-0.75
PCs	-0.02	**0.98**	0.01
SCs	-0.40	**0.89**	-0.07
RCs	**0.67**	**0.66**	0.15
RCs/SCs	**0.86**	-0.12	**0.31**
GCs	0.01	-0.07	**0.85**
Eigenvalue	2.61	2.21	1.43
Variance (%)	32.68	27.68	17.83
Cumulative variance (%)	32.68	60.35	78.18

PC = principal component, SCc = shoot carbon content, RCc = root carbon content, GCc = grain carbon content, PCs = total plant carbon stocks, SCs = shoot carbon stock, RCs = root carbon stock, RCs/SCs = root to shoot carbon stock ratio, GCs = grain carbon stock. The highest loading scores for each PC are highlighted in bold.

Characters such as RCc, PCs, SCs, RCs, RCs/SCs, and GCs were positively correlated. Negative correlations were observed between SCc, RCc, GCc, RCs, and RCs/SCs (Figure 2.2). A strong positive correlation was observed between RCc, RCs/SCs, and GCs. There was a strong correlation between PCs and SCs. A strong negative correlation was observed between GCc and RCs. The genotypes were equally scattered across both PC1 and PC2. Genotypes SS27, LP4403, and AS111 scored higher values for SCs, PCs, and RCs. Genotypes PAN8816 and ICSV92001 had a strong association with SCc.

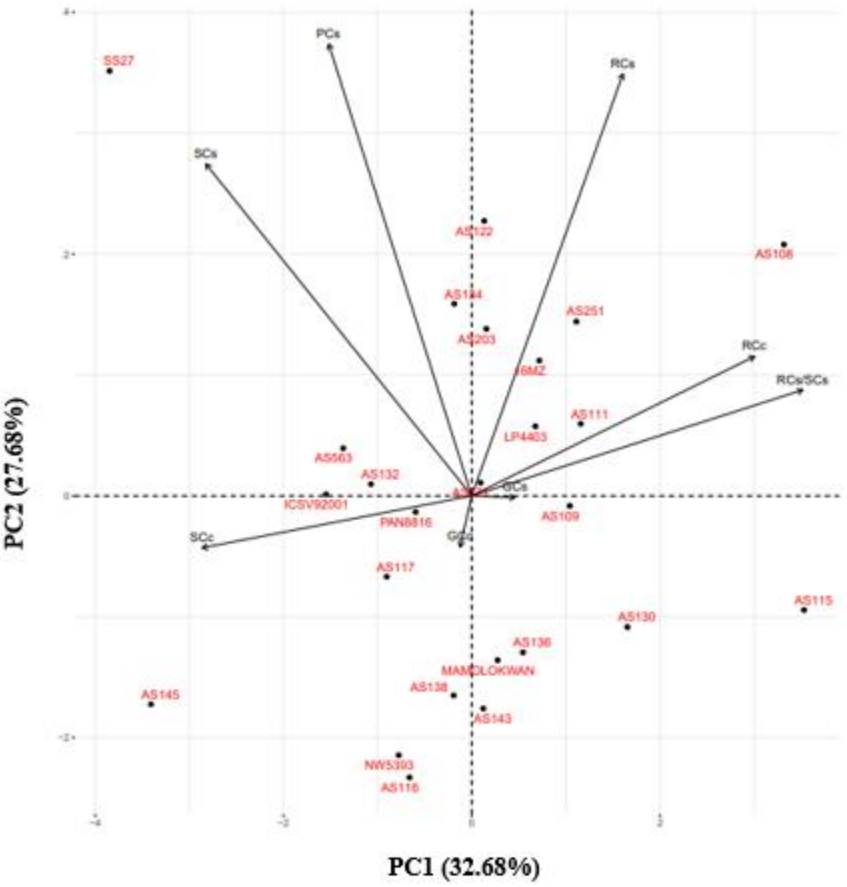

Figure 2.2 Principal component biplot displaying the relationship among carbon storage traits in the 25 selected sorghum genotypes. PC1 = first principal component, PC2 = second principal component, SCc = shoot carbon content, RCc = root carbon content, GCc = grain carbon content, PCs = total plant carbon stocks, SCs = shoot carbon stock, RCs = root carbon stock, RCs/SCs = root to shoot carbon stock ratio, GCs = grain carbon stock.

2.3.6 Principal component and biplot analyses for agronomic traits and carbon storage

The principal component analysis was performed to identify the most discriminative variables among the sorghum genotypes. A total of 82.51% of the variation explained by the agronomic traits and carbon storage traits were explained by the five principal components (Table 2.9). Generally, traits such as PB, PCs, SB, SCs, RCs/SCs, RS, GY, and GCc contributed much to the variations in the PCs. Nevertheless, PB, PCs, SB, SCs, and RB, had the highest contributions (with contributions of 0.90, 0.89, 0.82, 0.81, and 0.67, respectively) to PC1. Traits like RCs/SCs, RS, and RCs were highest (0.86, 0.78, and 0.70, respectively) positive contributors in PC2. The traits that contributed the most in PC3 were GY and GCs (0.78 and 0.74). The fourth principal component accounted for 76.43% of the total variation, with PH and Rcc (0.72 and 0.59) exhibiting the highest positive loadings for PC4. GCc was the only positive contributor to the observed variation on PC5 with a PC loading of 0.68.

Table 2.9 Principal components showing variation and contribution by agronomic traits and carbon storage traits among 25 selected sorghum genotypes.

Trait	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
DTH	-0.32	0.23	0.39	0.17	-0.25
DTM	0.18	-0.10	-0.35	-0.66	0.02
PH	-0.47	0.07	0.25	**0.72**	0.08
PB	**0.90**	0.23	0.16	0.12	-0.09
SB	**0.82**	-0.17	0.32	0.27	-0.18
RB	**0.67**	0.64	-0.10	-0.12	0.07
RS	-0.01	**0.78**	-0.35	-0.36	0.24
GY	-0.15	0.47	**0.78**	-0.13	0.04
HI	-0.48	0.48	0.58	-0.23	0.20
SCc	0.06	-0.30	0.55	-0.24	0.54
RCc	-0.17	0.43	-0.44	**0.59**	-0.02
GCc	-0.09	-0.31	-0.22	0.43	**0.68**
PCs	**0.89**	0.12	0.18	0.15	0.13
SCs	**0.81**	-0.25	0.37	0.07	0.10
RCs	0.57	**0.70**	-0.24	0.20	0.11
RCs/SCs	-0.20	**0.86**	-0.36	0.06	0.08
GCs	-0.23	0.54	**0.74**	0.03	-0.09
Eigenvalue	4.44	3.61	3.00	1.95	1.03
Variance (%)	26.11	21.23	17.65	11.45	6.08
Cumulative variance (%)	26.11	47.33	64.98	76.43	82.51

PC = principal component, DTH = days to 50% heading, DTM = days to 50% maturity, PH = plant height, PB = total plant biomass, SB = shoot biomass, RB = root biomass, RS = root to shoot biomass ratio, GY = grain yield, HI = harvest index, SCc = shoot carbon content, RCc = root carbon content, GCc = grain carbon content, PCs = total plant carbon stocks, SCs = shoot carbon stock, RCs = root carbon stock, RCs/SCs = root to shoot carbon stock ratio, GCs = grain carbon stock. The highest loading scores for each PC are highlighted in bold.

Biplots based on the principal component analysis were drawn for agronomic traits and carbon storage traits (Figure 2.3). High yielding and early flowering genotypes such as AS115, AS132, and AS203 had high GCs, RCc, RS, and RCs/SCs, while 16MZ, PAN8816, and SS27 were associated with SB and SCs. Grain yield had strong correlation with GCs, RCs, RS, RCs/SCs, RB, PB, and high yielding genotypes including AS138 and AS130. Shoot biomass was highly correlated with SCs and high carbon storage genotypes including SS27, 16MZ, and AS563

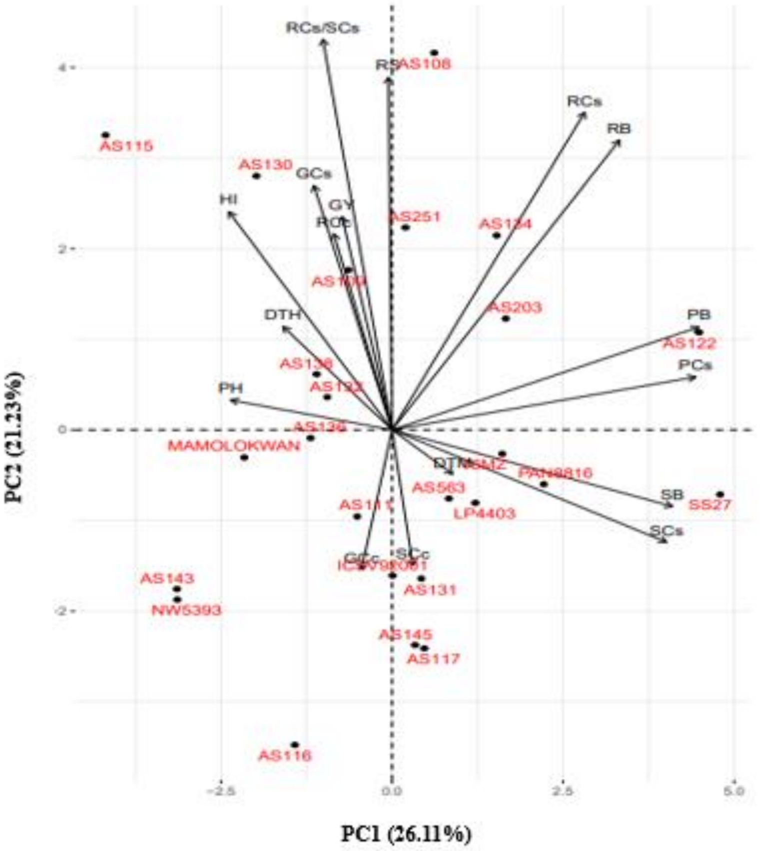

Figure 2.3 Principal component biplot displaying the relationship among agronomic traits and carbon storage traits in the 25 selected sorghum genotypes. PC1 = first principal component, PC2 = second principal component, DTH = days to 50% heading, DTM = days to 50% maturity, PH = plant height, PB = total plant biomass, SB = shoot biomass, RB = root biomass, RS = root to shoot biomass ratio, GY = grain yield, HI = Harvest index, SCc = shoot carbon content, RCc = root carbon content, GCc = grain carbon content, PCs = total plant carbon stocks, SCs = shoot carbon stock, RCs = root carbon stock, RCs/SCs = root to shoot carbon stock ratio, GCs = grain carbon stock.

2.3.7 Cluster analysis for agronomic traits

The assessment of the phenotypic diversity using agronomic traits delineated the genotypes into three distinct clusters (Figure 2.4). The second cluster had the highest number (25) of genotypes, while the first cluster had 15, and the third cluster had 10 genotypes. Nevertheless, all three clusters comprised a combination of landraces, breeding lines, cultivars, and origins in their genotype composition.

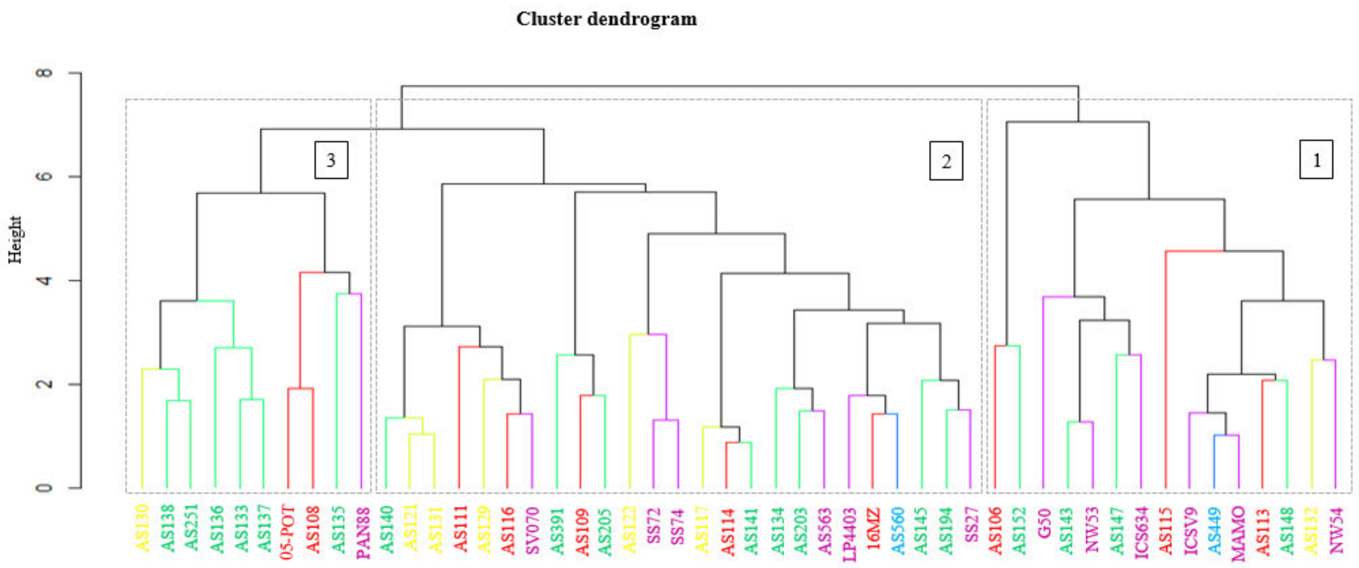

Figure 2.4 Hierarchical cluster dendrogram based on agronomic traits showing genetic similarity matrix of 50 sorghum genotypes evaluated in three locations in South Africa.

2.3.8 Cluster analysis for carbon storage

The results of cluster analysis for carbon storage are presented in Figure 2.5. Genotypes AS109, 16MZ, AS111, AS122, ICSV92001, AS563, LP4403, AS131, AS117, PAN8816, AS251, AS130, MAMOLOKWANE, AS136, NW5393, AS138, AS143, and AS116 were grouped together in the first cluster. The second cluster consisted of genotypes AS115 and AS108. The third cluster included genotypes AS134, AS132, AS203, SS27, and AS145.

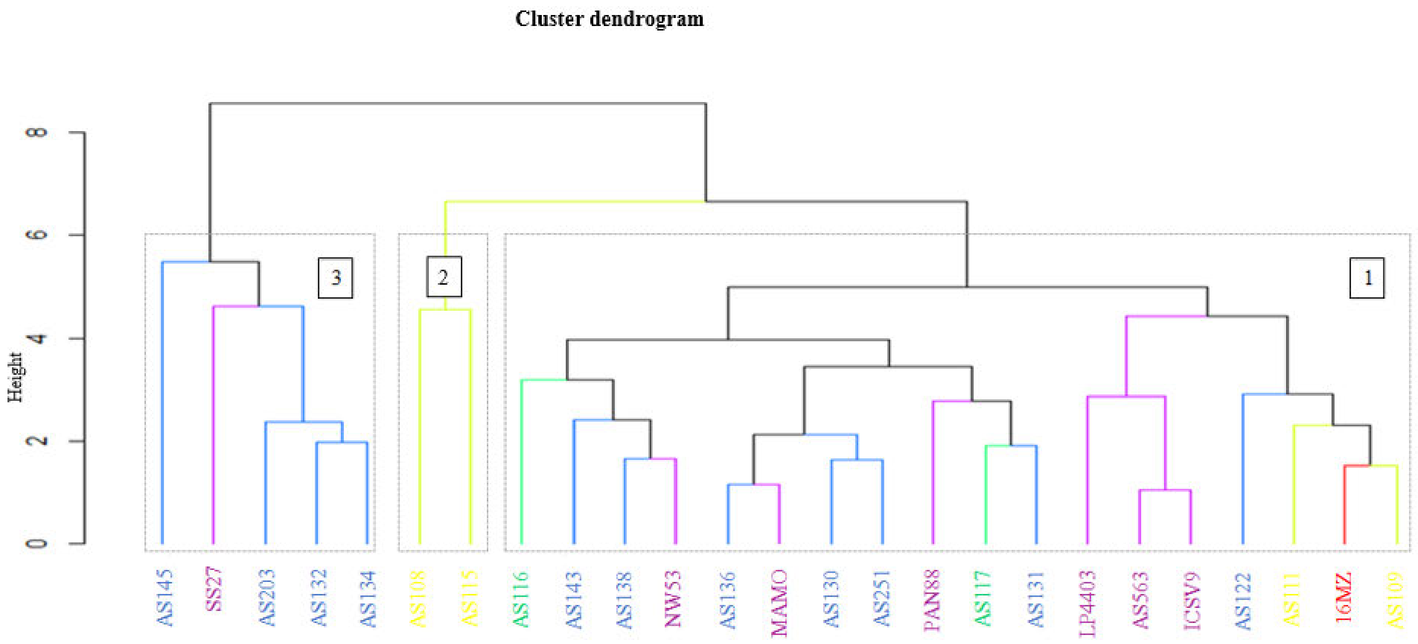

Figure 2.5 Hierarchical cluster dendrogram based on carbon storage traits showing genetic similarity matrix of the 25 selected sorghum genotypes.

