GENETIC DIVERSITY AND GENOME- WIDE ASSOCIATION STUDIES IN SORGHUM (*SORGHUM BICOLOR* (L) MOENCH) GENOTYPES FOR DROUGHT ADAPTATION IN ETHIOPIA

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Adama, Ethiopia

DECLARATION

I hereby declare that this dissertation entitled "**Genetic Diversity and Genome-Wide Association Studies in Sorghum (*Sorghum bicolor* (L) Moench) Genotypes for Drought Adaptation in Ethiopia**" is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials used for this thesis have been duly acknowledged through appropriate citations.

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RECOMMENDATON

I/we, the supervisor(s) of this dissertation, hereby certify that I/we have read and revised the dissertation entitled "**Genetic Diversity and Genome-Wide Association Studies in Sorghum (*Sorghum bicolor* (L) Moench) Genotypes for Drought Adaptation in Ethiopia**" prepared under my/our guidance by **Atnafu Kebede** submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Applied Biology (Specialization in Plant Biotechnology). Therefore, I/we recommend the submission of the dissertation to the department for further review and defense.

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I/we hereby certify that the recommendations and suggestions given by the board of examiners are appropriately incorporated into the final version of the dissertation entitled" **Genetic Diversity and Genome-Wide Association Studies in Sorghum (*Sorghum bicolor* (L) Moench) Genotypes for Drought Adaptation in Ethiopia**" by **Atnafu Kebede**.

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We, the under signed, members of the Board of Examiners of the dissertation open defense by **Atnafu Kebede** have read and evaluated the dissertation entitled "**Genetic Diversity and Genome- Wide Association Studies in Sorghum (*Sorghum bicolor* (L) Moench) Genotypes for Drought Adaptation in Ethiopia**" and examined the candidate during open defense. This is, therefore, to certify that the dissertation is accepted for partial fulfillment of the requirement of the degree of Doctor of Philosophy in Applied Biology (Specialization in Plant Biotechnology).

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Finally, approval and acceptance of the dissertation are contingent upon submission of its final copy to the Office of Postgraduate Studies (OPGS) through the candidate's Department Graduate Council (DGC) and College Graduate Committee (CGC).

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LIST OF ACRONYMS AND ABBREVIATIONS

AMOVA: Analysis of molecular variance

ANOVA: Analysis of variance

ChlC: Chlorophyll content

CSA: Central Statistics Agency

CTAB: Cetyl Trimethyl Ammonium Bromide

EBI: Ethiopian biodiversity institute

ECV: Environmental coefficient of variation

EMMA: Efficient mixed-model association

FASTmrEMMA: Factored spectrally transformed multi-locus random-SNP-effect EMMA

FASTmrMLM: Factored spectrally transformed multi-locus random-SNP-effect MLM

FTSW: Fraction of transpirable soil water

GA: Genetic advance

GAM: Genetic advance as percentage of the mean

GAPIT: Genomic Association and Prediction Integrated Tool

GBS: Genotyping by sequencing

GCV: Genotypic coefficient of variation

GLM: Generalized Linear Models

GWAS: Genome wide association studies

ICRISAT: International Crop Research Institute for Semi-Arid Tropics

ISIS EM-BLASSO: Iterative sure independence screening EM-Bayesian LASSO

ITr : Initial transpiration

LA: Leaf area

LD: Linkage disequilibrium

MaF: Major allele frequency

MAF: Minor allele frequency

MARC: Melkassa Agricultural Research Center

ML-GWAS: Multi-locus genome-wide association study

MLM: Mixed Linear Model

mrMLM.GUI: Multi-locus random-SNP-effect mixed linear model with graphical user interface

mrMLM: Multi-locus random-SNP-effect MLM

NJ: Neighbor Joining

PCA: Principal component analysis

PCoA: Principal coordinate analysis

PCV: Phenotypic coefficient of variation

PEG: Polyethylene glycol

PIC: Polymorphic information content

pKWmEB: Polygenic-background-control-based Kruskal–Wallis test plus empirical Bayes

pLARmEB: Polygenic-background-control-based least angle regression plus empirical Bayes

QTLs: Quantitative trait loci

QTN: Quantitative trait nucleotide

RAPDs: Random amplified polymorphic DNA

RCBDs: Randomized complete block design

RDM: Root dry mass

RFLPs: Restriction fragment length polymorphism

RSA: Root system architecture

RTR: Root to total dry mass ratio

SAS: Statistical Analysis System

SAT: Semi-arid tropical

SDM: Shoot dry mass

ShL: Shoot length

SNPs: Single nucleotide polymorphism

SSRs: Simple sequence repeats

TASSEL: Trait Analysis by Association, Evolution, and Linkage

TBE: Tris-Boric Acid-EDTA

TTr : Total transpiration

TWE: Total water extracted

WS: Water-Stress

WW: Well-Watered

ABSTRACT

Ethiopia, as a center of origin and diversity for sorghum, holds extensive collections that have been identified as valuable sources of genes to address production challenges and enhance yield; however, detailed genetic characterization and effective utilization in breeding programs remain limited. Moreover, sorghum productivity in Ethiopia is below the global average due to various biotic and abiotic constraints, with drought being a major one. This study aimed to evaluate the genetic diversity of the Ethiopian sorghum [Sorghum bicolor (L.) Moench] genotypes and identify genomic regions linked to drought responsive seedling traits using SNPs markers. A total of 158 sorghum genotypes from various regions of Ethiopia were assessed for fifteen phenotypic traits under greenhouse conditions using a high-throughput phenotyping platform. Significant variations ($p < 0.001$) were observed for all traits, with strong and positive correlations among root traits ($r = 0.76$-0.99, $p < 0.001$) and shoot traits ($r = 0.27$-0.98, $p < 0.001$). Cluster analysis based on the 15 traits grouped the genotypes into four genetic groups. Furthermore, 40 genotypes (20 with narrow root angles and 20 with wide root angles) were evaluated for growth and transpiration responses under well-watered (WW) and water-stress (WS) conditions in a greenhouse. Significant differences ($p < 0.01$) in growth and transpiration responses were observed among the genotypes under both WW and WS conditions. Under WS conditions, shoot dry mass, root dry mass, total transpiration, and total water extraction were significantly lower ($p < 0.05$) in wide root-angled genotypes compared to narrow root-angled genotypes, while the mean difference in fraction of transpirable soil water (FTSW) was lower in narrow root-angled genotypes. Conversely, these attributes were higher under WW conditions, except for root growth and total water extraction, which showed non-significant variation ($p > 0.05$) between narrow and wide root angled genotypes. Genotypic data were used in a genome-wide association study (GWAS) to identify significant single nucleotide polymorphism (SNP) markers associated with 13 phenotypic traits. The GWAS, conducted using the "mrMLM.GUI" R package, employed 127,804 high-quality SNP markers. A total of 49 stable quantitative trait nucleotides were consistently identified by multiple models. Candidate genes associated with root and shoot traits included ALUMINUM-ACTIVATED MALATE TRANSPORTER (ALMT), F-BOX domain, FAR1, RING ZINC FINGER, CHLORIDE CHANNEL (CLC), WRKY domain, and DEAD-box RNA helicase. Population structure analysis categorized the genotypes into five sub-populations. The genetic diversity of the sorghum collection was also assessed, with gene diversity (He) values ranging from 0.30 to 0.63 (average 0.51) and observed heterozygosity (Ho) values from 0.02 to 0.81 (average 0.13). PIC values ranged from 0.06 to 0.50 (average 0.32), and genetic distance varied from 0.13 to 0.95 (average 0.69), indicating high genetic distance among genotypes.Cluster and PCoA analyses showed clustering irrespective of regions of origin. AMOVA revealed 99% genetic variation within structured populations and regions. Fixation index (Fst) values (0.006 and 0.008) indicated low genetic differentiation among regional and structure sorghum populations. In conclusion, this study highlights the genetic basis of seedling root and shoot traits, supporting breeding strategies for drought-resilient sorghum. Further investigation of genetic mechanisms and candidate genes can advance the development of cultivars adapted to drought conditions.

Key words: *Drought, genetic diversity, GWAS, population structure, single nucleotide polymorphism*

CHAPTER ONE

1. INTRODUCTION

1.1. Background of the study

Sorghum [*Sorghum bicolor* (L.) Moench] is an annual C4 plant and largely self-pollinated species. Sorghum was named because of its height from the Latin word "*surgo*" which means 'arise' (Karper & Quinby, 1947). Sorghum belongs to the tribe of *Andropogoneae* and the family of *Poaceae*. It is particularly adapted to drought prone areas: hot, semi-arid tropical (SAT) regions of the world (Doggett, 1988). *S. bicolor* encompasses all cultivated sorghum varieties, as well as semi-wild and wild plants (Mutegi et al., 2011). Both the cultivated and wild types are diploid (2n = 2x = 20) and have a genome size of approximately 730 Mbp (Paterson et al., 2009). The cultivated sorghum has been categorized into five races: *Durra, Bicolor, Caudatum, Kafir, and Guinea*, as well as ten intermediates (Harlan & de Wet, 1972). All of these races, except for Kaffir, are present in Ethiopia (Teshome et al., 1997; Girma et al., 2020).

Sorghum with annual total production of 57,298 is the fifth most important cereal crop after maize (1,241,558), rice (799,999), wheat (798,975), and barley (145,759) in terms of production. Sorghum is produced worldwide on 39,851,368 hectares with an average yield of 1,437.8 kg/ha which is much lower than that of maize (5,962.3kg/ha), rice (4,751.8 kg/ha), and wheat (3,625 kg/ha) (FAOSTAT, 2023). Ethiopia is the fifth top sorghum producing country in the world following the United States of America, Nigeria, Mexico, and Brazil (FAOSTAT, 2023). The most important producers are the United States of America (8,071), Nigeria (6,402), Mexico (4,816), Brazil (4,498) , Ethiopia (4,010), India (3,814), Sudan (3,055), China (2,969), Australia (2,326), Burkina Faso (1,772) and Argentina (1,610) (FAOSTAT, 2023). Figures in parenthesis indicate production in thousand tonnes. It is a staple food crop for over half a billion people in developing countries, mostly in arid and semi-arid regions where drought stress is a major limiting factor (Abreha et al., 2022).

Ethiopia, as the native land of sorghum, showcases remarkable diversity encompassing both domesticated and wild relatives, positioning its status as the center of origin and diversity for the crop (Vavilov, 1951; Adugna, 2007; Mekbib et al., 2009). This multipurpose crop holds

considerable importance in the Ethiopian diet, being a primary component in traditional foods like "*injera*" and "*porridge*," as well as beverages such as "*tella*," "*borde*," and "*areki*." Beyond its dietary uses, sorghum biomass is vital for animal feed, construction, biofuel production, and fencing for rural farmers, often considered as important as the grain itself, indicating a growing demand for more sorghum in the future (Taye et al., 2016; Derese, Shimelis, Laing, et al., 2018; Amsalu & Endashaw, 2000; Ananda et al., 2020). In dry lowland areas of Ethiopia, sorghum is a significant crop for food and feed, as it can withstand drought better than other crops (Tirfessa et al., 2023).

Despite its resilience, sorghum primarily experiences yield loss due to drought stress during both pre- and post-anthesis stages, more than from other abiotic and biotic factors like shoot fly, stem borer, and striga (Wortmann et al., 2009; Amelework et al., 2015). Drought stress during the vegetative and reproductive stages results in a decrease of over 36% and 55% in grain yield, respectively (Assefa et al., 2010). Drought stress during the booting and flowering stages can cause 87% reduction in grain yield. However, such a significant yield loss only occurs when drought stress at the vegetative stage is much longer and more severe (Craufurd & Peacock, 1993).

Ethiopia is a key center for the origin and diversity of sorghum, contributing significantly to global genetic advancements in this crop (Chala, 2018; Mola, 2021). The country's germplasm contains a wide range of genetic variations, though the genetic structure of many traits remains poorly understood (Girma et al., 2019; Olani et al., 2021; Siraw Belay, 2022). Ethiopia has provided valuable germplasm collections to the International Crop Research Institute for Semi-Arid Tropics (ICRISAT) (Prasada Rao et al., 1989; Upadhyaya et al., 2008), highlighting its rich source of sorghum landraces, which are crucial for modern plant breeding (Harlan, 1992). These collections have been identified as sources of valuable genes for various agronomic traits (Cuevas et al., 2017; Girma et al., 2020; Wondimu et al., 2023) benefiting global agriculture through traits like drought tolerance, high lysine content (Singh & Axtell, 1973), cold tolerance (Singh, 1985), and resistance to diseases and insects (Doggett, 1988). Characterization of germplasm collections is essential for crop breeding, aiding in the analysis of genetic variability, identification of diverse parental combinations, introgression of desirable genes, and facilitate classification of genotypes and identification of core subsets for specific breeding purposes

(Upahyaya, 2015; Bhandari et al., 2017; Lema, 2018; Zhang et al., 2021; Paczos-Grzęda et al., 2023).

However, evaluation using phenotypic traits alone does not reliably reflect the real genetic variation, as it is influenced by the environment and genotype by environment (GxE) interaction effects. Whereas, molecular tools offer plant breeders the potential of making genetic progress more precisely and more rapidly (Nadeem et al., 2018). In recent years, the number of molecular tools available for application in this area has increased dramatically (Hasan et al., 2021). Various DNA markers have been utilized to assess genetic diversity in sorghum, which include restriction fragment length polymorphism (RFLPs) (Ahnert et al., 1996), random amplified polymorphic DNA (RAPDs) (Iqbal et al., 2010), Amplified fragment length polymorphism (AFLP) (Medraoui et al., 2024), simple sequence repeats (SSRs) (Emebet, 2021; Nemera et al., 2022; Mamo et al., 2023; Genet et al., 2024) and single nucleotide polymorphism (SNP) markers (Mengistu et al., 2020; Enyew et al., 2022; Yahaya et al., 2023; Kasule et al., 2024). With the advances in marker technology, single nucleotide polymorphism (SNP) markers have become the choice due to their low cost per data point, high genomic abundance, locus-specificity, co-dominance, the potential for high throughput analysis and lower genotyping error rates (Raza et al., 2016; Mengesha et al., 2017).

Currently, SNP markers are widely acknowledged for their usefulness in assessing population structure and genome-wide association studies in sorghum genotypes (Menamo et al., 2023; Wondimu et al., 2023; Enyew et al., 2025).Various Multi-locus GWAS (ML-GWAS) methodologies, such as Factored spectrally transformed multi-locus random-SNP-effect EMMA (FASTmrEMMA), Iterative sure independence screening EM-Bayesian LASSO (ISIS EM-BLASSO), Multi-locus random-SNP-effect MLM (mrMLM), Factored spectrally transformed multi-locus random-SNP-effect MLM (FASTmrMLM), Polygenic-background-control-based least angle regression plus empirical Bayes (pLARmEB), and Polygenic-background-control-based Kruskal–Wallis test plus empirical Bayes (pKWmEB) have been developed as effective tools for genome-wide association analysis (Chourasia, 2017; Cui et al., 2018). However, the application of ML-GWAS in sorghum research has been limited (Hu et al., 2021; Wondimu et al., 2023). Therefore, conducting association mapping studies using high-density markers (SNPs) and multi- locus-GWAS method provides a unique opportunity to

understand the genetic basis of important traits and accurately identify quantitative trait nucleotides (QTNs) associated with them (Wen et al., 2018; Zhang et al., 2019; Abreha et al., 2022; Wondimu et al., 2023). Therefore, this study aimed to assess sorghum genotypes for seedling shoot and root traits, investigate the genetic control of these traits using different multi-locus genome-wide association study approaches, and also intended to evaluate the population structure and genetic diversity of sorghum genotypes collected from various regions of Ethiopia.

1.2. Statement of the problem

Climate change is widely considered to be a major threat to future food security (Van Oort & Zwart, 2018). There is indication that climate change may lead to a change in the frequency and severity of drought events. For instance, by 2050, water shortages are expected to affect 67% of the world's population (Wagaw, 2019). Drought stress, the foremost barrier to agricultural productivity, impacts one-third of the globe's cultivable land and is anticipated to exacerbate due to continuing climate changes.Consequently, cultivating crops with enhanced drought tolerance will be essential to sustain land productivity in the future, at least partially. Extensive research has been conducted on drought tolerance thus far, yet the outcomes have not matched the demand for agricultural production. This discrepancy may be attributed to the high unpredictability and intricate nature of drought stress impacts (Kholová, 2010). Moreover, as water resources for agricultural uses become more limiting (Biswas et al., 2025), the development of drought tolerant lines will become increasingly important.