2.4 Discussion

2.4.1 Combined analysis of variance for agronomic traits

The current study assessed 50 sorghum genotypes across three different locations for their performance in terms of growth, yield stability, and adaptability, with the aim of identifying key genetic traits that contribute to resilience and productivity under diverse environmental conditions. A combined analysis of variance revealed that genotypes showed significant variation in agronomic traits (Table 2.3), indicating the presence of marked genetic variability in developing new sorghum cultivars with high grain and biomass yields (Shamuyarira *et al.,* 2022). Mulima *et al.,* (2018) reported significant variations in agronomic traits among sorghum genotypes obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) gene bank. Genotype performances were also impacted by significant genotype x location interactions, indicating that the performance of genotypes varied across different environments. Enyew *et al.,* (2021) reported a significant genotype x environment (G x E) interaction on yield and yield-components of sorghum genotypes. Environmental influences play a significant role in phenotypic variations, and the differential responses of genotypes to environmental conditions contribute to the observed variability (Mutava *et al.,* 2011). Understanding the effect of G x E and predicting the phenotypic response to various environments are vital to improving the selection efficiency in sorghum breeding programs. Phenotypic expression provides a critical screening and breeding method to exploit genetic variability. The significant variation observed in biomass and grain yield production among genotypes could be attributed to water availability, temperature, humidity, and soil fertility (Gratani, 2014). Furthermore, variation in agronomic performances may be influenced by the amount and distribution of rainfall, leading to adequate moisture and favorable temperature during panicle development and flowering. Low CVs were recorded for DTM, PB, RB, GY, and HI, indicating that these traits could be prioritized for assessing sorghum genotypes.

2.4.2 Analysis of variance for carbon content and carbon stocks variables

The significant differences among the sorghum genotypes for GCc, PCs, SCs, RCs, RCs/SCs, and GCs (Table 2.4), indicating the availability of sufficient genetic diversity in the test genotypes for carbon sequestration. Related results have been reported in sorghum genotypes (Datta *et al.,* 2018; Ahmed *et al.,* 2020). Genotypes that exhibit a wide range of trait values may be better equipped to thrive in diverse growing conditions (Boyles *et al.,* 2019).

2.4.3 Mean yield and yield component performance

The higher grain yield was recorded for genotypes SS27, AS203, AS145, AS563, and AS134 possibly due to their higher number of grains per head, likely influenced by their high shoot biomass (Table 2.5 and Figure 2.1). This aligns with the findings of George-Jaeggli *et al.,* (2011) which emphasized that seed number is the most crucial yield component associated with increases in sorghum yields. The genotypes AS115, AS251, AS203, and AS138 consistently produced the highest yields in all three environments (Table 2.5), suggesting the stable performance of the genotypes in diverse growing environments. The four genotypes expressed tall plant stature across the environments. These findings are consistent with the results reported by George-Jaeggli *et al.,* (2011), where increased plant height positively affected grain yield via an effect on shoot biomass. High-shoot biomass increases grain production through increasing leaf area for light absorption and carbon assimilation to facilitate grain filling (Reynolds *et al.,* 2005). Breeders can select these genotypes as they have demonstrated to be less sensitive to changes in environmental conditions. They can maintain reasonable yields even in adverse conditions, such as excessive rainfall or drought (Haussmann *et al.,* 2012). Drought stress affects the photosynthetic rate of sorghum through various physiological mechanisms including reduced stomatal conductance and transpiration rate, lowered quantum yield, increased leaf temperature, decreased chlorophyll content and ribulose-1,5-bisphosphate carboxylase, increased oxygen evolution, and reduced phosphoenolpyruvate photosynthesis activity (Abreha *et al.,* 2022). Previous findings indicated that drought-tolerant sorghum genotypes exhibit a significant increase in chlorophyll fluorescence and photosynthetic rate under drought stress conditions (Fracasso *et al.,* 2016; Sukumaran *et al.,* 2016). Getnet *et al.,* (2016) reported that drought tolerant sorghum genotypes exhibit an increased photosynthetic rate, supplying the required raw material and energy for growth and development, enabling them to sustain their grain yield under drought stress. Genotypes AS74 and AS72 ranked second and third for RB production, respectively, and these genotypes were among the genotypes with slightly high GY. These results are supported by the strong association between root biomass and grain yield (Figure 2.1), demonstrating the significance of root traits in enhancing productivity. Increased root growth improves plant capacity and efficiency in acquiring nutrients and moisture and enhancing agro-ecosystem resilience (Haussmann *et al.,* 2012). This is particularly vital under drought conditions when there is less water in the soil profile, and a deeper and larger root system can forage for water (Figueroa-Bustos *et al.,* 2019). Though larger root systems in crops are beneficial, particularly in arid areas, they may be inefficient or even result in a production penalty in wet seasons or regions with enough water and capacity to provide additional irrigation (Chai *et al.,* 2016). However, evidence from this study suggests that root biomass positively affects productivity. These results are consistent with the ones reported by Fang *et al.,* (2017), who pinpointed the contribution of deeper and more profuse root growth to grain production. These larger root diameters can be used to boost soil carbon in agricultural soils. Identification of genotypes based on biomass production and allocation will allow for a more effective explanation of differences between individual genotype (Cunniff *et al.,* 2015). The harvest index (HI) indicates the proportion of grain production to above-ground plant biomass. HI plays a vital role in selecting high-yielding genotypes. A high HI reflects a genotype's ability to efficiently convert biological yield into economic yield (Kusalkar *et al.,* 2003). Genotypes AS111 and AS147 had the lowest GY due to their low biomass allocation to the roots and shoots, respectively. Genotype AS111 had the lowest HI of 4.84% (Table 2.5). Preventing losses in grain production per plant requires a combination of minimal biomass reduction and an increased HI (George-Jaeggli *et al.,* 2011).

Grain yield formation in sorghum is often sink-limited rather than source-limited (Borrás *et al.*, 2004).

2.4.4 Carbon allocation to roots and shoots

Genotypes NW5393, AS116, and AS115 sequestered less carbon as they accumulated the lowest total plant carbon stocks (Table 2.6). This was associated with their low production of biomass. Genotypes SS27, AS122, AS134, AS203, and AS563 had high biomass production, which increased their capacity to sequester more C (Singh *et al.*, 2014). All test genotypes stored more carbon in their shoots, indicating that roots are weaker C sinks than shoots. Because C is only exported to other sinks when supply exceeds local demand, SCs are higher than RCs. A wide range of variation was recorded among the test genotypes for root biomass. This variation can be exploited in breeding sorghum genotypes for productivity and carbon sequestration. Root biomass could be an accurate indicator of crop C intake into the soil (Monti and Zatta, 2009). Despite sorghum genotypes in the current study allocating less C into their root system than the shoot, certain sorghum genotypes developed more root volume, increasing their competitiveness for nutrients. Root-to-shoot biomass ratio (RS) and root-to-shoot carbon stock ratio (RCs/SCs) increased as PB and PCs decreased, respectively (Figures 2.1 and 2.2). This might be due to increased root biomass regulated by growth hormones like trans-zeatin riboside. Andreas *et al.*, (2020) reported that cytokinins played an important role in plant growth. These results aligned with the study conducted by Qi *et al.*, (2019), who reported a negative correlation between RS and PB. Brassard *et al.*, (2009) reported that the RCs/SCs regulates carbon partitioning within shoots and roots.

Increased carbon allocation to the roots, higher root-to-shoot ratios may result in significantly less carbon storage in above-ground biomass. When a plant allocates more carbon to its roots, it invests a larger proportion of its resources below ground (Klimešová *et al.*, 2018). Due to the sorghum genotypes genetic make-up, different genotypes may exhibit different RS and RCs/SCs. Genotypes AS108, AS115, and AS130 might have evolved to allocate C more efficiently to their roots, influencing C storage patterns (Prasad *et al.*, 2008). Genotype AS108 ranked fifth and first for exhibiting the highest RS and RCs/SCs, respectively, but relatively producing low yields, these results are supported by Larson *et al.*, (2020), who also reported that if the trade-off oscillates too much towards root development, an excessively high RS may result in diminished above-ground growth and, eventually, lower grain yields. An increase in total plant biomass production was associated with high grain yield, it can be concluded that instead of changing RS; increasing PB can increase yield without reducing root carbon sequestration capability. It is feasible to increase root and shoot biomass simultaneously to attain high PB, as demonstrated by the balanced production of biomass in genotype AS251. This study confirmed that most sorghum genotypes can be C sink in soils. The amount of C stock in soil by several sorghum genotypes is affected by land-use change and sorghum management practices (Tolbert *et al.*, 2002).

2.4.5 Principal component analysis for agronomic traits

The principal component analysis facilitated the recognition of significant agronomic traits that displayed substantial variability among the evaluated genotypes. The present study identified PB, SB, RB, RS, and GY as the most crucial traits, given their substantial contributions to PC1 and PC (Table 2.7). Abraha *et al.,* (2015) and Mangena *et al.,* (2018) confirmed the significance of grain yield, dry matter, and biomass to sorghum improvement. Furthermore, the analysis of principal components revealed that the diversity observed in the test genotypes cannot be fully explained (accounts for 86.21 % of the total variation) by a limited set of characteristics. This suggests that numerous traits explain the overall variance among the accessions. In descending order of significance DTM, DTH, PH, RS, and HI were identified as the major contributors to explaining a substantial proportion of the entire phenotypic diversity. These findings are confirmed by Ayana and Bekele, (1999), who reported these traits in contributing to the overall diversity of sorghum landraces.

2.4.6 Principal component analysis for carbon content and carbon stocks variables

The current study identified RCs/Scs, RCc, RCs, PCs, and SCs as the most important traits, given their significant contribution to both PC1 and PC2 (Table 2.8). These findings suggest the importance of these traits for selection. Accessions exhibiting increased, and desirable mean performances in these targeted traits would be selected for further enhancement. The genotypes were equally scattered across both PC1 and PC2, this indicates that there is an even distribution of genotypic characteristics along the associated axes. This could suggest that the genetic variations shown by both components are not contributing significantly to the observed variation among genotypes. Maximum weight should be given to traits with strong positive loadings, notably GCs, in the third component. According to Upadhyaya *et al.,* (2007) and Soroj *et al.,* (2013), trait contribution to various PCs differs with genetic diversity within the assessed germplasm and the number of traits evaluated. The current study suggested that the above traits could play a significant role in atmospheric carbon sequestration.

2.4.7 Cluster analysis for agronomic traits

The cluster analysis outlined the genotypes into three distinct groups, containing significantly different numbers of genotypes (Figure 2.4). The cluster analysis was able to group the genotypes based on flowering period. The first cluster consists of early flowering genotypes, the second of intermediate maturing genotypes, and the third of late flowering genotypes. This analysis demonstrated that genotype information on the flowering period may be relevant in identifying parents with various maturing groups (Mulima *et al.,* 2018). The traits PB, SB, RB, and GY are most distinguished between the clusters. Grouping the genotypes by traits may reveal that the genotypes are similar in one or more traits. Promising genotypes can be found using cluster means of assessed traits (Seetharam and Ganesamurthy, 2013). Billot *et al.,* (2013) reported that the breeder must better understand the genetics of agronomic traits to maximize the efficiency of selecting more diverse and suited parents for cultivar development.

2.4.8 Cluster analysis for carbon content and carbon stocks variables

The cluster analysis delineated the genotypes into three groups (Figure 2.5). The grouping of genotypes into distinct clusters highlights the diversity in the pedigree of the test genotypes, as these clusters represent relatedness within the genetic lineage of the genotypes (Sorkheh *et al.*, 2007). The analysis suggests a high level of genetic diversity among genotypes for carbon storage. The genotypic variation present in the germplasm provides potential for sorghum improvement by selecting the best performing genotypes from various clusters to retain genetic diversity, which is essential for breeding (Nevo and Chen, 2010).

2.5 Conclusions

The genotypes displayed varying agronomic traits and carbon sequestration capacities, which can be exploited in breeding sorghum genotypes with high productivity and carbon sequestration potential. Some genotypes accumulated more carbon in their biomass, implying their high capacity of the genotypes to absorb more carbon from the atmosphere. Ten sorghum genotypes C by increasing root biomass production and root depth. Deeper and larger roots are important to improve carbon sequestration, soil fertility, and crop productivity. Further, the study found a high root-to-shoot ratio of carbon as a priority trait in estimating carbon sequestration capacity in sorghum. Overall, genotypes such as AS251, SS27, AS134, AS203, and AS563 were selected for their high biomass production, grain yield, and C sequestration potentials. The selected sorghum genotypes are recommended for production or further breeding and variety release adapted to various agroecologies in South Africa. Selecting genotypes with high C storage ability is a practical strategy for climate change mitigation, as it reduces land degradation. This approach also ensures soil health and sustainable productivity, thereby addressing the issue of food insecurity.

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Appendix 2.1 Mean values for nine agronomic traits of the 50 sorghum genotypes evaluated at Silverton (STN), Ukulinga (UKN) and Bethlehem (BTH) in 2022 and 2023 growing seasons in South Africa.