Therefore, there is an urgent need to accelerate plant breeding and mining of novel traits for increased yield potential and improving drought tolerance of food crops to secure the food availability and meet the future demand for agricultural production (Abdelrahman et al., 2017). Amelework et al. (2015) reviewed that drought is one of the most important factors that affect crop production worldwide. Many sorghum-growing areas in Ethiopia are subject to recurring droughts due to rainfall scarcity and/or unequal distribution. Rain comes late or ends early in sorghum producing regions, resulting in a crop growing time that is too short, leading to crop failures (Yali and Begna, 2022). The yield potential of the crop is significantly limited due to drought stress within the tropics and subtropics necessitating sorghum breeding for drought

tolerance (Derese et al., 2018; Ray et al., 2018; Wagaw, 2019; Assefa et al., 2020; Abreha et al.,2022).

Seedling traits for drought tolerance like nodal root angle (Singh et al., 2011; Mace et al., 2012; Tebeje et al., 2020; Menamo et al., 2023), root fresh weight and root dry weight (Mace et al., 2012; Tebeje et al., 2020), root length and root to shoot ratio (Rajkumar et al., 2013; Tebeje et al., 2020) have been identified and several QTLs associated with these traits were mapped in sorghum. However, most of these QTLs were identified using bi-parental linkage mapping and using low density markers (Abreha et al., 2022). Hence, population characterization and association mapping studies using high density markers such as single nucleotide polymorphisms (SNPs) create an unprecedented opportunity to dissect the genetic basis of complex traits and accurately identify QTLs associated with the traits (Beissinger et al., 2013; Sa et al., 2021). Limited research has focused on seedling-stage traits such as root angle under drought conditions, despite their significance in breeding for drought tolerance (Mace et al., 2012; Ali et al., 2015; Girma et al., 2020; Menamo et al., 2023).

Despite, Ethiopia's huge sorghum germplasm collection (11,353) (Tesfaye, 2017) and rich sorghum diversity characterization of sorghum landraces for drought adaptation traits at the seedling stage and identification of associated genomic regions or QTL/QTNs using SNP marker systems remain limited (Besufekad & Bantte, 2013; Adugna, 2014; Chala, 2018; Girma et al., 2019; Tebeje et al., 2020). Hence, knowledge of genetic variability for shoot and root traits at seedling stage can be the key component in selecting better parents that withstand drought for the future breeding program. Moreover, as water resources for agricultural uses become more limiting (Biswas et al., 2025), the development of drought tolerant lines will become increasingly important. Ingeneral, despite the genetic richness of Ethiopian landraces, previous studies have largely relied on single-locus GWAS models, with limited attention given to early-stage traits under drought conditions. This study integrates multi-locus GWAS with seedling trait phenotyping in Ethiopian landraces offering a novel approach to identify drought-resilience traits not well explored before. Therefore, breeders must effectively harness the potential of Ethiopian sorghum genotypes as valuable sources of breeding material. In view of the above-mentioned reasons, the present research was proposed to achieve the following objectives:

1.3. Objectives

General objectives

- To assess the genetic variation of Ethiopian sorghum [*Sorghum bicolor* (L.) Moench] genotypes and identify SNP markers linked to drought responsive traits at seedling stage

Specific objectives

- To evaluate Ethiopian sorghum [*Sorghum bicolor* (L.) Moench] genotypes for phenotypic traits at seedling stage.
- To evaluate the variation in growth and transpiration responses of selected sorghum genotypes under progressive soil drying at their vegetative growth stage
- To identify genomic regions associated with phenotypic traits in Ethiopian sorghum (*Sorghum bicolor* (L.) Moench) genotypes using single nucleotide polymorphism (SNP) markers
- To assess the genetic diversity of Ethiopian sorghum [*Sorghum bicolor* (L.) Moench] genotypes using single nucleotide polymorphisms (SNPs) markers.

1.4. Significance of the study

Sorghum is a native crop of Ethiopia, with a significant amount of diversity present in the country (Adugna, 2007). This diversity includes both domesticated and wild relatives, indicating Ethiopia as the center of origin and diversity (Vavilov, 1951; Mekbib et al., 2009).

Knowledge from this research helps to have a deeper comprehension of the genetic foundation of drought adaptation in sorghum landraces and provides breeders and geneticists with the essential for identifying genotypes that are tolerant to drought or in selecting breeding materials to be utilized in sorghum breeding programs, from the center of origin. Besides, might be used to the other related Gramineae crops.

Furthermore, the information generated from this study will be of great importance in the selection of appropriate parents for sorghum improvement and in managing the existing genetic diversity in sorghum collections by avoiding redundancy in germplasm conservation in the Ethiopian Biodiversity institute (EBI).

1.5. Scope of the study

The scope of the study is to characterize sorghum genotypes by employing phenotypic traits and molecular markers, specifically Single Nucleotide Polymorphisms (SNPs). This dual approach aims to elucidate the genetic diversity and relationships among various sorghum genotypes. Additionally, the study seeks to identify associations between single nucleotide polymorphisms (SNPs) and the root and shoot traits at the seedling stage of sorghum genotypes. This research is specifically confined to the investigation of seedling traits, providing a focused analysis on this critical developmental stage.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Sorghum origin and domestication

The origin and domestication of sorghum are thought to have occurred in northeastern Africa or near the Egyptian-Sudanese border around 5,000–8,000 years ago (Mann et al., 1983). On the other hand, some reports indicate that sorghum made its way from Ethiopia to Egypt around 300 BC during the period when cereal crops were being domesticated (House, 1983). The wide variety of both wild and cultivated sorghum found in East Central Africa suggests that domesticated sorghum likely originated in this region, in or around Ethiopia or Sudan (Fetene et al., 2011; Olembo et al., 2010). The Indian subcontinent is considered to be the secondary center of origin for sorghum, with archaeological evidence of early cereal cultivation at a site in western Rojdi (Saurashtra) dating back to about 4,500 years (Vavilov & Dorofeev, 1992; Damania, 2002).

2.2. Sorghum taxonomy and classification

In 1753, Linnaeus gave the genus Sorghum its initial classification as Holcus, which included three species: Holcus sorghum, Holcus saccaratus, and Holcus tricolour. According to Venkateswaran et al. (2019), Moench distinguished sorghum from the genus Holcus in 1794. Domesticated sorghum was officially acknowledged as Sorghum bicolor (L.) Moench after these classifications Moench (Venkateswaran et al., 2014; Hariprasanna & Patil, 2015). As to Hariprasanna & Patil (2015), sorghum is classified within the kingdom Plantae, division Magnoliophyta, class Liliopsida, order Cyperales, family Poaceae, tribe Andropogoneae, subtribe Sorghinae, and genus Sorghum.

Sorghum is a diploid plant (2n = 2x = 20) that primarily undergoes self-pollination. Sorghum is a C4 plant known for its superior photosynthetic capacity and enhanced resilience to environmental stressors. This crop consists of 25 distinct species, which are classified into five taxonomic subgroups: Chaetosorghum, Eu-Sorghum, Para-Sorghum, Heterosorghum, and Stiposorghum. *Sorghum bicolor* subsp. *bicolor*, *Sorghum halepense* (Johnson's grass), a weedy species and *Sorghum arundinaceum*, the recognized ancestor of *Sorghum bicolor* is has been discovered in *Eu-Sorghum* subgenera (Ananda et al., 2020).

Five basic races *bicolor, durra, guinea, caudatum, and kafir and ten intermediate races guinea-bicolor, caudatum-bicolor, durra-bicolor, kafir-bicolor, guinea-caudatum, guinea-durra, guinea-kafir, durra-caudatum, durra-kafir, and kafir-caudatum* are found in cultivated sorghum (Harlan and de Wet, 1972). These races' panicle and spikelet characteristics, which are preserved by geographical and ethnological isolation, give them a distinctive morphology. The traits of both parents are mixed together in the ten intermediate races. Wherever these produced varieties are sympatric, they also hybridize with their wild counterparts (within the same geographic area) resulting in subsp. *drummondii*. Agronomically, cultivated sorghums can also be categorized as Sudan grass, broomcorn, grain sorghum, sweet sorghum, etc. (Venkateswaran et al., 2019).

Race *kafir*, a Bantu race, is predominantly cultivated in Southern Africa (Kimber et al., 2013). *Caudatum* is the race found across eastern Nigeria, Chad, Sudan, and Uganda, but is less common in Ethiopia. Race *guinea*, morphologically distinct from other races, originates primarily from West Africa and likely evolved there (Burgarella et al., 2021). Race *durra* is morphologically distinctive, predominantly found in the Near East, and is widely grown in India and Pakistan (Morris et al., 2013). Race *bicolor* closely resembles wild forms and is considered the most primitive; it is now widely distributed and likely the progenitor of other races (Venkateswaran et al., 2019). In Ethiopia, four out of the five basic sorghum races are present, with *Kafir* being the exception (Teshome et al., 1997).

2.3. Biology of sorghum

The progression of sorghum's growth and development is categorized into three main phases: GS1, covering the time from sowing to the beginning of panicle formation; GS2, spanning from the initiation of the panicle to the blooming stage; and GS3, culminating in physiological maturity (Eastin, 1972). Conversely, Vanderlip & Reeves (1972) proposed a 0-9 numerical scale to describe the growth stages of sorghum, with specific designations for each stage: 0 (Emergence), 1 (3-leaf stage), 2 (5-leaf stage), 3 (Panicle initiation), 4 (Flag leaf), 5 (Boot stage), 6 (50% flowering), 7 (Soft dough stage), 8 (Hard dough stage), and 9 (Physiological maturity). The temporal duration of these developmental stages is known to exhibit variability influenced by factors such as cultivar characteristics, adaptation to particular environmental

conditions, prevailing climatic patterns, the timing of planting, and ambient temperature (Hariprasanna & Patil, 2015).

The primary mode of propagation for *Sorghum bicolor* is through seeds produced by self-pollination (Aruna & Audilakshmi, 2008). Nevertheless, outcrossing occurs at rates ranging from below 2% to 10% (Sobetski, 2015). Wind is a crucial vector for achieving substantial outcrossing, with insects also potentially contributing to cross-pollination (Schmidt & Bothma, 2005). Sorghum seed development occurs in distinct stages, beginning with fertilization, where pollen grains fertilize the ovules within the panicle. This leads to embryo formation, initiating the growth of the seed. The next phase is grain filling, where starch, proteins, and other nutrients accumulate, determining the final seed size and quality. Finally, the seed reaches physiological maturity, marked by the cessation of nutrient flow and the hardening of the seed coat, preparing it for harvest (Espinoza & Kelley, 2004).

2.4. Global sorghum production

Sorghum is a crucial economic and staple food crop for more than 500 million people in developing nations, particularly in areas with scarce water resources and frequent droughts, which underscores its resilience and ability to thrive in difficult conditions (Abreha et al., 2022; ICRISAT, 2023). It is grown worldwide on 39,851,368 hectares, yielding 57.3 million tonnes in the 2023 season (FAOSTAT, 2023). Over the years, global sorghum production has shown an upward trend (Figure 2.1). Presently, sorghum ranks as the fifth most significant cereal crop, following maize, rice, wheat, and barley. The leading producer of sorghum is the United States of America, followed by Nigeria and Mexico. Other notable producers include Brazil, Ethiopia, India, Sudan, China, Australia, Burkina Faso, and Argentina. The global average yield of sorghum in 2023 was only 1,437.8 kg/ha, which is much lower than that of maize (5,962.3kg/ha), rice (4,751.8 kg/ha), and wheat (3,625 kg/ha) (FAOSTAT, 2023).

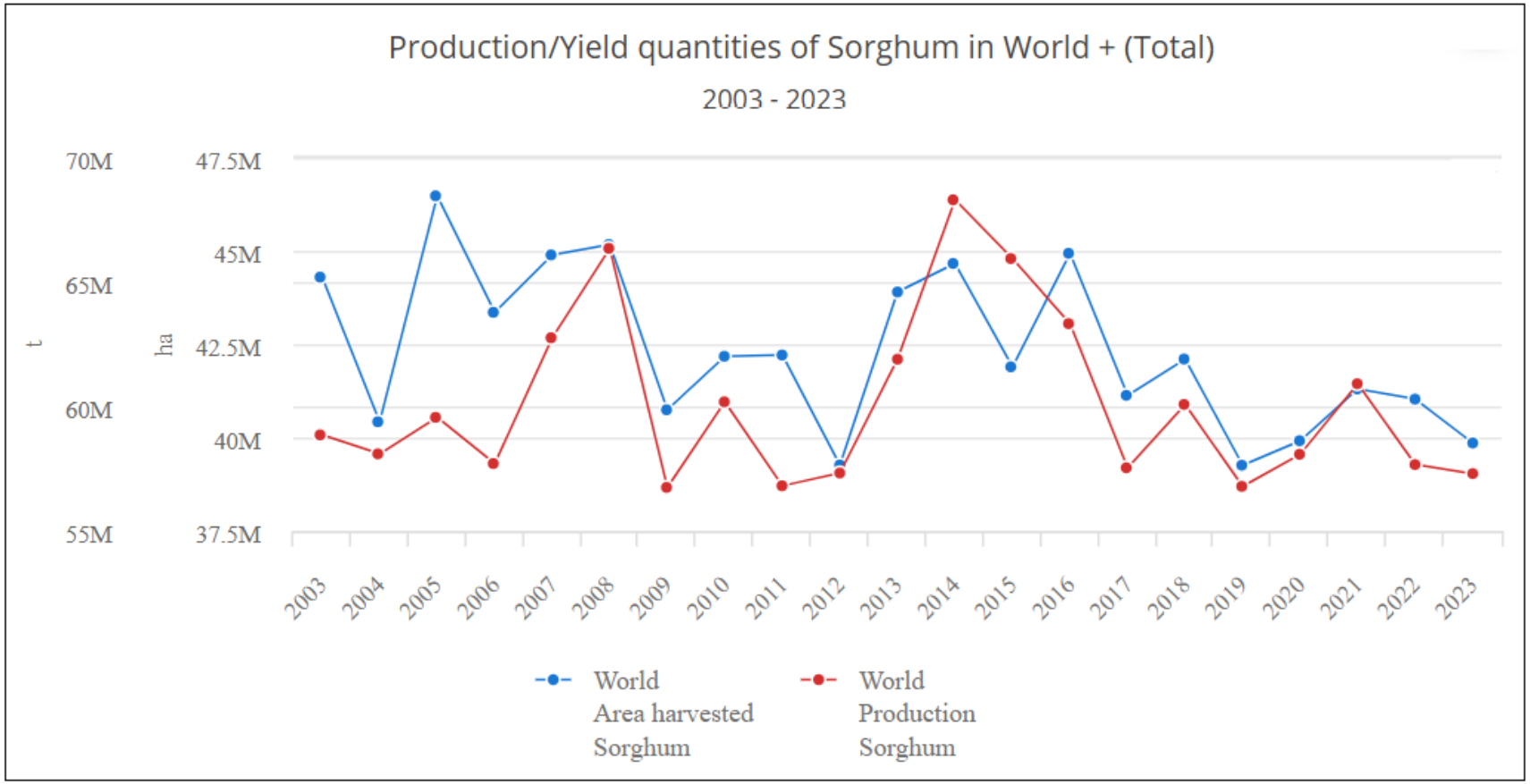


Figure 2.1. Global harvested area and production/yield quantities of sorghum (measured in million tonnes and hectares)

Source: FAOSTAT, March 17, 2025

2.5. Sorghum cultivation and production in Ethiopia

As one of the earliest countries to cultivate sorghum, Ethiopia showcases a wealth of genetic diversity, evident in the various morphological types cultivated across the nation and its extensive agro-ecological spectrum (Doggett, 1988). According to CSA (2021) data, In Ethiopia, cereals occupy about 81.19% (10,538,341.91 hectares) of the grain cultivation area, with sorghum constituting 12.94% (1,679,277.06 hectares) of this expanse. Regarding output, cereals contribute 88.36% (around 30,205,426.058 tonnes) to the total grain production. Specifically, sorghum accounts for 13.22% (4,517,350.218 tonnes) of the grain production. Thus, sorghum is the 3rd most important cereal crop after maize (*Zea mays*) and teff (*Eragrostis tef*); both in terms of planted area and production size (CSA, 2021; USDA, 2024). Oromia, Amhara, Tigray, and SEPR are the major sorghum-producing regions in Ethiopia, accounting for 42%, 34%, 13%, and 5% of the total production, respectively (USDA, 2024). It is grown in 13 out of 18 main agro-ecological zones and in 41 out of 49 sub-agro-ecological zones (Tirfessa, 2019), including intermediate, wet lowland, highland, and dry lowland areas (Gorfu & Ahmed, 2012; Temeche et al., 2021). The highland regions of Ethiopia, which have high altitudes (> 1900 m a.s.l.), significant rainfall (>1000mm), and low temperatures, only account for 10% of the total sorghum production in the country (Seyoum et al., 2020). In Ethiopia, sorghum production has shown a steady decline over the years (Figure 2.2).