Genotype	**DTH**			**DTM**			**PH**			**PB**			**SB**			**RB**			**RS**			**GY**			**HI**		
	STN	**UKN**	**BTH**	**STN**	**UKN**	**BTH**	**STN**	**UKN**	**BTH**	**STN**	**UKN**	**BTH**	**STN**	**UKN**	**BTH**	**STN**	**UKN**	**BTH**	**STN**	**UKN**	**BTH**	**STN**	**UKN**	**BTH**	**STN**	**UKN**	**BTH**
05-POTCH-138	77	68	76	154	139	139	107.67	112.83	145.33	27.14	27.56	24.07	9.79	7.78	9.14	17.35	19.78	14.93	1.85	2.54	1.71	6.50	8.17	7.33	40.23	51.07	44.81
16MZ	91	65	61	142	140	142	155.50	168.67	161.33	34.21	34.47	33.71	22.75	23.02	22.24	11.46	11.45	11.46	0.51	0.50	0.51	12.11	12.11	12.11	35.05	34.48	35.26
AS106	87	83	73	139	136	136	170.00	166.50	174.83	30.14	28.80	29.39	8.52	8.37	8.37	21.62	20.43	21.02	2.54	2.44	2.55	7.55	8.44	9.34	46.81	50.21	52.76
AS108	79	85	69	134	139	148	136.00	143.17	148.50	32.79	25.63	30.68	12.99	10.66	13.29	19.80	14.98	17.39	1.52	1.38	1.32	8.92	10.00	7.84	40.75	48.73	36.93
AS109	103	82	107	130	132	133	237.50	142.33	211.83	34.50	32.72	33.61	19.50	18.97	19.23	15.00	13.75	14.38	0.88	0.73	0.75	10.67	15.66	13.16	36.57	45.23	40.63
AS111	83	97	73	162	134	136	151.00	244.17	161.83	28.89	29.71	11.07	16.86	17.58	7.48	12.03	12.13	3.59	0.71	0.71	0.49	2.39	2.68	2.53	12.41	13.38	25.27
AS113	91	73	81	135	146	131	205.00	125.50	232.17	28.60	34.52	25.30	16.69	24.00	12.50	11.91	10.52	12.81	0.71	0.44	1.63	10.56	15.09	11.71	38.75	38.54	53.09
AS114	91	65	66	151	131	133	126.33	140.00	136.67	21.85	30.34	24.87	17.65	22.04	17.54	4.20	8.31	7.34	0.24	0.39	0.42	13.46	15.07	11.85	43.36	40.88	40.19
AS115	**89**	**81**	**95**	**141**	**125**	**123**	**126.83**	**145.67**	**275.00**	**16.90**	**18.07**	**29.22**	**7.48**	**12.92**	**17.22**	**9.42**	**5.14**	**12.00**	**1.26**	**0.41**	**0.70**	**29.66**	**15.80**	**29.78**	**79.86**	**55.15**	**63.36**
AS116	69	94	69	134	149	131	152.83	150.83	122.67	17.86	24.22	22.49	14.45	15.45	14.78	3.41	8.78	7.71	0.24	0.57	0.53	4.11	5.22	3.73	22.16	24.99	20.56
AS117	65	68	77	140	141	136	132.17	92.17	197.50	29.07	24.74	30.44	20.99	17.33	22.97	8.08	7.41	7.48	0.39	0.43	0.32	11.28	12.32	10.23	34.96	41.40	31.00
AS121	72	94	87	134	135	132	164.33	156.33	172.33	28.95	26.03	23.12	18.72	18.72	18.72	10.23	7.31	4.40	0.55	0.41	0.24	6.81	7.43	10.23	24.34	28.93	35.34
AS122	69	65	73	142	142	133	128.00	147.67	143.33	42.65	44.84	43.75	23.90	23.90	23.90	18.76	20.94	19.85	0.79	0.88	0.83	8.50	8.50	8.50	26.51	26.23	26.22
AS129	83	83	88	142	148	140	122.67	114.33	159.83	35.43	13.98	31.27	22.89	7.48	21.86	12.54	6.50	9.42	0.55	0.87	0.43	3.78	2.89	3.18	14.17	27.87	12.69
AS130	**81**	**65**	**85**	**138**	**148**	**148**	**163.83**	**128.17**	**198.00**	**20.56**	**24.78**	**31.36**	**9.90**	**9.90**	**14.08**	**10.66**	**14.88**	**17.28**	**1.08**	**1.50**	**1.23**	**21.33**	**21.33**	**12.83**	**68.30**	**67.58**	**47.69**
AS131	89	69	86	132	143	134	170.33	110.00	199.17	30.65	28.18	28.71	20.13	20.13	20.13	10.53	8.05	8.58	0.52	0.40	0.43	7.67	7.00	8.34	27.95	25.40	29.24
AS132	**106**	**98**	**80**	**136**	**136**	**126**	**238.17**	**103.83**	**171.17**	**32.54**	**26.48**	**18.69**	**20.69**	**14.45**	**11.69**	**11.85**	**12.03**	**7.00**	**0.61**	**0.89**	**0.60**	**21.57**	**21.36**	**7.75**	**51.74**	**60.09**	**39.87**
AS133	69	82	81	162	138	136	89.80	159.33	154.17	14.92	27.64	17.28	11.43	15.73	9.93	3.49	11.91	7.34	0.31	0.75	0.76	12.88	15.07	14.33	52.97	48.93	59.28
AS134	**83**	**85**	**65**	**135**	**135**	**136**	**195.33**	**132.83**	**160.00**	**39.17**	**42.23**	**25.17**	**21.04**	**20.58**	**19.48**	**18.13**	**21.65**	**5.70**	**0.86**	**1.05**	**0.29**	**26.31**	**27.71**	**10.23**	**55.57**	**57.35**	**34.50**
AS135	65	79	69	170	143	141	144.67	96.75	165.17	23.71	23.24	21.49	16.63	13.84	12.29	7.08	9.40	9.20	0.44	0.68	0.75	5.20	5.20	5.20	24.11	27.32	29.76
AS136	**90**	**87**	**98**	**138**	**162**	**149**	**126.67**	**147.50**	**135.83**	**21.55**	**27.07**	**29.95**	**11.25**	**16.93**	**20.21**	**10.30**	**10.14**	**9.75**	**0.92**	**0.60**	**0.48**	**15.07**	**16.06**	**9.35**	**57.26**	**48.51**	**31.67**
AS137	88	76	66	141	136	168	123.92	129.33	141.17	23.13	31.06	19.98	15.42	20.82	13.31	7.71	10.24	6.67	0.50	0.50	0.49	14.12	8.57	12.18	47.80	29.49	47.76
AS138	**65**	**91**	**77**	**139**	**149**	**134**	**156.67**	**141.67**	**140.17**	**19.72**	**22.01**	**43.90**	**11.93**	**12.97**	**20.19**	**7.79**	**9.04**	**23.71**	**0.65**	**0.70**	**1.20**	**18.54**	**18.89**	**14.92**	**60.84**	**59.36**	**43.16**
AS140	65	96	81	130	134	138	151.33	128.50	189.50	24.11	25.31	26.50	19.24	19.04	18.83	4.88	6.27	7.67	0.24	0.33	0.43	4.70	4.70	4.70	20.10	19.80	20.60
AS141	63	79	66	132	147	131	174.08	102.67	142.50	23.06	25.86	25.15	18.77	18.77	18.77	4.29	7.09	6.38	0.23	0.38	0.35	13.91	13.91	13.91	42.56	42.17	44.45
AS143	71	78	81	136	138	133	219.67	121.17	209.17	18.41	18.53	15.00	12.10	10.13	10.36	6.31	8.40	4.64	0.53	0.85	0.45	8.75	9.16	8.34	42.04	47.47	44.63
AS145	**69**	**100**	**89**	**140**	**147**	**136**	**155.33**	**159.33**	**158.33**	**27.09**	**36.03**	**33.49**	**21.92**	**20.38**	**23.56**	**5.17**	**15.66**	**9.93**	**0.24**	**0.78**	**0.42**	**15.31**	**29.93**	**13.04**	**40.90**	**59.50**	**35.60**

Appendix 2.1 Continued

Genotype	**DTH**			**DTM**			**PH**			**PB**			**SB**			**RB**			**RS**			**GY**			**HI**		
	STN	**UKN**	**BTH**	**STN**	**UKN**	**BTH**	**STN**	**UKN**	**BTH**	**STN**	**UKN**	**BTH**	**STN**	**UKN**	**BTH**	**STN**	**UKN**	**BTH**	**STN**	**UKN**	**BTH**	**STN**	**UKN**	**BTH**	**STN**	**UKN**	**BTH**
AS147	88	77	76	132	146	137	165.50	116.67	153.50	15.82	14.86	17.91	7.51	6.17	8.84	8.31	8.69	9.06	1.11	1.48	1.06	4.69	4.57	4.63	38.44	43.18	34.93
AS148	99	73	85	140	142	132	174.00	151.83	187.00	30.38	28.46	22.67	16.23	13.47	9.90	14.15	14.99	12.77	0.88	1.11	1.29	12.58	13.08	21.33	44.05	48.58	68.31
AS152	81	66	89	129	129	133	120.17	115.17	147.33	26.39	24.60	26.86	6.49	5.58	7.40	19.90	19.02	19.46	3.07	3.46	2.63	9.30	8.36	10.23	58.90	59.98	58.03
AS194	66	80	90	148	141	139	136.83	90.67	173.17	37.20	41.27	17.44	25.38	27.48	10.90	11.82	13.79	6.55	0.47	0.50	0.64	13.30	15.48	18.89	34.32	35.47	63.47
AS203	**94**	**88**	**65**	**138**	**141**	**114**	**177.67**	**185.17**	**160.67**	**35.28**	**42.65**	**36.65**	**20.54**	**27.91**	**21.91**	**14.74**	**14.74**	**14.74**	**0.73**	**0.52**	**0.68**	**16.63**	**16.63**	**16.63**	**45.02**	**37.72**	**43.20**
AS205	93	105	69	134	136	133	261.50	194.00	214.33	31.91	34.42	33.22	20.01	20.00	20.06	11.91	14.42	13.16	0.60	0.72	0.66	12.65	15.07	10.23	38.74	43.45	33.78
AS251	**86**	**83**	**87**	**148**	**143**	**136**	**144.33**	**120.83**	**129.00**	**26.31**	**27.44**	**29.05**	**14.72**	**14.35**	**14.82**	**11.59**	**13.09**	**14.24**	**0.79**	**0.91**	**0.96**	**21.83**	**21.83**	**21.83**	**59.75**	**59.79**	**59.57**
AS391	80	87	86	129	130	132	253.33	178.00	177.17	42.18	37.54	44.22	24.14	22.41	23.27	18.04	15.13	20.95	0.75	0.74	0.90	12.22	12.22	12.14	33.63	36.02	34.26
AS449	74	73	93	136	145	137	155.17	122.17	243.67	16.67	17.68	29.25	9.67	10.68	17.57	7.00	7.00	11.68	0.73	0.66	0.66	10.56	9.96	21.79	51.17	48.22	55.35
AS560	69	80	85	137	139	136	178.33	126.00	140.67	33.01	30.57	35.73	20.84	18.64	23.32	12.17	11.93	12.41	0.58	0.63	0.54	11.68	14.30	12.99	35.81	43.42	35.93
AS563	**86**	**84**	**82**	**136**	**138**	**132**	**179.67**	**124.67**	**217.50**	**34.68**	**26.13**	**38.25**	**23.11**	**20.70**	**20.12**	**11.57**	**5.43**	**18.13**	**0.50**	**0.26**	**0.90**	**13.28**	**12.31**	**24.91**	**36.48**	**37.29**	**55.27**
AS72	66	75	66	131	142	127	169.50	177.83	128.00	37.99	37.90	36.89	19.53	15.92	17.72	18.46	21.98	19.17	0.95	1.37	1.08	13.59	12.92	14.26	41.16	44.94	44.57
AS74	77	69	83	136	132	138	149.67	139.50	152.50	41.99	38.37	33.12	15.79	13.48	23.27	26.20	24.89	9.85	1.66	1.89	0.44	14.83	14.75	14.39	48.43	52.60	38.33
G50	65	69	69	122	132	132	147.83	165.83	175.33	19.16	22.23	19.16	7.56	7.56	7.56	11.60	14.67	11.60	1.53	1.94	1.63	9.08	8.50	9.66	54.57	52.93	56.45
ICS634	73	74	69	134	139	142	79.68	131.67	154.83	14.37	14.07	13.78	8.14	8.14	8.14	6.23	5.93	5.64	0.77	0.73	0.70	9.83	9.64	10.03	54.70	54.21	55.45
ICSV92001	77	87	74	142	142	138	150.83	124.17	187.83	33.20	23.29	19.41	23.21	12.38	10.12	9.99	10.92	9.29	0.43	0.88	0.98	7.50	15.07	15.07	24.46	55.00	60.23
LP4403	76	76	86	145	135	131	185.33	173.67	157.67	31.77	35.85	36.43	19.17	22.08	25.00	12.60	13.77	11.43	0.67	0.62	0.46	7.39	8.80	8.09	28.04	28.39	24.45
MAMOLOKWANE	84	69	103	144	136	140	191.83	99.17	203.00	20.77	26.92	19.14	12.33	16.00	10.65	8.44	10.92	8.49	0.68	0.68	0.80	12.28	15.07	10.23	49.98	48.50	49.01
NW5393	69	80	70	133	135	136	169.50	218.00	143.83	18.53	17.50	16.49	11.54	11.54	11.54	6.99	5.96	4.94	0.61	0.52	0.44	13.00	13.00	13.00	52.97	52.97	53.60
NW5430	101	99	86	136	123	141	173.17	140.50	158.08	23.54	22.49	21.44	16.08	16.08	16.08	7.45	6.41	5.36	0.47	0.40	0.33	7.35	8.79	10.23	31.39	35.34	38.88
PAN8816	76	69	81	132	146	142	117.83	96.33	107.00	30.30	36.19	34.27	16.61	17.64	18.15	13.69	18.55	16.12	0.82	1.05	0.90	3.75	3.75	3.75	17.89	17.56	17.28
SS27	**66**	**106**	**65**	**142**	**151**	**133**	**139.83**	**109.25**	**158.67**	**55.91**	**31.92**	**28.12**	**41.86**	**16.55**	**16.21**	**14.05**	**15.36**	**11.91**	**0.47**	**1.04**	**0.73**	**17.58**	**17.58**	**17.58**	**29.58**	**51.51**	**52.03**
SV07002	71	85	77	142	138	136	167.50	188.83	107.50	25.54	24.72	29.82	16.38	15.83	19.38	9.17	8.89	10.44	0.57	0.57	0.54	6.50	6.31	7.03	28.38	28.50	26.55
Mean	79.14	80.60	78.81	138.92	139.25	135.86	160.29	140.02	167.72	28.21	28.22	27.38	16.81	16.05	16.08	11.40	12.17	11.30	0.79	0.90	0.81	11.66	12.33	11.71	40.52	42.71	41.71
SD	11.31	10.92	10.49	8.78	6.92	7.49	37.33	32.13	33.58	8.45	7.42	7.86	6.26	5.42	5.27	5.00	4.90	4.96	0.55	0.62	0.51	5.69	5.84	5.55	14.00	12.57	13.14
SE	1.60	1.54	1.48	1.24	0.98	1.06	5.28	4.54	4.75	1.20	1.05	1.11	0.89	0.77	0.75	0.71	0.69	0.70	0.08	0.09	0.07	0.81	0.83	0.78	1.98	1.78	1.86
Skewness	0.41	0.45	0.43	1.44	0.30	1.17	0.66	0.89	0.85	0.61	0.20	0.13	0.98	-0.01	-0.12	0.66	0.63	0.61	2.25	2.17	1.90	0.94	0.73	0.94	0.26	-0.34	-0.04
kurtosis	-0.72	-0.50	-0.19	3.03	1.25	6.82	0.95	1.15	1.10	0.84	-0.31	-0.44	3.57	-0.55	-1.29	0.35	-0.26	-0.38	6.51	5.75	4.39	1.31	0.93	1.48	0.10	-0.61	-0.68

SD = standard deviation, SE = standard error, DTH = days to 50% heading, DTM = days to 50% maturity, PH = plant height (cm), PB = total plant biomass (g plant^{-1}), SB = shoot biomass (g plant^{-1}), RB = root biomass (g plant^{-1}), RS = root to shoot biomass ratio, GY = grain yield (g plant^{-1}), HI = harvest index (%). The top ten performing genotypes at each location are highlighted in bold.

CHAPTER 3: Genetic Variation of Yield, Yield Components, and Carbon Storage in Sorghum (*Sorghum bicolor* [L.] Moench) Genotypes

Abstract

Trait heritability and the response to selection depend on genetic variation, a prerequisite to developing sorghum varieties with desirable agronomic traits and high carbon sequestration for sustainable crop production and soil health. The present study aimed to assess the extent of genetic variability among agronomic and carbon storage traits in selected sorghum genotypes to identify the best candidates for production or breeding. Fifty genotypes were evaluated at Ukulinga, Bethlehem and Silverton sites in South Africa during the 2022/23 growing season. The experiments were laid out in a 5 x 10 alpha lattice design with two replications. Data were collected on days to 50% heading (DTH), days to 50% maturity (DTM), plant height (PH), total plant biomass (PB), shoot biomass (SB), root biomass (RB), root-to-shoot biomass ratio (RS), grain yield (GY), harvest index (HI), grain carbon content (GCc), shoot carbon content (SCc), root carbon content (RCc), total plant carbon stock (PCs), shoot carbon stock (SCs), root carbon stock (RCs), and root-to-shoot carbon stock ratio (RCs/SCs), and grain carbon stock (GCs). Variance components, heritability, genetic advance were computed. High phenotypic coefficient of variations (PCVs) were recorded for PH (68.91%), followed by GY (51.8%), RB (50.51%), RS (41.96%), RCs/SCs (44.90%), and GCs (41.90%), and high genotypic coefficient of variations (GCVs) for GY at 45.92%, RB (39.24%), RCs/SCs (38.45), and RCs (34.62). The high PCVs and GCVs values indicate the availability of genetic variability among the test genotypes for the assessed traits. High broad-sense heritability and genetic advance were recorded for HI (83.76 and 24.53%), GY (78.59 and 9.98%), PB (74.14 and 13.18%) and PCs (53.63 and 37.57%), respectively, suggesting a marked genetic contribution to the traits. These traits could be used in selection to develop sorghum cultivars with high biomass production and carbon sequestration. High broad-sense heritability combined with increased genetic advance in the assessed traits indicate that these traits are primarily controlled by additive genetic effects. This suggests that selecting within the germplasm should result in rapid improvement of these traits.

Keywords: carbon sequestration, genetic advance, genetic variability, broad-sense heritability, organic carbon, sorghum

3.1 Introduction

Sorghum (*Sorghum bicolor* [L.] Moench) is a resilient crop adapted to grow in diverse agro-ecologies, including semi-arid, subtropical, tropical and temperate climates globally. It is a primary staple food crop for most of Africa's semi-arid regions, including South Africa. It thrives under drought conditions where other major cereal crops fail (Dossou-Aminon *et al.,* 2016; Sharma *et al.,* 2015; Yohannes *et al.*, 2015). Sorghum seeds are rich sources of nutrients such as carbohydrates (65-76%), proteins (8-12%), dietary fibre (2%), vitamin B, and minerals (e.g. iron, magnesium, phosphorus, and potassium) (Abah *et al.,* 2020). Sorghum stover contains crude protein (8-12%), digestible organic matter and metabolizable energy (70-75%) (Amuda and Okunlola, 2023). Sorghum's nutritional content and resilience to grow under harsh growing conditions make it an ideal dual-purpose crop in a marginal and mixed livestock-cropping system.

Sorghum has a C4 photosynthetic pathway with high photosynthesis efficiency and biomass production which are vital under limited water and soil nutrients facilated by its extended root length, density, and water-use efficiency. It can reach a height of 4 meters and produce fresh biomass yields ranging from 45 to 112 tons per hectare, depending on genotype and growing environments (Shukla *et al.,* 2017). The high biomass production potential of sorghum contributes to increased carbon sequestration by efficiently capturing and storing atmospheric carbon dioxide (CO_2) through photosynthesis, reducing and compensating for emissions.

Previous studies have reported variable carbon sequestration rates for different crops. For example, Jarecki *et al.* (2005) reported carbon sequestration potential of 379 kg C ha yr^{-1} in corn. Srinivasarao *et al.* (2012) reported a significant soil organic carbon sequestration, recording 14.4 Mg C ha^{-1} in sorghum and reporting that for every ton increase in root carbon stock, there was 0.09 Mg ha^{-1} increase in grain yield of sorghum. Growing high carbon-sequestering potential crops could significantly reduce atmospheric carbon dioxide levels. It mitigates climate change by sequestering carbon in the soil and biomass and enhancing soil fertility, soil tilth, crop production, and overall soil sustainability (West and Post, 2002). Druille *et al.* (2020) reported that changes in plant carbon allocation patterns due to climate change can significantly impact agricultural productivity and food security. Crop genetic resources with adequate genetic variation are vital to developing new cultivars with desirable agronomic traits and high carbon sequestration for sustainable crop production and soil health. Phenotypic variation depends on the test population's genetic constitution and the growing conditions.

Continued development of sorghum cultivars is crucial to mitigate future climate change impacts and sustainably feed a growing global population. This can be accomplished by effectively selecting genetically superior and resilient genotypes based on the degree of diversity in the source material. The success of any crop improvement relies on the extent of genetic variability present in the source material and the effectiveness of selection (Ranjith *et al.,* 2017). The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) provide information on the relative degree of phenotypic and genotypic variation in distinct traits of sampled populations (Rashid *et al.,* 2017). Heritability is predictive in indicating the reliability of phenotypic value as a guide to breeding value (Falconer and Mackay, 1996). High values of heritability indicate that the phenotypic expression of the genotype is a good indicator of its genetic potential. The degree of genetic gain acquired in an economic trait under a specific selection pressure is explained by genetic advance. Selection progress depends on genetic variability for yield and yield contributing traits and their heritability (Begna, 2021). High genetic advance and heritability estimates demonstrate the genetic effects of conditioning economic trait, indicating the efficiency of selection in crop improvement programs (Mofokeng *et al.,* 2019).

In this regard, A multi-location and -seasonal assessment of sorghum genotypes is essential to develop the best-performing varieties for yield components and carbon storage. Genetically diverse sorghum accessions were collected from major producing countries, including Ethiopia, Tanzania, and South Africa, for selection under South African conditions. From this gene pool, 50 accessions were sampled based on their high grain yield, biomass, and ethanol production (Mangena *et al.,* 2018). The objective of this study was to assess the extent of genetic variability among agronomic and carbon storage traits in selected genetically diverse sorghum genotypes to select contrasting traits for production or breeding.

3.2 Materials and Methods

3.2.1 Plant materials

This study used fifty sorghum genotypes obtained from South Africa (Agricultural Research Council, African Centre for Crop Improvement and Department of Agriculture), Zimbabwe, Ethiopia, and Tanzania were used in this study. A detailed description of genotypes used in this study was presentented in Chapter 2, Section 2.2.1.

3.2.2 Study sites

The 50 genotypes were evaluated in three locations: Ukulinga research farm at the University of KwaZulu-Natal in Pietermaritzburg, Bethlehem in Free State, and Silverton in Pretoria during the 2022/23 growing season. This was described in Chapter 2, Section 2.2.2.

3.2.3 Experimental design and field trial establishment

The experiments were laid out in a 5 x 10 alpha lattice design with two replications. This was described in Chapter 2, Section 2.2.3.

3.2.4 Data collection

3.2.4.1 Agronomic traits

Data was collected on nine agronomic traits at all three locations. Details of the data collected were summarised in Chapter 2, Section 2.2.4.1.

3.2.4.4 Carbon stocks detemination

Among the 50 sorghum genotypes, 25 were selected from the Silverton trials based on their grain yield performance. The 25 lines were subjected to carbon analysis using two replications. This was described in Chapter 2, Section 2.2.4.2.

3.2.5 Data analysis

Data were subjected to analysis of variance using the Statistical Analysis System (SAS) software version 9.4 program with the lattice procedure (Guide, 2014). A combined analysis of variance was conducted after testing the homogeneity of variance using Levene’s test (1960). Variance components for agronomic and carbon storage traits were estimated based on combined and single environment analyses, respectively.

3.2.5.1 Estimation of phenotypic and genotypic variance

Phenotypic and genotypic variance for agronomic traits were calculated from the results of combined analysis of variance according to Rahimi and Hernandez (2022):

$$\sigma_e^2 = MSe$$

$$\sigma_{ge}^2 = \left(\frac{MSge - MSe}{r}\right)$$

$$\sigma_g^2 = \left(\frac{MSg - MSge}{r * e}\right)$$

$$\sigma_p^2 = \sigma_g^2 + \sigma_{ge}^2 + \sigma_e^2$$

Where; σ_e^2 is the environmental variance of a particular trait; σ_{ge}^2 is the genotype x environment interaction variance of a particular trait; σ_g^2 is the genotypic variance of a particular trait; σ_p^2 is the phenotypic variance of a particular trait; MS_e is the mean square of the environment; MSg_e is the mean square of genotype x environment; MS_g is the mean square of genotype; r = number of replications; and e = number of environments

Phenotypic and genotypic variance for carbon storage traits were calculated from the results of a single environment analysis of variance, as suggested by Burton and Devane (1953).

$$\sigma_\varepsilon^2 = MS\varepsilon$$

$$\sigma_g^2 = \frac{MSg - MS\varepsilon}{r}$$

$$\sigma_p^2 = \sigma_g^2 + \sigma_\varepsilon^2$$

Where $MS\varepsilon$ is the mean square error and σ_ε^2 is the error variance component.

3.2.5.2 Estimation of coefficient of variability

Phenotypic coefficient of variation (PCV) and Genotypic coefficient of variation (GCV) were computed according to Burton and Devane (1953):

$$PCV = \left(\frac{\sigma_p}{\bar{x}}\right) x\ 100$$

$GCV = (\frac{\sigma_g}{\bar{x}}) \times 100$

Where σ_g is the genotypic standard deviation of a particular trait; σ_p is the phenotypic standard deviation of a particular trait; and $\bar{x}$ is the mean performance of a particular trait.

3.2.5.3 Heritability and genetic advance

The broad-sense heritability of a given trait at a single environment was calculated according to Alvarado *et al.* (2020):

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_\varepsilon^2 / r}$$

Where σ_g^2 is the genotypic variance; σ_ε^2 is the error variance component; and r is the number of replications, whereas regarding combined analyses, broad-sense heritability was calculated as:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{ge}^2 / e + \sigma_\varepsilon^2 / (r * e)}$$

Where σ_{ge}^2 is the genotype x environment interaction and e is the number of environments.

Genetic advance (GA) and the genetic advance as a percent of the mean (GAM) were computed using the following formulae (Allard, 1999):

$$GA = K\, H^2\, \sigma_p$$

$$GAM = (\frac{GA}{\bar{x}}) * 100$$

Where K is 2.06 at 5% selection intensity

3.3 Results

3.3.1 Combined analysis of variance for agronomic traits

A combined analysis of variance showing the mean square values and significant tests is presented in Table 3.1. Significant ($p < 0.05$) differences were calculated among genotypes for all the assessed agronomic traits except DTM. Highly significant ($p < 0.01$) genotypes by location interactions were recorded for DTH, DTM, PH, PB, SB, RB, RS, and GY.

Table 3.1 Combined analysis of variance and significance tests for agronomic traits of 50 sorghum genotypes across three locations in South Africa.

Source of variation	**DF**	**DTH**	**DTM**	**PH**	**PB**	**SB**	**RB**	**RS**	**GY**	**HI**
Location	2	91.83	349.41	20546.17***	20.14	18.49	22.83**	0.004	13.70**	50.34
Replication	3	479.85	519.62**	28833.24***	9.61	7.93	0.37	0.01	7.43*	18.37
Block	24	167.77	111.44	2757.55	25.17	11.53	3.31	0.005	4.42*	21.91
Genotype	49	307.42*	130.78	3194.9**	349.57***	151.72***	129.22***	0.26***	181.77***	1031.47***
Genotype x location	98	207.06**	111.63*	2257.43**	18.36***	14.44**	4.5*	0.004*	2.57***	15.34
Error	123	195.51	135.71	1826.3	15.99	14.52	5.06	0.004	2.73	17.83

*, ** and *** denote significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. DF = degrees of freedom, DTH = days to 50% heading, DTM = days to 50% maturity, PH = plant height (cm), PB = total plant biomass (g plant^{-1}), SB = shoot biomass (g plant^{-1}), RB = root biomass (g plant^{-1}), RS = root to shoot biomass ratio, GY = grain yield (g plant^{-1}), HI = harvest index (%).

3.3.2 Analysis of variance for carbon storage traits

The analysis of variance revealed highly significant differences ($p < 0.001$) among genotypes for GCc, PCs, SCs, RCs, RCs/SCs, and GCs (Table 3.2).

Table 3.2 Analysis of variance and significance tests for carbon storage of the 25 selected sorghum genotypes at Silverton during 2022 growing season

Source of variation	**DF**	**SCc**	**RCc**	**GCc**	**PCs**	**SCs**	**RCs**	**RCs/SCs**	**GCs**
Replication	1	1.24*	5.21	0.03	1.34	2.85	0.29	0.004	0.86
Block	8	0.28**	7.45**	0.01**	10.88	13.41	0.38	0.05*	0.21
Genotype	24	0.65	12.77	0.49***	29.02**	20.13*	5.47***	0.13***	8.44**
Error	16	0.32	8.03	0.01	8.76	7.61	0.42	0.02	0.34

*, ** and *** denote significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. DF = degrees of freedom, CV = coefficient of variation, SCc = shoot carbon content (%), RCc = root carbon content (%), GCc = grain carbon content (%), PCs = total plant carbon stocks (g plant^{-1}), SCs = shoot carbon stock (g plant^{-1}), RCs = root carbon stock (g plant^{-1}), RCs/SCs = root to shoot carbon stock ratio, GCs = grain carbon stock (g plant^{-1}).

3.3.3 Genotype response for grain yield and component traits and carbon storage

The mean grain yield of the 50 genotypes observed for the genotypes was 11.9 g plant^{-1} (Table 3.3). The following high yielding genotypes were selected: AS115, AS251, AS134, AS145, and AS130 with mean yields of 25.08 g plant^{-1}, 21.83 g plant^{-1}, 21.42 g plant^{-1}, 19.43 g plant^{-1}, and 18.50 g plant^{-1}, respectively. DTM ranged from 128 to 151 days. The study selected early maturing genotypes, such as AS115 and AS230 with 130 and 131 maturation days, respectively. Genotypes AS115 and AS111 had tall plant stature with a mean PH of 182.5 cm and 185.67 cm, respectively. The genotypes that had the highest SB and RB were among the top performing genotypes. Genotype SS27 had the highest PB and SB with mean values of 38.65 g plant^{-1} and 24.87 g plant^{-1}, in that order. The highest RB was recorded for genotypes AS134 and AS130, with mean values of 15.16 g plant^{-1} and 14.27 g plant^{-1}, respectively. Genotype AS130 had the highest root-to-shoot biomass ratio (1.26) compared to all the genotypes.

The mean total plant carbon stock for the test genotypes was 12.95 g plant^{-1} (Table 3.4). The highest PCs were recorded for SS27, AS122, AS134, and AS203 with values of 24.64 g plant^{-1}, 18 g plant^{-1}, 16.48 g plant^{-1}, and 15.55 g plant^{-1}, respectively. The four genotypes were also among the highest grain yield producers. The genotypes that allocated the highest carbon in shoots were SS27, AS122, ICSV92001, and AS563 at19.04 g plant^{-1}, 10.42 g plant^{-1}, 10.34 g plant^{-1}, 10.33 g plant^{-1}. Almost all the genotypes stored more carbon in the shoots than in the roots. The genotype that stored more carbon in the roots compared to shoots was AS108, with the RCs value of 8.87 g plant^{-1} and the highest RCs/SCs of 1.56.

3.3.4 Variance components, heritability, and genetic advance for agronomic traits

Phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (H^2), genetic advance (GA), and genetic advance as a percentage of the mean (GAM) for agronomic traits are presented in Table 3.3. PB varied from 14.07 to 43.75 g plant^{-1}, and GY ranged from 2.53 to 25.08 g plant^{-1}. The phenotypic variance was the highest for PH (1158.05), followed by DTM (233.71), and HI (202.2). HI and PH had the highest genotypic variance of 169.36 and 156.25, respectively. The highest environmental variance was recorded for PH (20546.17) and DTM (349.41). The trait that exhibited the highest genotype by location interaction variance was DTH, with a value of 57.62, while PH exhibited the lowest genotype by environment interaction of -9144.37. The highest PCV values were recorded for PH (68.91%), followed by GY (51.8%), RB (50.51%), and RS (41.96%). The highest GCV values were recorded for GY (45.92%), RB (39.24%), and HI (31.38%).

The highest heritability values were recorded for HI, GY, PB, and RB at 83.76%, 78.59%, 74.14%, and 60.34%, respectively. Moderate heritability values were computed for shoot biomass (58.15%) and RS (37.87%). The highest genetic advance values were recorded for HI (24.53 g plant^{-1}), followed by PB (13.18 g plant^{-1}), and GY (9.98 g plant^{-1}). The highest GAM values were computed for GY (83.87%) and RB (62.78%). Moderate GAM values were recorded for HI, RS, PB, and SB with values of 59.16%, 50.86%, 47.17%, and 46.07%, respectively. The lowest GAM was recorded for DTH (3.36%), DTM (0.31%), and PH (1.92%).

Table 3.3 Mean performances among the ten best and five bottom sorghum genotypes ranked based on grain yield and genetic parameters for agronomic traits in 50 genotypes evaluated at three locations during the 2022/23 growing seasons in South Africa.

Genotype	**DTH**	**DTM**	**PH**	**PB**	**SB**	**RB**	**RS**	**GY**	**HI**
Top ten genotypes									
AS115	88	130	182.50	21.39	12.54	8.85	0.71	25.08	66.66
AS251	85	142	131.39	27.60	14.63	12.97	0.89	21.83	59.88
AS134	78	135	162.72	35.52	20.37	15.16	0.74	21.42	51.26
AS145	86	141	157.67	32.20	21.95	10.25	0.47	19.43	46.95
AS130	77	145	163.33	25.56	11.29	14.27	1.26	18.50	62.09
SS27	79	142	135.92	38.65	24.87	13.77	0.55	17.58	41.41
AS138	78	140	146.17	28.54	15.03	13.51	0.90	17.45	53.73
AS132	94	133	171.06	25.90	15.61	10.29	0.66	16.89	51.98
AS563	84	135	173.94	33.02	21.31	11.71	0.55	16.83	44.12
AS203	82	131	174.50	38.19	23.45	14.74	0.63	16.63	41.49
Bottom five genotypes									
AS147	80	138	145.22	16.20	7.51	8.69	1.16	4.63	38.13
AS116	77	138	142.11	21.52	14.89	6.63	0.45	4.35	22.62
PAN8816	75	140	107.06	33.58	17.47	16.12	0.92	3.75	17.68
AS129	84	143	132.28	26.89	17.41	9.49	0.54	3.28	15.87
AS111	84	144	185.67	23.22	13.97	9.25	0.66	2.53	15.34
Mean	81.23	138.7	155.8	31.74	17.44	11.25	0.59	10.69	30.39
SD	14.37	11.17	57.94	8.26	7.375	4.57	0.25	6.31	13.92
SE	1.52	1.18	6.11	0.87	0.78	0.48	0.03	0.66	1.47
Overall statistics of the 50 genotypes									
Mean	79.52	138.01	156.01	27.94	16.31	11.62	0.8	11.9	41.47
$\boldsymbol{\sigma_p^2}$	166.17	233.71	11558.05	74.45	39.35	34.45	0.11	38	202.2
$\boldsymbol{\sigma_g^2}$	16.73	3.19	156.25	55.2	22.88	20.79	0.04	29.87	169.36
$\boldsymbol{\sigma_e^2}$	91.83	349.41	20546.17	20.14	18.49	22.83	0.05	13.7	50.34
$\boldsymbol{\sigma_{ge}^2}$	57.62	-118.89	-9144.37	-0.89	-2.03	-9.17	0.02	-5.57	-17.5
PCV (%)	16.21	11.08	68.91	30.88	38.46	50.51	41.96	51.8	34.29
GCV (%)	5.14	1.29	8.01	26.59	29.33	39.24	25.82	45.92	31.38
H^2 %	10.07	1.37	1.35	74.14	58.15	60.34	37.87	78.59	83.76
GA (%)	2.67	0.43	2.99	13.18	7.51	7.3	0.41	9.98	24.53
GAM	3.36	0.31	1.92	47.17	46.07	62.78	50.86	83.87	59.16

SD = standard deviation, SE = standard error, CV = coefficient of variation, DTH = days to 50% heading, DTM = days to 50% maturity, PH = plant height (cm), PB = total plant biomass (g plant^{-1}), SB = shoot biomass (g plant^{-1}), RB = root biomass (g plant^{-1}), RS = root to shoot biomass ratio, GY = grain yield (g plant^{-1}), HI = harvest index (%), σ^2_p = phenotypic variance, σ^2_g = genotypic variance, σ^2_e = environmental variance, σ^2_{ge} = genotype by environment interaction variance, PCV = phenotypic coefficient of variation, GCV = genotypic coefficient of variation, H^2 = heritability, GA = genetic advance, GAM = genetic advance as a percentage of the mean.