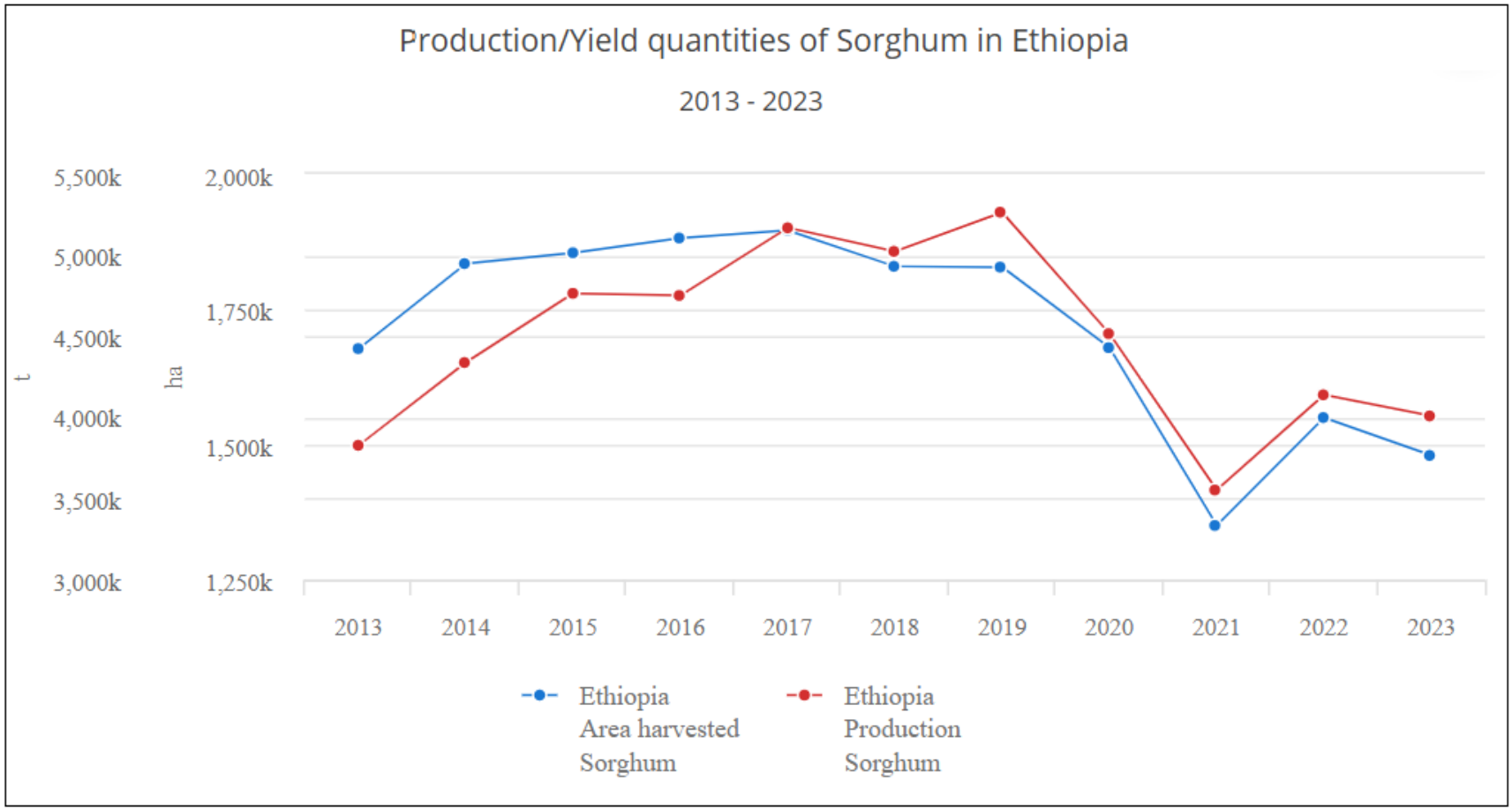


Figure 2.2. Area harvested and production/yield quantities (in million tonnes and hectares) for sorghum in Ethiopia.

Source: FAOSTAT, March 17, 2025

2.6. Sorghum production constraints

Sorghum production faces a myriad of challenges that hinder optimal productivity. Drought poses a significant challenge to sorghum production, emerging as the primary cause of yield decline in agricultural crops, especially in water-scarce regions (Wortmann et al., 2009; Amelework et al., 2016). Drought can adversely impact sorghum at any developmental stage, from seedling establishment to pre-flowering, flowering, and grain filling, with the most severe consequences for crop yields (Verma et al., 2018). Seedling-stage drought significantly hinders plant establishment (Assefa et al., 2010), while drought during pre-flowering, flowering, or grain filling periods can lead to substantial yield reductions or even complete crop failure (Blum, 2011). Drought tolerance research has focused on both pre- and post-flowering stress, revealing diverse genotypic responses mediated by numerous genetic pathways (Goldsbrough, 2000). In drought prone areas, farmers often resort to using drought-tolerant, low-yielding landraces, which worsens the problem (Amelework et al., 2016).

Insect pests such as shoot flies, midges, stem borers, and head bugs are severe insect pests, causing damage of up to 85% in some cases (Okosun et al., 2021). Additionally, parasitic weeds such as Striga heavily infest sorghum fields, further limiting crop yields (Gebretsadik et al., 2014; Olkeba et al., 2021). In Ethiopia, approximately 30% of low altitude (1500 m.a.sl) areas where sorghum is the primary staple crop are infested with striga, resulting in yield losses ranging from 50% to 100% (Bejiga, 2019; Teferi, 2019; Teressa, 2019).

The poor soil fertility (nutrient deficiency) in many sorghum-growing regions also contributes to reduced productivity (Habte et al., 2020). Socio-economic constraints also play a significant role in limiting sorghum production in Ethiopia (Degefa et al., 2023). Farmers face challenges such as limited access to improved seeds, fertilizers, and other agricultural inputs (Amelework et al., 2016; Degefa et al., 2023). The adoption rate of improved sorghum varieties remains low due to the lack of resources and awareness among smallholder farmers. Moreover, market access and price fluctuations pose significant hurdles, discouraging farmers from investing in improved practices and technologies that could enhance sorghum productivity (Degefa et al., 2023). Environmental and climatic factors also heavily influence sorghum production. The impact of climate change, characterized by unpredictable rainfall patterns and increased

incidences of drought, hampers sorghum cultivation. The dependence on rain-fed agriculture exacerbates the vulnerability of sorghum crops to these climatic variations (Habte et al., 2020).

2.7. Drought tolerance mechanisms

Drought tolerance refers to a plant's ability to survive, recover, and reproduce under conditions of water scarcity (Luo, 2010; Muhammad Ameer, 2024). Plants respond, adapt to, and endure drought stress by triggering various morphological, physiological, and molecular mechanisms. The expression of one or a combination of these mechanisms defines the plant's ability to thrive under limited moisture conditions (Khan et al., 2018).