3.3.5 Variance components, heritability, and genetic advance for carbon storage traits

PCs, SCs and RCs values ranged from 7.52 to 24.64 g plant^{-1}, 3.25 to 19.04 g plant^{-1}, and 1.37 to 8.87 g plant^{-1}, respectively (Table 3.4). The highest phenotypic variance was recorded for PCs, SCs, and GCs, with values of 18.89, 13.87, and 4.39, respectively. The same trend was observed for genotypic variance. Higher PCV and GCV values were observed for PCs (33.98 and 24.88%), SCs (45.42 and 30.51%), RCs (37.39 and 34.62%), RCs/SCs (44.90 and 38.45 %), and GCs (41.90 and 40.25%), respectively. The SCc values had PCV of 34.02% and GCV of 1.58 %, RCc (7.85 and 3.75 %), and GCc (1.15 and 1.12%), respectively.

High heritability values were recorded for GCc, GCs, RCs, and RCs/SCs with 96%, 92.26%, 85.74%, and 73.33%, respectively (Table 3.4). PCs and SCs had moderate heritability estimates of 53.63% and 45.13%, respectively. Low heritability (< 50%) estimates were computed for SCc and RCc with 34.02%, and 22.79%, respectively.

The highest GA estimates were calculated for PCs (4.8 g plant^{-1}), followed by GCs (3.98 g plant^{-1}), SCs (3.46 g plant^{-1}), and RCs (3.03 g plant^{-1}) (Table 3.4). SCc, RCc, GCc, and RCs/SCs had very low GA estimates with values of 0.49 g plant^{-1}, 1.51 g plant^{-1}, 0.99 g plant^{-1}, and 0.41 g plant^{-1}, respectively. The GAM estimates were the highest for GCs (79.64%), RCs/SCs (67.82%), and RCs (66.03%). PCs and SCs had GAM estimates of 37.54% and 42.23%, respectively. Very low GAM estimates were recorded for SCc, RCc, and GCc, with values of 1.1%, 3.68%, and 2.27%, respectively.

Table 3.4 Mean performances among the ten best and five bottom sorghum genotypes ranked based on their total plant carbon stock and genetic parameters for carbon storage traits in 25 selected sorghum genotypes at Silverton during the 2022/23 growing season.

Genotype	**SCc**	**RCc**	**GCc**	**PCs**	**SCs**	**RCs**	**RCs/SCs**	**GCs**
Top ten genotypes								
SS27	45.48	39.92	43.27	24.64	19.04	5.61	0.29	7.61
AS122	43.62	40.39	43.43	18	10.42	7.58	0.73	3.69
AS134	44.41	39.34	43.25	16.48	9.34	7.13	0.76	11.38
AS203	44.56	43.41	43.47	15.55	9.15	6.4	0.7	7.23
AS563	44.7	40.26	44.49	14.99	10.33	4.66	0.45	5.91
AS251	43.51	43.26	42.89	14.97	8.48	6.49	0.76	4.57
16MZ	43.2	44.41	43.24	14.92	9.83	5.09	0.52	5.24
AS108	43.74	44.79	43.96	14.55	5.68	8.87	1.56	3.92
ICSV92001	44.55	39.94	44.65	14.33	10.34	3.99	0.39	3.35
LP4403	43.73	43.76	45.31	13.89	8.38	5.51	0.66	3.35
Bottom five genotypes								
AS138	44.87	40.72	43.36	8.52	5.35	3.17	0.59	8.04
AS143	44.68	43.67	44.24	8.16	5.41	2.76	0.51	3.87
NW5393	45.09	39.4	44.07	7.96	5.2	2.75	0.53	5.73
AS116	43.78	40.12	43.34	7.69	6.33	1.37	0.22	1.78
AS115	43.44	45.34	43.56	7.52	3.25	4.27	1.31	12.92
Mean	44.25	41.86	43.78	12.95	8.32	4.63	0.62	5.23
SD	0.81	2.74	0.65	5.56	4.57	2.31	0.35	2.21
SE	0.15	0.5	0.12	1.02	0.83	0.42	0.06	0.40
Overall statistics of the 25 genotypes								
Mean	44.18	41.1	43.65	12.65	7.98	4.67	0.65	5.84
$\boldsymbol{\sigma_p^2}$	0.49	10.4	0.25	18.89	13.87	2.95	0.08	4.39
$\boldsymbol{\sigma_g^2}$	0.17	2.37	0.24	10.13	6.26	2.53	0.06	4.05
PCV (%)	1.58	7.85	1.15	33.98	45.42	37.39	44.9	41.9
GCV (%)	0.92	3.75	1.12	24.88	30.51	34.62	38.45	40.25
H^2 (%)	34.02	22.79	96	53.63	45.13	85.74	73.33	92.26
GA (%)	0.49	1.51	0.99	4.8	3.46	3.03	0.41	3.98
GAM (%)	1.1	3.68	2.27	37.54	42.23	66.03	67.82	79.64

SD = standard deviation, SE = standard error, CV = coefficient of variation, SCc = shoot carbon content (%), RCc = root carbon content (%), GCc = grain carbon content (%), PCs = total plant carbon stocks (g plant^{-1}), SCs = shoot carbon stock (g plant^{-1}), RCs = root carbon stock (g plant^{-1}), RCs/SCs = root to shoot carbon stock ratio, GCs = grain carbon stock (g plant^{-1}), σ^2_p = phenotypic variance, σ^2_g = genotypic variance, PCV = phenotypic coefficient of variation, GCV = genotypic coefficient of variation, H^2 = heritability, GA = genetic advance, GAM = genetic advance as a percentage of the mean.

3.4 Discussion

3.4.1 Genetic parameters for the agronomic and carbon storage traits

The results suggest that the test genotypes displayed adequate genetic variation for selection for enhanced biomass production, grain yield, and carbon allocation to roots and shoots. In agreement with the present results, Abraha *et al.* (2015) reported high PCV and GCV for sorghum grain yield, biomass, and carbon storage traits. The highest PCV and GCV values were recorded for GY, followed by RB, RCs/SCs, and RCs supporting the agronomic traits (Tables 3.3 and 3.4). Narrow differences between PCV and GCV values were recorded for SB, RB, GY, HI, SCc, GCc, and GCs, indicating the minimal impact of the test locations on genotype selection (Barton and Keightley, 2002). These results are consistent with those of Haussmann *et al.* (2002) and Ayele (2011), who found the same trend for sorghum grain yield and those of Kishore and Singh (2005) for biomass yield. Ayana *et al.* (2000) and Munzbergova *et al.* (2017) reported that genotypic variation is influenced by rainfall, temperature, and growing site gradients. McGuigan and Sgro (2009) argued that phenotypic expression can reveal genetic heterogeneity and is subject to environmental influences.

Heritability refers to the proportion of phenotypic variance attributed to genetic variance. High to moderate broad sense heritability values were computed for PB, GY, HI, RB, GCc, GCs, RCs, RCs/SCs, SB, RS, PCs (Tables 3.3 and 3.4). The results suggest that these traits will have higher response to selection, being less influenced by environmental effects. Related results were observed in sorghum for grain yield and biomass (Abraha *et al.,* 2015). Days to 50% heading, days to 50% maturity, and plant height, shoot carbon content, and root carbon content showed low heritability. Phenotypic selection for low heritable traits can be less effective, needing indirect selection via traits with higher heritability or the use of complementary high throughput molecular markers. These results conform with the findings of Gebregergs and Mekbib (2020) who reported low heritability in sorghum for days to 50% maturity. Low heritability traits can be selected using molecular markers linked to quantitative trait loci (QTLs) for the target traits, allowing individuals to be graded based on their genetic makeup rather than their phenotypic characteristics (Mackay *et al.,* 2009). Previous findings have indicated a complex inheritance pattern for days to 50% heading and days to 50% maturity, conditioned by dominant and recessive genes (Zhai *et al.,* 2014). A putative dwarfing locus was found through genome-wide association studies (GWAS) (Li *et al.,* 2015), and the locus has been linked to higher total plant biomass and root biomass

(Dossa *et al.,* 2021). These findings indicate that the locus may have a pleiotropic effect on carbon storage and partitioning (Kumar *et al.,* 2022). Heritability value helps determine the success of selection via phenotypic traits (Al-Tabbal and Al-Fraihat, 2012). The high heritability estimate observed for RCs/SCs suggests the effectiveness of selection using the root-shoot ratio for enhancing carbon allocation and genetic gain in roots and shoots.

Characters with high heritability can easily be successfully selected, resulting in quick genetic progress. However, it has been accentuated that heritability alone has less practical importance without genetic advancement due to the reliance on genetic variability and the possibility of unexpected environmental interactions altering characteristic expression over time (Aman, 2021). Genetic advance (GA) refers to the degree of gain obtained in a trait under a particular selection pressure. High GA was recorded for PB, GY, and HI in this study (Table 3.3). Similar results were reported by Mofokeng *et al.* (2019) in sorghum for grain yield and thousand seed weight. The highest heritability and genetic advance as a percentage of the mean (GAM) was recorded for RB, followed by GY, HI, RCs, RCs/SCs, and GCs. High GAM is associated with effective selection of sorghum genotypes with high yield and carbon storage. High heritability and genetic advance values indicate the presence of additive gene action, which are highly heritable and suggesting that crop improvement can be achieved by selecting such traits (Bello *et al.,* 2012; Ogunniyan and Olakojo, 2014; Janaki *et al.,* 2015; Malbhage *et al.,* 2020). Estimates of heritability and genetic advance are more reliable and informative (Slater *et al.,* 2014; Hika *et al.,* 2015). Priority should be given to traits that displayed high heritability and genetic advances to develop accurate selection indices for developing sorghum genotypes with high grain yield and carbon sequestration potential.

3.5 Conclusions

The current study examined the genetic variation and associations among agronomic and carbon storage traits in sorghum genotypes and revealed a considerable genetic variation among the test genotypes. The findings suggest the opportunity for selection and breeding programs to develop improved sorghum cultivars for enhanced yield production and carbon sequestration. The following traits were highly heritable with higher genetic advance values: PB, RB, GY, HI, RS, GCs, RCs, and RCs/SCs, making them ideal traits for selection.

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CHAPTER 4: Association of Agronomic Traits and Carbon Storage on Grain Yield Response in Sorghum

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Abstract

Multi-trait selection is crucial for developing ideotypes with desirable product profiles in plant breeding programs. Correlation and path coefficient analyses reveal the trend and magnitude of associations of several traits to guide simultaneous selection for enhanced grain yield, its components and carbon storage. This study aimed to assess the trend and magnitude of relationships between agronomic and carbon storage traits in sorghum to identify grain yield and carbon storage contributing traits. Fifty sorghum genotypes were evaluated at three locations during the 2022/23 growing season. At each location, the experiment was laid out in a 5 x 10 alpha lattice design with two replications. The assessed traits were days to 50% heading (DTH), days to 50% maturity (DTM), plant height (PH), total plant biomass (PB), shoot biomass (SB), root biomass (RB), root-to-shoot biomass ratio (RS), grain yield (GY), harvest index (HI), grain carbon content (GCc), shoot carbon content (SCc), root carbon content (RCc), total plant carbon stock (PCs), shoot carbon stock (SCs), root carbon stock (RCs), and root-to-shoot carbon stock ratio (RCs/SCs), and grain carbon stock (GCs). Grain yield exhibited significant ($P < 0.05$) and positive phenotypic and genotypic correlations with HI at $r = 0.79$ and $r = 0.76$, DTH ($r = 0.31$ and $r = 0.13$), PH ($r = 0.27$ and $r = 0.1$), PB ($r = 0.02$ and $r = 0.01$), RB ($r = 0.06$ and $r = 0.05$), respectively. Further, the path analysis revealed significant positive direct effects of SB (0.61) and RB (0.46) on GY. The RS exerted a positive and significant indirect effect (0.23) on GY through SB. The overall association analyses revealed that PB, SB, RB, RS, RCs, and RCs/SCs significantly influenced GY performance and are the principal traits when selecting sorghum genotypes with high carbon storage capacity.

Keywords: Agronomic traits, carbon storage, correlation coefficient, path analysis, sorghum

4.1 Introduction

Sorghum (*Sorghum bicolor* [L.] Moench, 2n=2x=20) is the world's fifth most widely cultivated cereal crop, following wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), maize (*Zea mays* L.), and barley (*Hordeum vulgare* L.). It is an essential crop in the semi-arid tropics, alongside maize and pearl millet (*Pennisetum glaucum* L.). Sorghum is a multipurpose crop used for food, feed, and biofuel production. Sorghum grain is primarily used as a food source supporting more than 500 million people in Africa and Asia (Tonapi *et al.,* 2020). It is grown in at least 86 countries, covering 38 million hectares, and producing approximately 58 million tons of grain annually. The average yield of traditional unimproved sorghum varieties in sub-Saharan African countries is 1.5 t ha^{-1}, far lower than 5.7 t ha^{-1} reported in improved sorghum hybrids (Khalifa and Eltahir, 2023). The sorghum biomass yield varied from 22.2 to 37.5 t ha^{-1} (Bartzialis *et al.,* 2020), making it an ideal crop for forage and biofuel production. Reports indicated that, on average, sorghum can sequester approximately 0.5 to 1.5 Mg C ha^{-1} yr^{-1} (Srinivasarao *et al.,* 2012). Hence, there is a need to breed new-generation sorghum varieties with desirable product profiles, including high yield and biomass production for carbon harvest and to improve soil health.

Sorghum is relatively tolerant to drought, salinity, heat, and flooding (Ejeta and Knoll, 2007). However, severe drought stress that occurs during post-flowering, significantly affects the productivity of sorghum (Tuinstra *et al.,* 1997; Kebede *et al.,* 2001). Further, in sub-Saharan Africa (SSA), sorghum is also affected by poor soil fertility due to degradation caused by high soil carbon (C) loss through intensive production, poor soil management and crop residue removal (Corbeels *et al.,* 2019). Other constraints to sorghum production include bird damage and limited product development and marketing.

Sorghum is a C4 crop belonging to the grass family and is characterized by its high radiation and water use efficiency and biomass production (Sage, 2004). Crop biomasses represent the most substantial carbon source with approximately 45% available for incorporation into the soil via carbon sequestration, thereby enhancing soil health and fertility (Meena *et al.,* 2020). Assessing associations between biomass allocation to plant roots, shoots, and agronomic traits helps forecast genetic gains attainable through breeding and evaluate the impact of plant biomass on ecosystem benefits, including carbon, water, and nutrient cycles, which significantly affect crop productivity (Allard *et al.,* 2013). Approximately 80% of soil carbon originates from the activity and turnover of roots (Pausch and Kuzyakov, 2018). Allocating carbon to roots significantly impacts carbon transfer to soil, since it helps ameliorate climate change and improves the quality of the soil and health (Khan *et al.,* 2021). Breeding sorghum genotypes with increased root biomass allocation has the potential to promote carbon sequestration and contribute to yield stability in SSA (Poffenbarger *et al.,* 2019).

Crops cultivated in dryland or rainfed environments have substantial water fluctuations in the soil profile due to rainfall variability and recurring drought. In response to these fluctuations, plants alter their biomass allocation patterns (Daryanto *et al.,* 2017). The most unique strategy by which plants alter biomass allocation during drought stress is to increase root-to-shoot ratios, which enables plants to allocate more carbohydrates to roots (Zhang *et al.,* 2020). The build-up of carbohydrates in the roots promotes deeper root growth as plants hunt for moisture to sustain growth and development (Lynch *et al.,* 2012). Several research studies have studied root architecture including root size and length of different cultivars of major cereals such as sorghum (Ahmed *et al.,* 2020; Demissie *et al.,* 2023), wheat (El Hassouni *et al.,* 2018), rice (Henry *et al.,* 2011), and maize (Trachsel *et al.,* 2011), and agronomic traits like plant height, panicle length, panicle width, shoot biomass, and root biomass in the identification of contrasting parental genotypes possessing optimal growth, biomass allocation, and grain yield (Cuevas and Prom, 2020; Habyarimana *et al.,* 2020; Enyew *et al.,* 2022).

Multi-trait selection is crucial for developing ideotypes with desirable product profiles in plant breeding programs. Correlation and path coefficient analyses reveal the trend and magnitude of several traits to guide simultaneous selection for enhanced grain yield, its components and carbon storage. Selection based on phenotypic performance may not result in expected genetic advancement due to genotype-by-environment interactions. In this regard, a multi-location and -seasonal assessment of sorghum genotypes is essential to minimize the environmental effects and select the best-performing varieties with stable and high grain yield and carbon storage. Correlation analysis provides information on the degree of relationship between essential traits. Understanding the trend and magnitude of the relationship between yield and yield components is crucial for improving the selection efficiency of complex traits such as grain yield and carbon sequestration (Shimelis, 2006; Gurmu et al. 2018; Kwame et al. 2022). Consequently, opting for agronomic traits with strong correlations with grain yield can increase genetic gains. Path coefficient analysis is used to partition the correlation between yield and component traits into direct and indirect effects, identify the cause-effect relationship, and devise effective selection strategies (Chandra *et al.*, 2020). Genetically diverse sorghum accessions were collected from major producing countries, including Ethiopia, Tanzania, and South Africa, for selection under South African growing conditions. From this gene pool, 50 accessions were sampled based on their high grain yield, biomass, and ethanol production (Mangena *et al.*, 2018). Therefore, the objective of this study was to assess the trend and magnitude of associations between agronomic and carbon storage traits in sorghum to identify grain yield and carbon storage contributing traits.

4.2 Materials and methods

4.2.1 Plant materials

The study used and screened 50 sorghum genotypes under South African growing conditions. A detailed description of genotypes used in this study was presented in Chapter 2, Section 2.2.1.

4.2.2 Study sites

The experiments were conducted at three locations: Ukulinga research farm at the University of KwaZulu-Natal in Pietermaritzburg, Bethlehem in Free State, and Silverton in Pretoria during the 2022/23 growing season. This was described in Chapter 2, Section 2.2.2.

4.2.3 Experimental design and field trial establishment

The experimental design was a 5 x 10 alpha lattice with two replications. This was described in Chapter 2, Section 2.2.3.

4.2.4 Data collection

4.2.4.1 Agronomic traits

Data on nine agronomic traits were collected at all three locations. Details of the data collected were summarised in Chapter 2, Section 2.2.4.1.

4.2.4.2 Carbon stocks determination

From the 50 sorghum genotypes, 25 were selected from the Silverton trials based on their grain yield performance. These selected genotypes were replicated twice and underwent carbon analysis. This was described in Chapter 2, Section 2.2.4.2.

4.2.5 Data analysis

4.2.5.1 Phenotypic and genotypic correlation analyses

The phenotypic and genotypic correlation coefficients for agronomic and carbon storage traits were computed based on the procedure of Dabholkar (1992).

Phenotypic correlation coefficients were estimated as follows:

$$rph = COVph\,(xy) / \sigma ph\,(x) * \sigma ph\,(y)$$

While genotypic correlation coefficients were estimated as follows:

$$rg = COVg\,(xy) / \sigma g\,(x) * \sigma g\,(y)$$

Where,

rph is the phenotypic correlation coefficient; rg is the genotypic correlation coefficient; COV_{ph} (xy) and COV_{g} (xy) are the phenotypic and genotypic covariances of two variables (x and y), respectively; σ_{ph} (x) and σ_{ph} (y) are the phenotypic standard deviations of variables, x and y, respectively; and σ_{g} (x) and σ_{g} (y) are the genotypic standard deviations for variables, x and y, respectively.

The significance of phenotypic and genotypic correlation coefficients were tested using procedures proposed by Singh and Chaudhary (1977).

4.2.5.2 Path coefficient analysis

Path coefficient analysis involved estimating the phenotypic and genotypic direct and indirect effects of independent traits on the dependent trait (grain yield) and carbon storage using methods reported by Dewey and Lu (1959).

$$r_{ij} = P_{ij} + \Sigma r_{ik} P_{kj}$$

Where, r_{ij} is the association between the independent variable (i) and dependent variable (j) as measured by correlation coefficient; P_{ij} is the component of the direct effect of the independent variable (i) on the dependent variable (j) as measured by path coefficient; and $\sum r_{ik} P_{kj}$ is the summation of components of indirect effects of a given independent variable (i) on a given dependent variable (j) via all other independent variables. The residual factor (P^2R) was estimated as described in Dewey and Lu (1959):

$$1 \quad = P^2R + \sum P_{ij}\, r_{ij}$$

Phenotypic and genotypic path coefficient analysis diagrams were developed in R software using structural equation modelling (Team, 2013) to visualize trait associations.

4.3 Results

4.3.1 Performance of sorghum genotypes for agronomic traits

The mean performance of sorghum genotypes for nine agronomic traits evaluated in three locations is shown in Table 4.1. The top ten genotypes, distinguished by their superior grain yield production, are highlighted in bold fonts. The mean DTH and DTM were 80 and 138 days, respectively. Genotype G50 was the earliest to reach 50% heading and maturity at 61 and 114 days, respectively, followed by AS72 (69 and 133 days), AS122 (69 and 139 days), AS141 (69 and 137 days), and AS117 (70 and 139 days). Extended flowering and maturity periods were recorded for genotypes AS136 (92 and 71 days) and AS135 (149 and 151 days). Plant height ranged from 107.06 cm to 223.28 cm. The mean plant height for the assessed genotypes was 156.01 cm. The tallest genotypes with mean plant height greater than 180 cm were AS205, AS391, AS109, AS113, and AS111. The shortest genotype across the testing locations was PAN8816. The mean total plant biomass of the evaluated genotypes was 27.94 g plant^{-1}. The total plant biomass of the genotypes varied between 14.07 g plant^{-1} to 43.75 g plant^{-1}, with genotypes AS122 (43.75 g plant^{-1}), AS391 (41.31 g plant^{-1}), SS27 (38.65 g plant^{-1}), AS203 (38.19 g plant^{-1}), and AS74 (37.83 g plant^{-1}) exhibiting the highest total plant biomass. The mean shoot biomass varied from 6.49 to 24.87 g plant^{-1}, with a grand mean of 16.31 g plant^{-1}. The most productive genotypes with the highest shoot biomass were SS27 with the mean value of 24.87 g plant^{-1}, followed by AS122 (23.90 g plant^{-1}), AS203 (23.45 g plant^{-1}), and AS391 (23.27 g plant^{-1}). The grand mean root biomass for the evaluated genotypes was 11.62 g plant^{-1}, ranging from 5.92 to 21.02 g plant^{-1}. The genotypes that amassed the highest shoot biomass were AS106, AS74, AS72, AS122, and AS152 with mean values of 21.02 g plant^{-1}, 20.31 g plant^{-1}, 19.87 g plant^{-1}, 19.5 g plant^{-1}, 19.46 g plant^{-1}, and 18.04 g plant^{-1}, respectively. The root-to-shoot biomass ratio varied from 0.32 to 3.00. The highest root-to-shoot biomass ratio of 3.00, 2.50, and 1.95 was observed in genotypes AS152, AS106, and 05-POTCH-138, respectively; this indicates that the mentioned genotypes allocated more biomass to their roots than their shoots. The wide genetic variation in grain yield spanned from 2.53 to 25.08 g plant^{-1}, averaging 11.90 g plant^{-1}. Genotypes AS115, AS251, AS134, AS145, and AS130 were the five best-performing genotypes with mean yields of 25.08 g plant^{-1}, 21.83 g plant^{-1}, 21.42 g plant^{-1}, 19.43 g plant^{-1}, 18.50 g plant^{-1}, respectively. The harvest index ranged from 15.34% to 66.66%. Genotypes AS115, AS130, and AS251 exhibited the highest harvest index $\geq$ 60%.

Table 4.1 Mean values for nine agronomic traits of the 50 sorghum genotypes evaluated at three sites in South Africa.

Genotype	**DTH**	**DTM**	**PH**	**PB**	**SB**	**RB**	**RS**	**GY**	**HI**
05-POTCH-138	73	144	121.94	26.25	8.90	17.35	1.95	7.33	45.16
16MZ	72	141	161.83	34.13	22.67	11.46	0.51	12.11	34.82
AS106	81	137	170.44	29.44	8.42	21.02	2.50	8.44	50.06
AS108	77	140	142.56	29.70	12.31	17.39	1.41	8.92	42.01
AS109	97	132	197.22	33.61	19.23	14.38	0.75	13.16	40.63
AS111	84	144	185.67	23.22	13.97	9.25	0.66	2.53	15.34
AS113	82	137	187.56	29.47	17.73	11.75	0.66	12.45	41.26
AS114	74	138	134.33	25.69	19.07	6.61	0.35	13.46	41.37
AS115	**88**	**130**	**182.50**	**21.39**	**12.54**	**8.85**	**0.71**	**25.08**	**66.66**
AS116	77	138	142.11	21.52	14.89	6.63	0.45	4.35	22.62
AS117	70	139	140.61	28.08	20.43	7.65	0.37	11.28	35.57
AS121	84	133	164.33	26.03	18.72	7.31	0.39	8.16	30.34
AS122	69	139	139.67	43.75	23.90	19.85	0.83	8.50	26.24
AS129	84	143	132.28	26.89	17.41	9.49	0.54	3.28	15.87
AS130	**77**	**145**	**163.33**	**25.56**	**11.29**	**14.27**	**1.26**	**18.50**	**62.09**
AS131	81	136	159.83	29.18	20.13	9.05	0.45	7.67	27.59
AS132	**94**	**133**	**171.06**	**25.90**	**15.61**	**10.29**	**0.66**	**16.89**	**51.98**
AS133	77	145	134.43	19.95	12.36	7.58	0.61	14.09	53.26
AS134	**78**	**135**	**162.72**	**35.52**	**20.37**	**15.16**	**0.74**	**21.42**	**51.26**
AS135	71	151	135.53	22.81	14.25	8.56	0.60	5.20	26.73
AS136	92	149	136.67	26.19	16.13	10.06	0.62	13.49	45.55
AS137	76	148	131.47	24.72	16.52	8.20	0.50	11.62	41.30
AS138	**78**	**140**	**146.17**	**28.54**	**15.03**	**13.51**	**0.90**	**17.45**	**53.73**
AS140	80	134	156.44	25.31	19.04	6.27	0.33	4.70	19.80
AS141	69	137	139.75	24.69	18.77	5.92	0.32	13.91	42.56
AS143	76	135	183.33	17.31	10.86	6.45	0.59	8.75	44.61
AS145	**86**	**141**	**157.67**	**32.20**	**21.95**	**10.25**	**0.47**	**19.43**	**46.95**
AS147	80	138	145.22	16.20	7.51	8.69	1.16	4.63	38.13
AS148	86	138	170.94	27.17	13.20	13.97	1.06	15.67	54.27
AS152	78	130	127.56	25.95	6.49	19.46	3.00	9.30	58.90
AS194	79	143	133.56	31.97	21.25	10.72	0.50	15.89	42.78
AS203	**82**	**131**	**174.50**	**38.19**	**23.45**	**14.74**	**0.63**	**16.63**	**41.49**
AS205	89	134	223.28	33.18	20.02	13.16	0.66	12.65	38.72
AS251	**85**	**142**	**131.39**	**27.60**	**14.63**	**12.97**	**0.89**	**21.83**	**59.88**
AS391	84	130	202.83	41.31	23.27	18.04	0.78	12.19	34.38
AS449	80	139	173.67	21.20	12.64	8.56	0.68	14.10	52.73
AS560	78	137	148.33	33.10	20.93	12.17	0.58	12.99	38.29

Table 4.1 Continued

Genotype	DTH	DTM	PH	PB	SB	RB	RS	GY	HI
AS563	**84**	**135**	**173.94**	**33.02**	**21.31**	**11.71**	**0.55**	**16.83**	**44.12**
AS72	69	133	158.44	37.59	17.72	19.87	1.12	13.59	43.40
AS74	76	135	147.22	37.83	17.51	20.31	1.16	14.66	45.56
G50	67	129	163.00	20.18	7.56	12.62	1.67	9.08	54.56
ICS634	72	138	122.06	14.07	8.14	5.93	0.73	9.83	54.71
ICSV92001	79	141	154.28	25.30	15.23	10.06	0.66	12.55	45.16
LP4403	79	137	172.22	34.68	22.08	12.60	0.57	8.09	26.82
MAMOLOKWANE	85	140	164.67	22.28	12.99	9.28	0.71	12.53	49.09
NW5393	73	135	177.11	17.50	11.54	5.96	0.52	13.00	52.97
NW5430	95	133	157.25	22.49	16.08	6.41	0.40	8.79	35.35
PAN8816	75	140	107.06	33.58	17.47	16.12	0.92	3.75	17.68
SS27	**79**	**142**	**135.92**	**38.65**	**24.87**	**13.77**	**0.55**	**17.58**	**41.41**
SV07002	77	139	154.61	26.70	17.20	9.50	0.55	6.61	27.77
Mean	79.52	138.01	156.01	27.94	16.31	11.62	0.8	11.9	41.47
SD	6.82	5	22.74	6.56	4.74	4.26	0.52	5.03	12.09
SE	0.97	0.71	3.22	0.93	0.67	0.6	0.07	0.71	1.71
Skewness	0.5	0.38	0.47	0.23	-0.26	0.6	2.48	0.25	-0.34
kurtosis	0.22	0.09	0.4	-0.25	-0.73	-0.55	7.41	-0.13	-0.3

SD = standard deviation, SE = standard error, DTH = days to 50% heading, DTM = days to 50% maturity, PH = plant height (cm), PB = total plant biomass (g plant^{-1}), SB = shoot biomass (g plant^{-1}), RB = root biomass (g plant^{-1}), RS = root to shoot biomass ratio, GY = grain yield (g plant^{-1}), HI = harvest index (%). The top ten performing genotypes at each location based on GY production are highlighted in bold.

4.3.2 Performance of sorghum genotypes for carbon allocation to roots and shoots

The mean performance of the 25 selected sorghum genotypes for carbon storage is presented in Table 4.2. Based on their high root carbon stock, the top ten genotypes are highlighted in bold fonts. All the carbon content variables ranged between 40 to 45%. The total plant carbon stocks ranged from 7.52 to 24.64 g plant^{-1}, with a mean of 12.65 g plant^{-1}. The genotypes that had the highest carbon sequestration potential with the highest total plant carbon stock were SS27 (24.64 g plant^{-1}), AS122 (18 g plant^{-1}), AS134 (16.48 g plant^{-1}), AS203 (15.55 g plant^{-1}), and AS563 (14.99 g plant^{-1}). The genotypes with lowest carbon sequestration potential were NW5393, AS116, and AS115 with the lowest total plant carbon stock of 7.96 g plant^{-1}, 7.69 g plant^{-1}, and 7.52 g plant^{-1}, respectively. The shoot carbon stock of the selected genotypes had a mean of 7.98 g plant^{-1}, spanning from 3.25 to 19.04 g plant^{-1}. The genotypes that allocated more carbon to the shoots with the highest shoot carbon stock were SS27 (19.04 g plant^{-1}), followed by AS122 (10.42 g plant^{-}

^{1}), ICSV92001 (10.34 g plant^{-1}), and AS563 (10.33 g plant^{-1}). The root carbon stocks ranged from 1.38 g plant^{-1} to 8.37 g plant^{-1}. The mean root carbon stock for the selected genotypes was 4.67 g plant^{-1}. The highest root carbon stock was recorded in genotypes AS108, AS122, AS134, AS251, and AS203 with mean values of 8.87 g plant^{-1}, 7.58 g plant^{-1}, 7.13 g plant^{-1}, 6.49 g plant^{-1}, and 6.40 g plant^{-1}, respectively. Genotypes AS145 and AS116 were among the genotypes with the lowest root carbon stock with 1.74 g plant^{-1} and 1.37 g plant^{-1}, respectively. The root-to-shoot carbon stock ratio varied from 0.18 to 1.56. The genotypes allocated more carbon to their shoots than their roots. The genotypes that allocated more carbon to their roots than their shoots were AS108 and AS115, with the highest ranking of root-to-shoot carbon stock ratio of 1.56 and 1.31, respectively. The grain carbon stocks ranged between 1.04 g plant^{-1} to 12.92 g plant^{-1}, with a grand mean of 5.84 g plant^{-1}. The genotypes AS115 and AS134 ranked first for the highest grain carbon stock, with mean values of 12.92 and 11.38 g plant^{-1}, respectively.

Based on the mean values, a marked difference existed between the test genotypes, enabling correlation and path coefficient analyses to discern the trend and magnitude of trait associations.

Table 4.2 Mean values for carbon storage traits of the 25 selected sorghum genotypes.