Sorghum exhibit drought escape, a strategy that involves rapid phenological development, developmental plasticity, and the movement of stored resources to the grain. Early-maturing sorghum varieties exhibit lower water loss due to their smaller leaf area compared to late-maturing types (Verma et al., 2018). Additionally, certain sorghum cultivars can escape drought by utilizing stored reserves in their stems (Begna, 2021). Sorghum also possesses developmental plasticity, enabling it to delay or halt development during stress and resume growth when rainfall returns. Furthermore, sorghum's ability to form deep and extensive root systems contributes to its drought escape mechanism (Tari et al., 2013).

Sorghum employs drought avoidance strategies to maintain sufficient water availability by either decreasing water loss or enhancing water extraction. Reduced water loss from shoots is achieved through adaptations such as leaf rolling and abscission, reduced leaf area, and higher stomatal resistance (Osmolovskaya et al., 2018). Sorghum genotypes frequently possess a thick waxy layer, which considerably restricts foliar water loss during arid conditions (Abreha et al., 2022). Tolerant sorghum lines exhibit increased leaf rolling under water deficit, lessening the uppermost leaf area by as much as 75% compared to sensitive lines (Mwamahonje et al., 2021).

Sorghum employs various physiological strategies to withstand drought conditions, including osmotic adjustment, osmoprotection, antioxidant defense mechanisms, and scavenging systems (Chourasia, 2017). Comparative studies of susceptible and resistant genotypes reveal that resistant varieties demonstrate traits such as early flowering, higher leaf water potential, cooler canopy temperatures, and improved stomatal conductance (Blum et al., 1989). In drought-tolerant sorghum cultivars, the antioxidant system operates more efficiently, with enzymes like

superoxide dismutase and catalase coordinating to mitigate oxidative stress (Guimarães et al., 2018). Additionally, sorghum conserves water effectively by minimizing transpiration and optimizing water use efficiency (Škeříková et al., 2018).

Molecular mechanisms of drought tolerance involve alterations in gene expression, including up regulation and down regulation of specific genes. At the molecular level, drought tolerance is a highly intricate phenomenon governed by the combined action of several genes (Oladosu et al., 2019). Studies have revealed that approximately 3.5% of the sorghum genome exhibited changes of more than twofold in response to drought stress (Johnson et al., 2014). Similarly, Buchanan et al. (2005) and Dugas et al. (2011) observed that 4% of the sorghum genome underwent changes when subjected to osmotic stress induced by polyethylene glycol (PEG) treatment.

2.8. Sorghum breeding in Ethiopia

Ethiopian farmers have been doing continuous selection and improvement of their landrace genotypes for years to meet their changing needs, climate and farming systems, such as intercropping (Mindaye et al., 2015) high-quality traits, including larger grain sizes and a range of colors for different end uses, as well as high plant biomass (Mekbib, 2006).

In Ethiopia, sorghum is well-suited to various agro-ecological conditions and is grown in three primary zones in the country: highlands (above 1900 m.a.s.l), intermediate areas (1600-1900 m.a.s.l), and lowland regions (below 1600 m.a.s.l)(Kebede & Menkir, 1987; Tesso et al., 2011). It is a crucial crop in the dry lowlands, comprising about 66% of the total cultivated area in the country (Geremew et al., 2004).

A breeding program for sorghum has been in place since the 1950's with 52 improved varieties (48 open pollinated and 4 hybrid varieties) of the crop released since then by various national and regional research institutions, as well as universities across the country (Kinfe & Tesfaye, 2018; Yali & Begna, 2022). Despite their availability, adoption rates for these improved varieties have remained low, largely due to a weak extension system (Cavatassi et al., 2011) and the fact that these improved varieties lack certain farmer-preferred traits such as plant height and grain size (Wubeneh & Sanders, 2006; Assefa, 2012; Taye Tadesse, 2016; Kinfe & Tesfaye, 2018).

Approximately eighty-five percent of the improved varieties for lowland and intermediate environments are characterized by shorter plant stature, early maturity, and smaller grain size (Adugna, 2007; Yitayeh, 2019). In contrast, all highland environment varieties have been pure lines derived from highland landrace collections. However, these improved varieties offer only a limited yield advantage compared to farmer-selected varieties or landraces, which is a key factor contributing to their lower adoption rate among farmers (Mekbib, 2006). Since the 1970s, there has been an ongoing effort to collect and conserve a vast number of sorghum landraces, resulting in more than ten thousand sorghum genotypes currently preserved at the Ethiopian Biodiversity Institute (EBI) and within the national sorghum improvement program at the Melkassa Agricultural Research Center (Kimber et al., 2013).

2.9. Sorghum genetic diversity in Ethiopia

Ethiopia is widely recognized as a key center for the origin and genetic diversification of sorghum, exhibiting extensive variability across both domesticated strains and their wild relatives. Ethiopia has played a significant role in global sorghum conservation by contributing a diverse array of germplasm to international gene banks, with its accessions accounting for approximately 12.5% of the collections at ICRISAT and 17.5% at the USNPGS repositories (Dahlberg et al., 1996; Yitayeh, 2019; Menamo et al., 2021; Wondimu et al., 2021). The genetic collections from Ethiopia encompass all major sorghum races (except kafir), with durra and bicolor, along with their respective sub-races, being particularly prevalent (Doggett, 1988; Reddy et al., 2002; Cuevas & Prom, 2013). Additionally, the zera zera landraces, native to the Ethiopia–Sudan border region and classified under the caudatum race, have played a significant role in enhancing traits such as disease resistance, productivity, and grain quality in numerous breeding initiatives (Prasada Rao & Mangesha, 1987).

Furthermore, the Ethiopian sorghum collections have been used as a source of genes for crucial agronomic traits worldwide, including stay-green genes for post-flowering drought tolerance (Kebede et al., 2001; Haussmann et al., 2002), superior grain quality and increased yield potential (Prasada Rao & Mengesha, 1981), high lysine and improved protein digestibility (Singh & Axtell, 1973), and starch digestibility (Gilding et al., 2013).

2.10. Phenotypic traits based genetic diversity studies in sorghum

In plant breeding, the initial step involves characterizing and identifying sorghum genotypes that possess key traits for enhancing the crop's genetic makeup. In earlier studies, researchers like Harlan & de Wet (1972), de Wet et al. (1976), Harlan & Stemler (1976), and de Wet (1978) utilized agronomic and phenotypic traits to characterize cultivated sorghum. Numerous genetic diversity studies have since focused on these phenotypic traits to analyze sorghum genotypes (Geleta et al., 2006; Tesso et al., 2011; Amelework et al., 2016; Girma et al., 2020; Sejake et al., 2020; Wondimu et al., 2021; Alemu & Demelash, 2022; Somu et al., 2024). Moreover, characterization of sorghum genotypes based on phenotypic traits at early growth stages could accelerate the development of drought adapted sorghum varieties (Menamo et al., 2023). Several genetic diversity studies in sorghum have been carried out based on seedling traits (Singh et al., 2011; Mace et al., 2012; Joshi et al., 2017; Menamo et al., 2023; Elias et al., 2023). However, the utility of agro-morphological traits for characterizing and analyzing the diversity of germplasm collections is often constrained by several factors. One major limitation is the time-intensive nature of phenotyping, as it requires meticulous observation and measurement of traits across multiple growth stages. Additionally, the expression of these traits is significantly influenced by environmental conditions, which can introduce variability and reduce the reliability of results (Avise, 2012; Amom & Nongdam, 2017).

2.11. Phenotyping methods used for drought-related traits at early stages

To assess drought-related traits in sorghum during early growth stages, researchers employ both field-based and controlled-environment methods. These approaches help characterize how plants respond to water stress through morphological, physiological, and developmental changes. Key root system features such as branching patterns, growth angle, and elongation are commonly analyzed using specialized tools like rhizoboxes, clear growth pouches, and column-based setups, allowing researchers to study root development without disturbing the plant (Ali et al., 2015; Mace et al., 2012; Varshney et al., 2021).