Genotype	SCc	RCc	GCc	PCs	SCs	RCs	RCs/SCs	GCs
16MZ	**43.20**	**44.41**	**43.24**	**14.92**	**9.83**	**5.09**	**0.52**	**5.24**
AS108	**43.74**	**44.79**	**43.96**	**14.55**	**5.68**	**8.87**	**1.56**	**3.92**
AS109	43.95	42.53	43.06	11.40	6.47	4.93	0.76	9.40
AS111	**43.91**	**45.40**	**43.64**	**12.86**	**7.40**	**5.46**	**0.74**	**1.04**
AS115	43.44	45.34	43.56	7.52	3.25	4.27	1.31	12.92
AS116	43.78	40.12	43.34	7.69	6.33	1.37	0.22	1.78
AS117	43.51	38.33	43.51	12.23	9.13	3.09	0.34	4.91
AS122	**43.62**	**40.39**	**43.43**	**18.00**	**10.42**	**7.58**	**0.73**	**3.69**
AS130	43.73	40.55	43.40	8.65	4.33	4.32	1.00	9.26
AS131	43.51	41.76	44.06	13.15	8.76	4.39	0.50	3.38
AS132	44.48	38.55	43.16	13.77	9.20	4.57	0.50	9.31
AS134	**44.41**	**39.34**	**43.25**	**16.48**	**9.34**	**7.13**	**0.76**	**11.38**
AS136	44.17	39.15	43.58	9.00	4.97	4.03	0.81	6.57
AS138	44.87	40.72	43.36	8.52	5.35	3.17	0.59	8.04
AS143	44.68	43.67	44.24	8.16	5.41	2.76	0.51	3.87
AS145	45.16	33.69	43.64	11.64	9.90	1.74	0.18	6.68
AS203	**44.56**	**43.41**	**43.47**	**15.55**	**9.15**	**6.40**	**0.70**	**7.23**
AS251	**43.51**	**43.26**	**42.89**	**14.97**	**8.48**	**6.49**	**0.76**	**4.57**
AS563	44.70	40.26	44.49	14.99	10.33	4.66	0.45	5.91
ICSV92001	44.55	39.94	44.65	14.33	10.34	3.99	0.39	3.35
LP4403	**43.73**	**43.76**	**45.31**	**13.89**	**8.38**	**5.51**	**0.66**	**3.35**
MAMOLOKWAN	44.35	41.17	43.44	8.94	5.47	3.47	0.64	5.33
NW5393	45.09	39.40	44.07	7.96	5.20	2.75	0.53	5.73
PAN8816	**44.44**	**37.71**	**43.24**	**12.54**	**7.38**	**5.16**	**0.70**	**1.62**
SS27	**45.48**	**39.92**	**43.27**	**24.64**	**19.04**	**5.61**	**0.29**	**7.61**
Mean	44.18	41.10	43.65	12.65	7.98	4.67	0.65	5.84
SD	0.61	2.76	0.56	3.96	3.14	1.77	0.31	3.02
SE	0.12	0.55	0.11	0.79	0.63	0.35	0.06	0.60
Skewness	0.35	-0.44	1.42	0.96	1.66	0.34	1.25	0.56
Kurtosis	-0.81	0.64	2.10	2.01	5.41	0.24	2.50	-0.07

SD = standard deviation, SE = standard error, SCc = shoot carbon content (%), RCc = root carbon content (%), GCc = grain carbon content (%), PCs = total plant carbon stocks (g plant^{-1}), SCs = shoot carbon stock (g plant^{-1}), RCs = root carbon stock (g plant^{-1}), RCs/SCs = root to shoot carbon stock ratio, GCs = grain carbon stock (g plant^{-1}). The top ten performing genotypes based on RCs are highlighted in bold fonts.

4.3.3 Correlations among agronomic traits

Estimates of phenotypic and genotypic correlation coefficients between pairs of agronomic traits are presented in Table 4.3. Grain yield had positive significant correlations based on phenotypic and genotypic values with DTH at r = 0.306 and r = 0.127, PH (r = 0.267 and r = 0.096), and PB (r = 0.015 and r = 0.01), respectively, while having negative non-significant correlations with DTM (r = -0.08 and r = -0.054), SB (r = -0.033 and r = -0.03), and RS (-0.02 and r = -0.021), respectively. Plant height had a positive significant phenotypic and genotypic correlations with DTH (r = 0.543 and r = 0.232) but had a negative correlation with DTM (r = -0.435 and r = -0.161), respectively. Plant height had a positive and significant genotypic correlation with SB (r = 0.117). Total plant biomass exhibited a positive and significant phenotypic and genotypic correlations with SB (r = 0.771 and r = 0.726), RB (r = 0.72 and r = 0.685), and GY (r = 0.015 and r = 0.01), respectively, and a negative significant correlation with HI (r = -0.388 and r = -0.424). Root biomass positively and significantly correlated with RS (r = 0.672 and r = 0.657) using phenotypic and genotypic correlations. Harvest index exhibited a positive and significant correlation with RS (r = 0.37 and r = 0.383) and GY (r = 0.786 and r = 0.764); and had a negative and significant correlation with SB (r = -0.579 and r = -0.516) using both phenotypic and genotypic correlations, respectively.

Table 4.3 Phenotypic (above diagonal) and genotypic (below diagonal) correlation coefficients among agronomic traits in 50 sorghum genotypes.

Traits	**DTH**	**DTM**	**PH**	**PB**	**SB**	**RB**	**RS**	**GY**	**HI**
DTH	**1**	-0.12	0.543***	0.043	0.074	-0.014	-0.078	0.306*	0.127
DTM	0.052	**1**	-0.435**	-0.152	-0.036	-0.199	-0.196	-0.08	-0.118
PH	0.232***	-0.161**	**1**	0.182	0.174	0.095	-0.073	0.267**	0.114
PB	0.033	-0.049	0.079	**1**	0.771***	0.720***	0.048	0.015***	-0.388**
SB	0.05061	-0.02821	0.117*	0.796***	**1**	0.113	-0.548***	-0.033	-0.579***
RB	-0.007	-0.046	-0.01	0.685***	0.105	**1**	0.672***	0.06*	0.026
RS	-0.049	-0.042	-0.08	0.013	-0.527***	0.657***	**1**	-0.02	0.37***
GY	0.127*	-0.054	0.096*	0.01**	-0.03	0.053***	-0.021	**1**	0.786***
HI	0.052	-0.051	0.026	-0.424***	-0.586***	0.008	0.383***	0.764***	**1**

*, ** and *** denote significance at P < 0.05, P < 0.01, and P < 0.001, respectively. DTH = days to 50% heading, DTM = days to 50% maturity, PH = plant height, PB = total plant biomass, SB = shoot biomass, RB = root biomass, RS = root to shoot biomass ratio, GY = grain yield, HI = harvest index.

4.3.4 Correlations among carbon storage variables

Phenotypic and genotypic correlation coefficients between carbon storage traits are shown in Table 4.4. The total plant carbon stock exhibited positive significant phenotypic and genotypic correlations with SCc (r = 0.185 and r = 0.094), SCs (r = 0.894 and r = 0.91), RCs (r = 0.687 and r = 0.593), and GCs (r = 0.086 and r = 0.077), respectively. Shoot carbon content had a negative significant phenotypic and genotypic correlation with RCc (r = -0.518 and r = -0.303), while exhibiting positive significant correlations with PCs (r = 0.185 and r = 0.094), SCs (r = 0.309 and r = 0.154), and GCs (r = 0.194 and r = 0.113), respectively. Root carbon content exhibited a positive significant phenotypic and genotypic correlation with RCs/SCs (r = 0.371 and r = 0.354). Grain carbon stock had a positive significant correlation with all the assessed traits except for RCc (r = -0.089 and r = -0.125) using both phenotypic and genotypic correlations.

Table 4.4 Phenotypic (above diagonal) and genotypic (below diagonal) correlation coefficients among carbon storage traits in 25 selected sorghum genotypes.

Traits	SCc	RCc	GCc	PCs	SCs	RCs	RCs/SCs	GCs
SCc	**1**	-0.518**	0.09	0.185**	0.309*	-0.106	-0.175	0.194**
RCc	-0.303*	**1**	0.14	-0.109	-0.273	0.211	0.371	-0.089
GCc	0.112	0.102	**1**	-0.086	-0.186	0.117	0.194	0.056***
PCs	0.094**	-0.129	-0.077	**1**	0.894***	0.687***	0.138	0.086*
SCs	0.154**	-0.257	-0.155	0.910***	**1**	0.289	-0.295	0.032**
RCs	-0.077	0.196	0.118	0.593***	0.207	**1**	0.773***	0.132*
RCs/SCs	-0.105	0.354**	0.187	0.021	-0.364**	0.758***	**1**	0.077*
GCs	0.113*	-0.125	0.057***	0.077**	0.03**	0.122*	0.057*	**1**

*, ** and *** denote significance at P < 0.05, P < 0.01, and P < 0.001, respectively. SCc = shoot carbon content, RCc = root carbon content, GCc = grain carbon content, PCs = total plant carbon stocks, SCs = shoot carbon stock, RCs = root carbon stock, RCs/SCs = root to shoot carbon stock ratio, GCs = grain carbon stock.

4.3.5 Path coefficient analysis for agronomic traits

The phenotypic direct and indirect effects of different agronomic traits on grain yield are presented in Table 4.5 and Figure 4.1. Positive and high direct effects on grain yield were recorded by HI (1.136), SB (0.393), RB (0.31), DTH (0.131), and DTM (0.04), while negative and high direct effect was recorded by PH (-0.037), PB (-0.043), and RS (-0.418). DTH had positive and indirect effects of 0.071 and 0.017 on grain yield, which can be selected through PH and HI. Shoot biomass (0.303) and root biomass (0.224) had the highest positive indirect effects on grain yield through PB. Negative indirect effects on grain yield through RB were observed for DTH (-0.004) and DTM (-0.062). Root-to-shoot biomass ratio had negative indirect effects on grain yield through PB (-0.02), RB (-0.281), and HI (-0.155). The residual effect for phenotypic path coefficient analysis was 0.066.

Table 4.5 Phenotypic direct effect (bold diagonal) and indirect effect (off-diagonal) of eight agronomic traits on grain yield of 50 sorghum genotypes.

Trait	DTH	DTM	PH	PB	SB	RB	RS	HI	rph
DTH	**0.131**	-0.005	-0.020	-0.002	0.029	-0.004	0.033	0.144	0.306
DTM	-0.015	**0.040**	0.016	0.007	-0.014	-0.062	0.082	-0.134	-0.080
PH	0.071	-0.017	**-0.037**	-0.008	0.068	0.029	0.031	0.130	0.267
PB	0.006	-0.006	-0.007	**-0.043**	0.303	0.224	-0.020	-0.440	0.015
SB	0.010	-0.001	-0.006	-0.033	**0.393**	0.035	0.229	-0.658	-0.033
RB	-0.002	-0.008	-0.003	-0.031	0.045	**0.310**	-0.281	0.030	0.060
RS	-0.010	-0.008	0.003	-0.002	-0.215	0.209	**-0.418**	0.421	-0.021
HI	0.017	-0.005	-0.004	0.017	-0.228	0.008	-0.155	**1.136**	0.786

DTH = days to 50% heading, DTM = days to 50% maturity, PH = plant height, PB = total plant biomass, SB = shoot biomass, RB = root biomass, RS = root to shoot biomass ratio, HI = harvest index, rph = phenotypic correlation for grain yield.

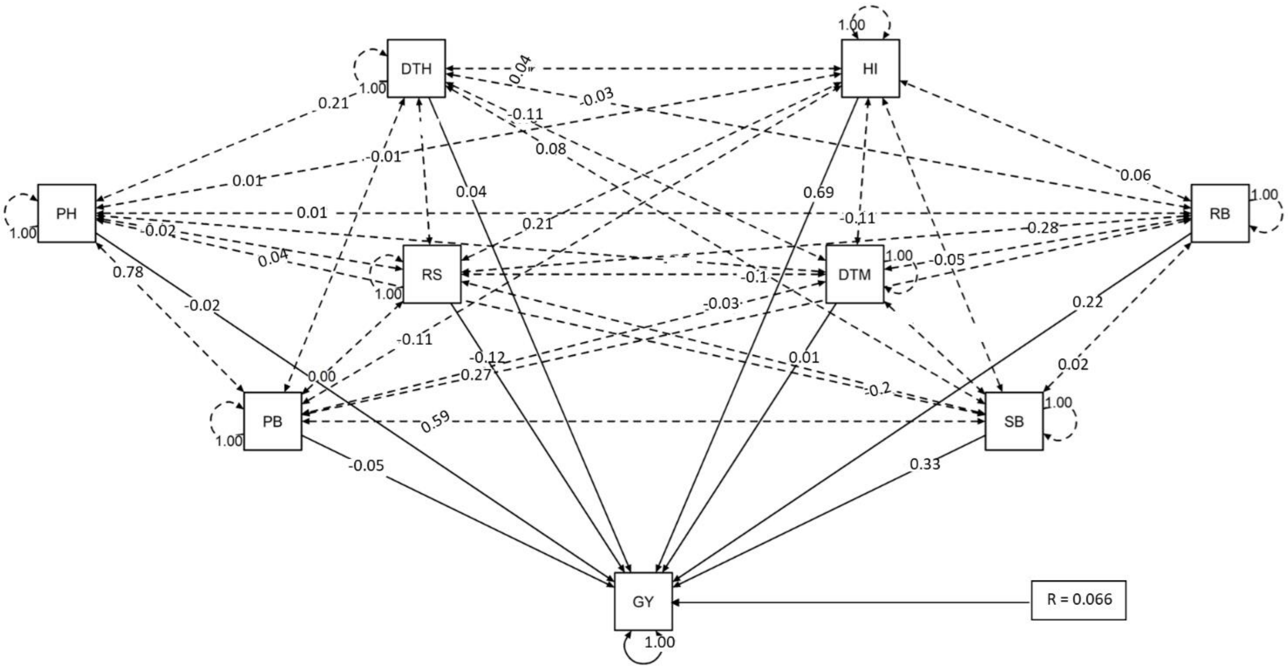

Figure 4.1 Path analysis model displaying causal relationships of eight agronomic traits on grain yield in 50 sorghum genotypes assessed in three locations using phenotypic correlations. DTH = days to 50% heading, DTM = days to 50% maturity, PH = plant height (cm), PB = total plant biomass (g plant^{-1}), SB = shoot biomass (g plant^{-1}), RB = root biomass (g plant$^{-1)}$, RS = root to shoot biomass ratio, HI = harvest index (%).

Path analysis using genotypic correlations showed positive and high direct effects on grain yield by HI (1.159), SB (0.607), RB (0.546), DTH (0.033), and DTM (0.003), while negative and high direct effects were observed by plant height (-0.024), PB (-0.347), and RS (-0.5) (Table 4.6 and Figure 4.2). The positive and high correlation coefficients of HI and DTH with grain yield were due to the positive indirect effects of DTH (0.002), PB (0.147), and RB (0.005) through HI on grain yield. Similarly, DTH and RB had strong positive correlations and positive direct effects on grain yield, indicating they are more significantly related to grain yield at the genotypic level. Total plant biomass (0.374) and RS (0.358) had the most significant positive indirect effects on grain yield through RB. Shoot biomass (-0.277) and root biomass (-0.238) had the highest negative indirect effects on grain yield through PB. Root biomass (-0.328), HI (-0.192), and PB (-0.007) exhibited negative and high indirect effects on grain yield through RS. Harvest index (0.445) exerted the positive and highest indirect effect on grain yield through RS. The residual effect for the genotypic path coefficient analysis was 0.095.

Table 4.6 Genotypic direct effect (bold-faced diagonal values) and indirect effect (off-diagonal) of eight agronomic traits on grain yield of 50 sorghum genotypes.

Traits	**DTH**	**DTM**	**PH**	**PB**	**SB**	**RB**	**RS**	**HI**	**rg**
DTH	**0.0327**	0.0002	-0.0055	-0.0114	0.0307	-0.0039	0.0243	0.0602	0.1273
DTM	0.0017	**0.0030**	0.0038	0.0169	-0.0171	-0.0251	0.0210	-0.0587	-0.0545
PH	0.0076	-0.0005	**-0.0239**	-0.0276	0.0707	-0.0055	0.0450	0.0299	0.0958
PB	0.0011	-0.0001	-0.0019	**-0.3474**	0.4830	0.3738	-0.0066	-0.4915	0.0102
SB	0.0017	-0.0001	-0.0028	-0.2767	**0.6065**	0.0572	0.2635	-0.6790	-0.0297
RB	-0.0002	-0.0001	0.0002	-0.2380	0.0636	**0.5456**	-0.3282	0.0098	0.0526
RS	-0.0016	-0.0001	0.0022	-0.0046	-0.3197	0.3583	**-0.4999**	0.4449	-0.0206
HI	0.0017	-0.0002	-0.0006	0.1473	-0.3554	0.0046	-0.1919	**1.1589**	0.7645

DTH = days to 50% heading, DTM = days to 50% maturity, PH = plant height, PB = total plant biomass, SB = shoot biomass, RB = root biomass, RS = root to shoot biomass ratio, HI = harvest index, rg = genotypic correlation with grain yield.