For physiological evaluation, scientists measure indicators like chlorophyll levels, stomatal behavior, and water-use efficiency using handheld sensors (e.g., SPAD meters), porometers, and weight-based techniques, which reveal how drought affects photosynthesis and plant

growth (Tardieu et al., 2020). Modern innovations, including aerial drones equipped with multispectral cameras and automated stress-response tracking systems, have significantly improved the speed and accuracy of data collection (Araus et al., 2022). By combining these phenotypic assessments with genetic analysis, breeders can more efficiently pinpoint drought-tolerant sorghum varieties, supporting the development of hardier crops (Bhat et al., 2023; Paul et al., 2024).

The fundamental processes governing plant water relations involve complex interactions between transpiration mechanisms and root water acquisition strategies. Stomatal behavior, controlled by both hormonal pathways and environmental factors, serves as the primary regulator of water vapor exchange while maintaining photosynthetic efficiency (Tardieu et al., 2018). Below ground, the spatial arrangement and morphological features of root systems significantly influence a plant's capacity to access soil moisture, with specialized structures like root hairs dramatically increasing absorption surface area (Lynch, 2022). Molecular components such as aquaporins facilitate the controlled movement of water molecules across cellular membranes, enabling precise regulation of hydraulic conductivity (Maurel et al., 2021). Advances in molecular genetics have identified key genomic regions associated with these drought-responsive characteristics in important cereal crops (Vadez et al., 2023). The combination of these physiological insights with modern phenomic technologies offers promising avenues for developing crops with enhanced water productivity (Comas et al., 2020).

2.12. Genome wide association studies

Association mapping, originally developed to investigate complex human diseases, has proven to be valuable in plant genetics, as shown by Thornsberry et al. (2001). Genome-wide association studies (GWAS) have effectively identified significant marker-trait associations in cereal crops, such as maize (Remington et al., 2001; Thornsberry et al., 2001), rice (Huang et al., 2010), and sorghum (Morris et al., 2013). The primary advantage of GWAS lies in its capability to identify novel genomic regions associated with traits, even in the absence of prior genetic knowledge.

Nevertheless, association mapping has limitations. It necessitates a large number of molecular markers and is influenced by population structure, has low power to detect rare alleles within

populations, and requires comprehensive statistical analysis to assess the relatedness of genotypes and the overall population structure (Kushwaha et al., 2017; Ibrahim et al., 2020). Additionally, the detection power of association mapping is dependent on the phenotype under study and the association of the marker locus (Álvarez et al., 2014).

Sorghum's extensive genetic diversity, predominantly self-pollinating nature, and the presence of top-tier genetic datasets, encompassing a reference genome, make it exceptionally suitable for association studies (Morris et al., 2013). Using a genome-wide association study (GWAS) on sorghum, Chen et al. (2017) discovered that variations in single nucleotide polymorphisms (SNPs) are significantly associated with the leaf-level response to heat stress, which impacts grain yield and quality. Gemenet et al. (2015) carried out a study on the genetic association of tolerance to low phosphorus levels in West African pearl millet utilizing DArT markers. Their findings revealed a prominent genomic region linked to phosphorus-related traits, including flowering time and grain yield under phosphorus-deficient conditions. They further reported significant genotype by phosphorus interactions conditions with both limited and elevated phosphorus availability in their association mapping study. Morris et al. (2013) studied the genetic basis underlying several agro-climatic traits using GWAS and mapped several QTL for plant stature and the arrangement of floral structures in sorghum.

Several authors reported the dissection of the genetic basis of sorghum root system architectural traits (Menamo et al., 2023; Elias et al., 2024; Enyew et al., 2025), grain yield components (Boyles et al., 2016), grain quality traits (Rhodes et al., 2014; Shakoor et al., 2016; Boyles et al., 2017; Rhodes et al., 2017) , inflorescence morphology and plant height (Zhang et al., 2015; Zhao et al., 2016), cold and heat stress (Chen et al., 2017; Chopra et al., 2017; Ortiz et al., 2017), anthracnose (*Colletotrichum sublineolum*) resistance (Cuevas et al., 2018), stalk rot diseases resistance (Adeyanju et al., 2015) and grain mold resistance (Cuevas et al., 2019), using the panel constructed by Morris et al. (2013).

Using 104,627 SNPs, researchers investigated the allelic associations with bioclimatic and soil gradients in a collection of 1,943 geo-referenced sorghum landraces. The results showed that environmental factors explained a significant portion of SNP variation, with genic SNPs being particularly enriched for associations related to environmental conditions, especially drought and aluminum toxicity (Lasky et al., 2015). In a separate study focused on precipitation

parameters, a genome-wide association mapping analysis revealed a modest but significant influence of climatic adaptation on nucleotide variation in Nigerian sorghum landraces. Furthermore, genes associated with morphological traits and flowering time were identified as key contributors to improved drought resilience (Olatoye et al., 2018). Faye et al. (2019) utilized 213,916 SNPs to explore the population structure and genomic diversity of 421 Senegalese sorghum landraces, uncovering genomic regions affected by climate adaptation. They found that both floral regulatory pathways and the stay-green trait played important roles in climate adaptation across the Sahelian and Sudanian regions. More recently, an investigation into the genetic basis of seed weight variations relative to precipitation gradients in 1,901 geo-referenced sorghum landraces revealed that seed mass variability corresponds to shifts in precipitation levels (Wang et al., 2020).

2.13. DNA markers

DNA markers are specific sequences of DNA located at particular sites within the genome, passed down through generations according to inheritance laws. Unlike functional genes, DNA markers typically have no direct biological effect (Moniruzzaman et al., 2014). They serve as tools for pinpointing locations within the genome and can be utilized to monitor the inheritance of both simple traits governed by a single gene and more intricate traits influenced by multiple genes. . There are two main types of markers pertinent to genetic studies: (1) linked markers and (2) direct markers. Linked markers are located in proximity to the genes associated with the traits on a chromosome, leading to the concurrent inheritance of alleles at both the marker and the gene responsible for the trait. Conversely, direct markers represent functional variations within a gene that contribute to differences in traits (Bidyananda et al., 2024). DNA markers are instrumental in associating alleles with quantitative traits and enhancing our understanding of quantitative variations, thereby aiding in the genetic improvement of crops. Various classes of DNA markers exist. Table 2.1 compares salient features of the common DNA markers (Amiteye, 2021).

Table 2.1. Comparison of key features of various classes of DNA markers

Genetic Marker	PCR Dependence	Inheritance Pattern	Genomic Site Specificity	Degree of Polymorphism	Assay Repeatability
AFLP	Yes	Dominant	No	High	Medium
CAPS	Yes	Co-dominant	Yes	High	High
CpSSR	Yes	Co-dominant	Yes	Low	High
DArT	No	Dominant	Yes	High	High
EST	Yes	Dominant	Yes	High	High
ISSR	Yes	Dominant	Yes	High	Medium
RAMP	Yes	Co-dominant	Yes	High	High
RAPD	Yes	Dominant	No	Medium-High	Low
RFLP	No	Co-dominant	Yes	Low-Medium	High
SAMPL	Yes	Co-dominant	Yes	High	High
SCAR	Yes	Co-dominant	Yes	High	High
SNP	Yes	Co-dominant	Yes	Extremely High	High
SRAP	Yes	Dominant	No	Medium-High	Low
SSCP	Yes	Co-dominant	Yes	High	High
SSR	Yes	Co-dominant	Yes	High	High

Source: Amiteye (2021) ; Nadeem et al. (2018)

2.13.1. Single nucleotide polymorphisms

Single nucleotide polymorphisms (SNPs) are genetic variations that result from specific differences in single base pairs within DNA sequences among individuals of the same species or across different species. SNPs are the most common type of DNA sequence polymorphism found in organisms, occurring more frequently than other genetic markers (Manivannan et al., 2021). They can be found in coding regions, non-coding regions, and intergenic areas of genomes, with a typical frequency of one SNP for every 100 to 300 base pairs of DNA (Fusari et al., 2008). A single nucleotide is the smallest unit of genetic inheritance, and SNP variations arise when one allele contains a recognition site for a restriction enzyme while the other allele lacks this site. The presence or absence of SNPs is the result of mutations such as transitions, transversions, insertions, or deletions due to nucleotide substitutions. Additionally, the presence or absence of restriction enzyme recognition sites leads to differences in the lengths of DNA fragments after enzyme digestion and gel electrophoresis.