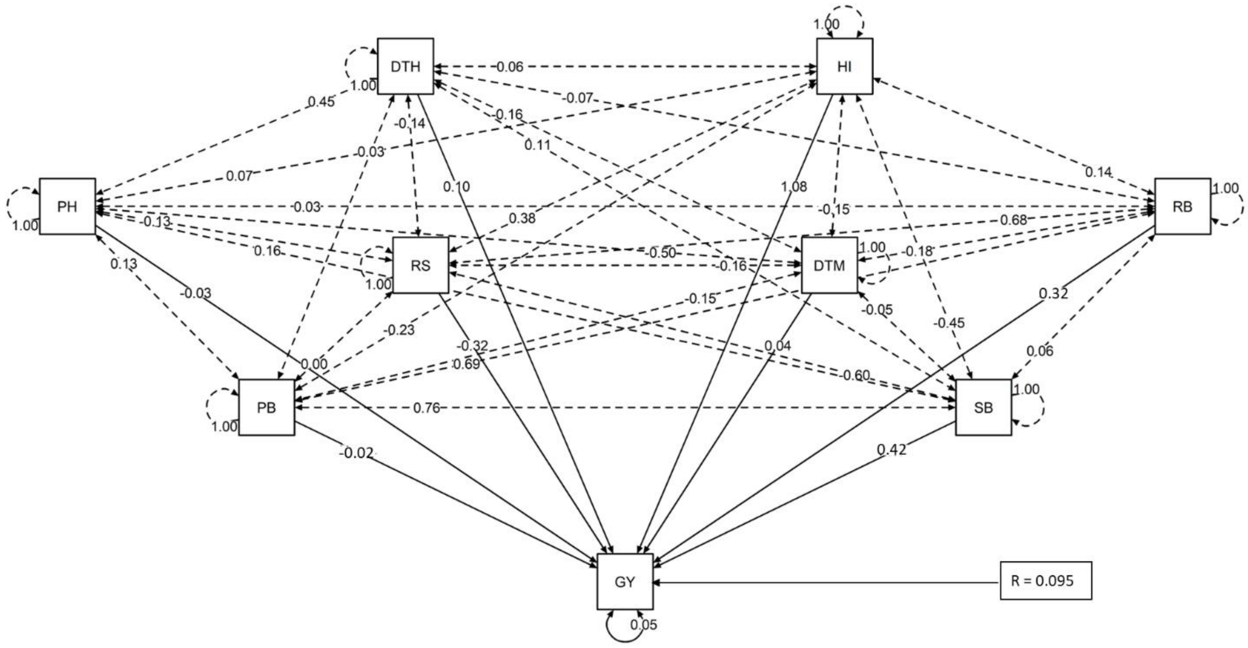

Figure 4.2 Path analysis model displaying causal relationships of eight agronomic traits on grain yield in 50 sorghum genotypes assessed in three locations using genotypic correlations. DTH = days to 50% heading, DTM = days to 50% maturity, PH = plant height (cm), PB = total plant biomass (g plant^{-1}), SB = shoot biomass (g plant^{-1}), RB = root biomass (g plant$^{-1)}$, RS = root to shoot biomass ratio, HI = harvest index (%).

4.4 Discussion

4.4.1 Correlations among agronomic and carbon storage traits

4.4.1.1 Agronomic traits

Grain yield is a complex polygenic trait influenced by various yield component traits. Assessing the association between these traits and their correlation with grain yield helps establish effective selection strategies (Saleh *et al.,* 2020). This study examined the trend and magnitude of relationships between sorghum agronomic and carbon storage traits to identify grain yield and carbon storage contributing traits. The results indicated a strong association among the assessed traits (Tables 3.3 and 3.4). Traits such as DTH ($r = 0.306$ and $r = 0.127$), PH ($r = 0.267$ and $r = 0.096$), and PB ($r = 0.015$ and $r = 0.01$), and HI ($r = 0.79$ and $r = 0.76$) positively and significantly correlated with grain yield (Table 4.3). These are proxy traits to be used for yield improvement in sorghum breeding programs. Plant height had a positive and significant correlation with DTH ($r = 0.54$ and $r = 0.23$) but exhibited a significant and negative correlation with DTM ($r = -0.435$ and $r = -0.161$) using phenotypic and genotypic correlations (Table 4.3). PH positively correlated with SB ($r = 0.17$ and $r = 0.12$) when using both phenotypic and genotypic correlations. Hence, improving PH, PB, and SB will significantly increase grain yield and carbon storage in sorghum genotypes. These results concord with the findings of Amare *et al.* (2015), who reported a positive and significant association between HI and grain yield in sorghum when phenotypic and genotypic correlations were assessed. Traits such as SB, RB, RS, and GY exhibited a positive and significant phenotypic and genotypic correlation with PB, signifying their importance in improving this trait. Similarly, positive and significant corrections were reported among grain yield, spikelet per spike, and fresh biomass in wheat genotypes (Shimelis *et al.,* 2019). Therefore, increasing the SB and RB can improve vegetative growth, resulting in increased plant biomass production and carbon storage, as increased plant biomass results in increased plant leaf area available for photosynthesis and thus increasing production of photo-assimilates needed for grain filling (Araus *et al.,* 2002). Grain yield had positive and significant correlations with all measured traits except for DTM, SB, and RS using both phenotypic and genotypic correlations (Table 4.3). These findings revealed that an increase in the performance of all these traits could increase grain yield and carbon sequestration potential. George-Jaeggli *et al.* (2011) reported that reduced shoot biomass can affect grain yield by decreasing grain size, and variations in carbon allocation in the shoot biomass contribute to trade-offs between structural and non-structural carbohydrate content (Calvino and Messing, 2012; Boatwright *et al.,* 2022). Carbon allocation is also influenced by environmental factors, such as drought stress, which causes plants to transition between vegetative and reproductive periods (Kakani *et al.,* 2011). There is a need to analyse carbon sinks in grain or biomass yields to understand the associations and trade-offs among traits related to carbon allocation. This involves macro-scale phenotyping of traits like grain yield, above-ground biomass and plant height and micro-scale evaluation of compositional traits using techniques such as near-infrared spectroscopy (Murray *et al.,* 2008).

4.4.1.2 Carbon storage traits

Root-to-shoot biomass ratio had a positive and highly significant correlation with RB (r = 0.67 and r = 0.66) but exhibited a negative and significant correlation with SB (r = -0.55 and r = -0.53) using both phenotypic and genotypic correlations (Table 4.3). In agreement with the present results, Mathew *et al.* (2018) and Shamuyarira *et al.* (2022) reported that RS exhibited negative correlations with all agronomic traits except for RB in wheat. According to optimal partitioning theory, plants allocate resources between shoots and roots to promote plant growth (Comas *et al.*, 2013). In this regard, plants may exhibit a specific root-to-shoot ratio, balancing resource limitations and implying genetic plasticity or responsiveness (Shipley and Meziane, 2002). Changes in the root-to-shoot ratio occur during plant growth and development and in response to resource limitations both above and below ground. Thus, it is essential to carefully manage and account for plant size and ontology to draw accurate conclusions about root-to-shoot allocation (Comas *et al.*, 2013). Root biomass had a positive significant correlation with PB (r = 0.72 and r = 0.69) and GY (r = 0.06 and r = 0.05) using the phenotypic and genotypic analysis (Table 4.3), indicating the importance of root system size in crop biomass, grain yield, and carbon storage. The results are consistent with previous studies in sorghum genotypes (Zegada-Lizarazu *et al.*, 2012; Sankarapandian *et al.*, 2013; Demissie *et al.*, 2023). Ehdaie *et al.* (2012) stated that a larger root biomass may benefit plants in water-limited conditions. Wasson *et al.* (2012) indicated that bigger root systems limit the amount of assimilates available for grain production. Some studies have reported that a carbon imbalance between root and shoot biomass results in lower sorghum yields (Prakasham *et al.*, 2014; Datta *et al.*, 2018). Increasing RS alone in pursuit of larger carbon inputs may negatively affect grain production. Therefore, considering the balance between root and shoot biomass is essential to optimize biomass production, grain yield, and carbon sequestration potential. There is a need to resolve the negative relationship between below-ground root characteristics and grain yield. This is feasible if contrasting genotypes with below-ground (e.g. deep and large roots) and above-ground (e.g. tall stature and increased leaf area) characteristics are inter-crossed, followed by a selection of best-recombined individuals (Richards *et al.,* 2010).

4.4.2 Path coefficient analysis of agronomic traits

The present findings found that GY had positive and significant correlations with DTH, PB, RB, and HI using path analysis through phenotypic and genotypic correlations (Tables 3.5 and 3.6). This revealed the actual contribution of agronomic traits to improving grain yield. The study found it crucial to partition the correlation between grain yield and component traits into direct and indirect effects. This allowed to dissect the intricate relationships between grain yield and its component traits to understand whether the influences reflect directly on yield or take some other pathway that ultimately impacts yield and use the most yield-contributing traits for direct and indirect selection. In the current study, positive and high direct effects were exerted on grain yield by DTH (r = 0.13 and r = 0.03), DTM (r = 0.04 and r = 0.003), SB (r = 0.39 and = 0.61), RB (r =

0.31 and r = 0.55), and HI (r = 1.14 and r = 1.16) while negative and high direct effect was exerted by PH (r = -0.04 and r = -0.02), PB (r = -0.04 and r = -0.35), and RS (r = -0.42 and r = 0.5) using both phenotypic and genotypic pathways (Figures 3.1 and 3.2). The slightly high and positive correlation coefficient between grain yield and DTH was due to its high indirect effects through PH and HI on grain yield (Figure 4.1). These results are consistent with Shivaprasad *et al.* (2019), who reported a slightly high positive correlation coefficient between grain yield and days to 50% flowering among mutants of sorghum. The residual factors of the phenotypic (0.066) and genotypic (0.095) path coefficient analysis indicate that traits included in the path analysis explained 93.4% and 90.5% of the total variability in grain yield at phenotypic and genotypic levels, respectively. This suggests the repeatability of selection in the present study.

4.5 Conclusions

The current study examined the associations among agronomic and carbon storage traits in sorghum genotypes. The findings suggest the opportunity for selection and breeding programs to develop improved sorghum cultivars for enhanced yield production and carbon sequestration. The path coefficient analysis revealed that most traits included in the path analysis displayed a positive phenotypic and genotypic direct effect, suggesting direct selection through these traits would be effective for improving grain yield. The study revealed that PB, SB, RB, RS, RCs, and RCs/SCs are the principal traits when selecting sorghum genotypes with high yield and carbon storage capacity.

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Overview of Research Findings and Implications of the Study

Sorghum (*Sorghum bicolor* [L.] Moench, 2n = 2x = 20) is a vital crop with unique nutritional value and adaptation to dryland conditions. Poor soil fertility, which results from soil degradation due to significant soil carbon (C) loss through intensive agricultural practices, inadequate soil management, and crop residue removal, threatens sustainable sorghum production in South Africa, including drought associated with climate change. Developing sorghum genotypes with increased biomass production and carbon sequestration potential is key to enhancing soil health, food security, and crop productivity and subsequently mitigating climate change. Crop genotypes that allocate more biomass to the root system can be selected for stress adaptability in breeding programs. Increased root biomass and size improve access to water deeper in the soil profile during drought stress. Allocating more plant biomass to roots can enhance soil carbon and nutrient levels through root activity and decay, eventually forming soil organic matter. This will increase soil health and prevent soil degradation from farming practices, resulting in more sustainable and resilient sorghum agroecologies. This chapter summarises major research findings and recommendations for the selection of sorghum genotypes with enhanced agronomic- and biomass-related traits, as well as C storage for further breeding and production.

The specific objectives of the study were:

i. to quantify the extent of variation in biomass allocation and C storage between major crops including sorghum for crop production, and C sequestration potential through a meta-analysis.
ii. to assess agronomic performance, biomass production and carbon accumulation in selected sorghum genotypes for production and breeding.
iii. to assess the extent of genetic variability for agronomic and carbon storage traits in selected sorghum genotypes to identify the best candidates for production or breeding.
iv. to assess the trend and magnitude of relationships between agronomic and carbon storage traits in sorghum to identify grain yield and carbon storage contributing traits and to guide future sorghum variety development and release.

Biomass Allocation and Carbon Storage in the Major Cereal Crops: A Meta-analysis

Metadata were gathered and analysed involving 40 global studies reporting on the allocation of plant biomass and C between roots and shoots of sorghum, maize, and wheat cultivars for comparative analysis. Data reporting on the following traits were collected: total plant biomass (PB), shoot biomass (SB), root biomass (RB), root-to-shoot biomass ratio (R/S), total plant carbon content (PCc), shoot carbon content (SCc), root carbon content (RCc), total plant carbon stock (PCs), shoot carbon stock (SCs), root carbon stock (RCs), and root-to-shoot carbon stock ratio (RCs/SCs). Descriptive statistics including standard deviations, coefficient of variation and mean values were used to assess the variability among sorghum, maize, and wheat cultivars for the traits collected from the global studies. Subsequently, boxplots, Pearson's correlation coefficients, and principal component analyses were computed. The major findings were as follows:

- Maize and sorghum had the highest variability for total plant biomass and plant carbon stock accumulation.
- Maize had the highest variability between cultivars for RB, and Wheat had the highest variability between cultivars for RCs.
- Negative associations were observed for variability in PB and SB with mean annual temperature (MAT) and mean annual precipitation (MAP), whereas positive associations for RB with MAT and MAP were observed.
- The principal component revealed that variability in PCs increased with increasing SCs.

Response of Sorghum (*Sorghum bicolor* [L.] Moench) Genotypes for Yield and Yield Components and Organic Carbon Storage in the Shoot and Root systems

Fifty sorghum genotypes were evaluated at three locations (Silverton, Ukulinga, and Bethlehem) in the 2022/23 growing season. The following data was collected: days to 50% heading (DTH), days to (DTM), PB, SB, RB, RS, GY, HI, GCc, SCc, RCc, PCs, SCs, RCs, RCs/SCs, and grain carbon stock (GCs). The mean performances, analysis of variance, rotated component matrix, principal component analysis (PCA) biplots, and a hierarchical cluster analysis were computed. The major findings of the study were as follows:

- Significant genotype x location ($p < 0.05$) interactions were observed for DTH, DTM, PH, PB, SB, RB, RS, and GY.
- Significant differences were also observed among the test genotypes for GCc, PCs, SCs, RCs, RCs/SCs, and GCs.
- Genotypes AS251, SS27, AS134, AS203, and AS563 were identified for their high grain yield, enhanced biomass production, and C sequestration potential.
- The PCA revealed that GY, DTH, PH, PB, SB, RB, RCs, and RCs/SCs are the most distinctive traits among the test genotypes.
- The cluster analysis revealed marked genetic variation among the test genotypes. This variation is beneficial for cultivar development, increased carbon storage, and sustainable sorghum production.

Genetic Variation of Yield, Yield Components, and Carbon Storage in Sorghum (*Sorghum bicolor* [L.] Moench) Genotypes

The above data set collected from the field experiments were used to calculate the variance components, heritability, and genetic advance using the combined and individual location analyses of variance. The main findings of the study were as follows:

- GY, RB, and RCs/SCs contributed to the highest phenotypic and genotypic coefficient of variations.
- Variance components, heritability, and genetic advance estimates revealed high values for PB, RB, GY, HI, RS, GCs, RCs, and RCs/SCs. Hence, these traits were primarily influenced by genetic effects, indicating that selection within the germplasm should rapidly improve these traits.

Association of Agronomic Traits and Carbon Storage on Grain Yield Response in Sorghum

Correlation and path coefficient analyses were computed to assess the trend and magnitude of associations of several traits to guide simultaneous selection for enhanced grain yield, its components and carbon storage. The following agronomic and carbon storage data: DTH, DTM, PH, PB, SB, RB, R/S, SCc, RCc, GCc, PCs, SCs, RCs, RCs/SCs, and GCs were subjected to correlation and path coefficient analyses. The major findings were as follows:

- Significant ($P < 0.05$) positive phenotypic and genotypic correlations were observed between GY and HI, DTH, PH, PB, and RB, respectively.
- Highly significant positive and genotypic correlations were exhibited by RCs with PCs, RCs/SCs, and GCs.
- RB had significant positive phenotypic and genotypic correlations with grain yield (0.06 and 0.05), respectively. This trait exerted high phenotypic (0.39) and genotypic (0.61) direct effects on gain yield.
- When selecting sorghum genotypes for high yield and carbon storage capacity, the key traits to focus on are PB, SB, RB, RS, RCs, and RCs/SCs.

Implications of findings for future breeding of sorghum genotypes with high yielding, biomass production, and carbon storage

- The meta-analysis highlights the need for standardizing study protocols and data reporting to reduce inconsistencies and biases. Future research should integrate traits like leaf area, root architecture, and photosynthetic efficiency to enhance understanding and optimization of biomass and carbon allocation in major cereal crops.
- Selected genotypes AS251, SS27, AS134, AS203, and AS563 with high carbon storage, substantial biomass production, and high grain yield should be advanced using the single seed descent selection method.
- Genotypes AS251, SS27, AS134, AS203, and AS563 should be further evaluated through root phenotyping to improve their root size, length, volume, and surface area for enhanced drought tolerance, nutrient uptake, and carbon sequestration into soils.
- High heritability and genetic advance values for HI, PB, and PCs indicates their importance for direct selection to improve yield and carbon storage in sorghum.