There are several methods for SNP genotyping that utilize techniques for distinguishing alleles and various detection platforms. Numerous SNP genotyping assays are currently available for

assessing genetic diversity and identification. Most of these assays rely on molecular mechanisms, including primer extension in PCR, invasive cleavage with restriction digestion, ligation of oligonucleotides, and allele-specific hybridization (Bruse et al., 2008). Historically, SNP detection was primarily performed using the Sanger dideoxy-sequencing method. However, Sanger sequencing has notable limitations, such as generating excessive data, inefficiency in detecting SNPs in heterozygous samples, and being time-consuming and costly. These issues have led to the rapid development of alternative gel-based SNP detection methods that are easier to implement. Techniques such as Cleaved Amplified Polymorphic Sequences (CAPS), Allele-Specific PCR (AS-PCR), and Single-Strand Conformation Polymorphism (SSCP) utilize electrophoresis of PCR products in agarose or polyacrylamide gels. However, the use of gels can limit high-throughput capabilities (Hashim & Al-Shuhaib, 2019; Amiteye, 2021).

To address the challenges associated with gel-based methods, high-throughput SNP genotyping platforms have emerged, employing techniques such as mini-sequencing, heteroduplex analysis, and allele-specific hybridization. Recent advancements include next-generation sequencing (NGS), Genotyping-by-Sequencing (GBS), chip-based NGS, and allele-specific PCR, which have been successfully used to identify informative SNPs. As a result, SNPs have become some of the most desirable markers compared to other molecular markers available for genotyping (Long et al., 2017). The discovery of SNPs involves creating sequence alignments and analyses using data stored in databases. Many current SNP techniques aim for large-scale analysis in a single run. Similar to traditional methods, these new approaches often require marker-specific amplification reactions, specific oligonucleotide primers, or probes like Taqman or Molecular Beacon (Ewart et al., 2019) along with sequencing. SNP markers are typically biallelic and stable over generations, making them suitable for automation and high-throughput applications, thus becoming a popular choice for molecular plant breeding.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Phenotypic characterization

The study aimed to assess the genetic diversity of sorghum genotypes based on their phenotypic traits.

3.1.1. Description of the experimental site

The experiment took place between 26 December and 10 March. 2022/23 in a greenhouse at the College of Agriculture and Veterinary Medicine, Jimma University, Jimma, Ethiopia. The mean daily air temperature ranged from 7.8 °C to 40.8 °C throughout the growing season in the greenhouse, while allowing 60% of the incoming photosynthetically active radiation to pass through. The elevation of the site is 1738 m a.s.l., with 7° 33' N latitude and 36° 57' E longitude. The annual amount of precipitation in Jimma is 1564 mm, with a maximum and minimum temperature of 28°C and 23°C, respectively (source: https://hikersbay.com/climate-conditions/ethiopia/jima).

3.1.2. Plant materials

A total of 158 genotypes were used in this study (Appendix 8.1). The genotypes comprised of genotypes obtained from different geographical regions and diverse agro-ecologies in Ethiopia including the Afar, Amhara, Benshangul Gumuz, Dire Dawa, Gambella, Oromia, SEPR, Somali, Tigray regions and some were with unidentified origins and one improved variety 'Dagim' developed by Melkassa Agricultural Research Center (MARC). The genotypes were subjected to one generation of self-pollination in order to reduce residual heterozygosity. The genotypes were originally collected by the Ethiopian Biodiversity Institute (EBI) and were obtained from Melkassa Agricultural Research Center (MARC). The geographical distribution of the sorghum genotypes is indicated in Figure 3.1.

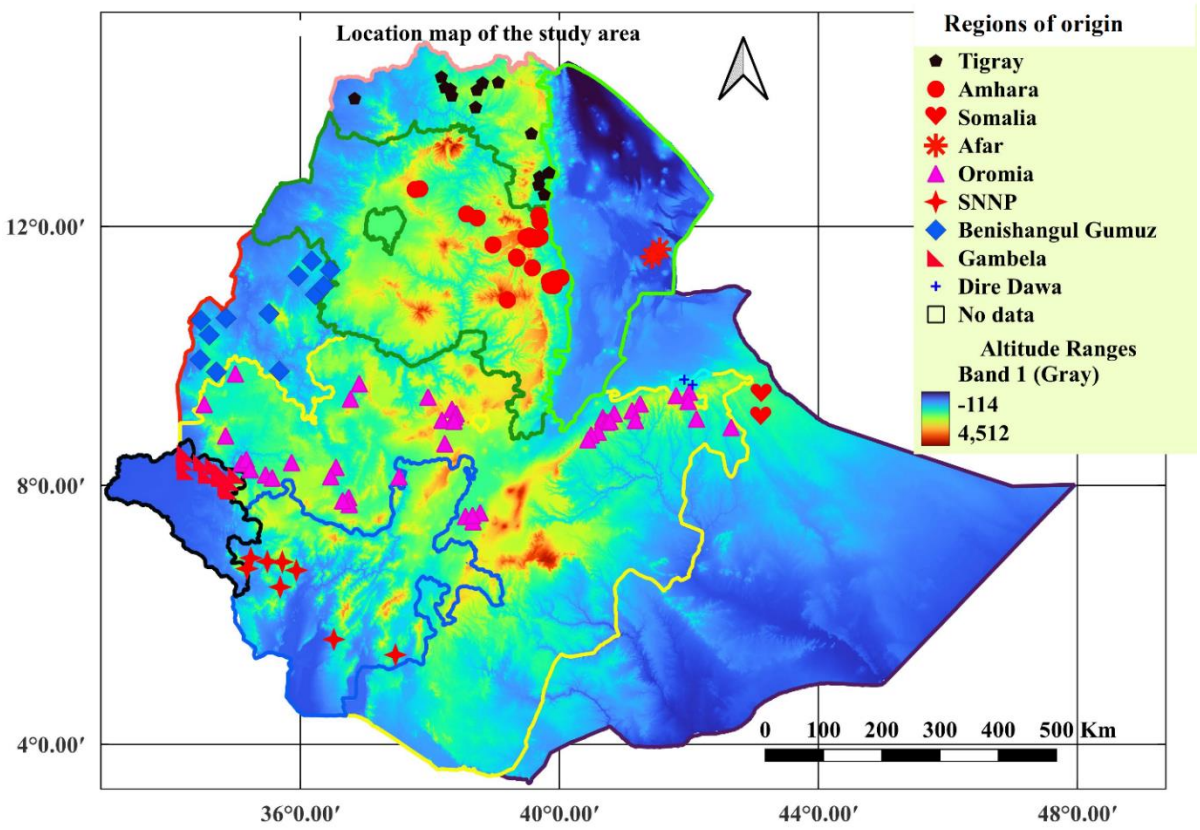


Figure 3.1. Map of Ethiopia showing the regions of origin, and their altitudinal ranges. Symbols represent sorghum genotypes (using QGIS3.36.2 software).

3.1.3. Experimental details and design

The greenhouse experiment was conducted using a high-throughput platform that uses specially designed root chambers. The experiment was conducted using a row-column design with three replicates (Figure 3.2). Each chamber consisted of two 40 cm high and 35 cm wide transparent Perspex sheets with a 3 mm gap. The two Perspex sheets were separated on three sides by 3 mm thick rubber which was held in place with fold back stainless-steel clips. There was a total of 10 tubs (columns), and each tub held 50 root chambers (rows) with a total of 500 root chambers. Root chambers (filled with red, fine-textured soil) were positioned vertically within 10 stainless steel tubes measuring 2 m in length, with the same height and width as the chambers, and covered with polycarbonate sheets to exclude light from the soil and roots,

leaving 5 cm long small slits for the seedlings to emerge. Each tub had holes at the base to allow drainage of excess water and nutrient solution. The soil-filled chambers were watered to field capacity before planting and left until soil water saturation.

Following seed treatment with ApronStar 42 W S fungicide, three seeds were sown in each chamber. At sowing, the seeds were oriented vertically, with the embryo facing downwards, and placed along one of the Perspex sheets to ensure clear visibility of the root system against at least one of the perspex sheets of the root chamber. Plant nutrients were applied in the form of diammonium phosphate (DAP, 1.5kg) prior to sowing and urea (1kg) when the plants reached the three-leaf stage (Demelash et al., 2021). Plants were watered twice per week.

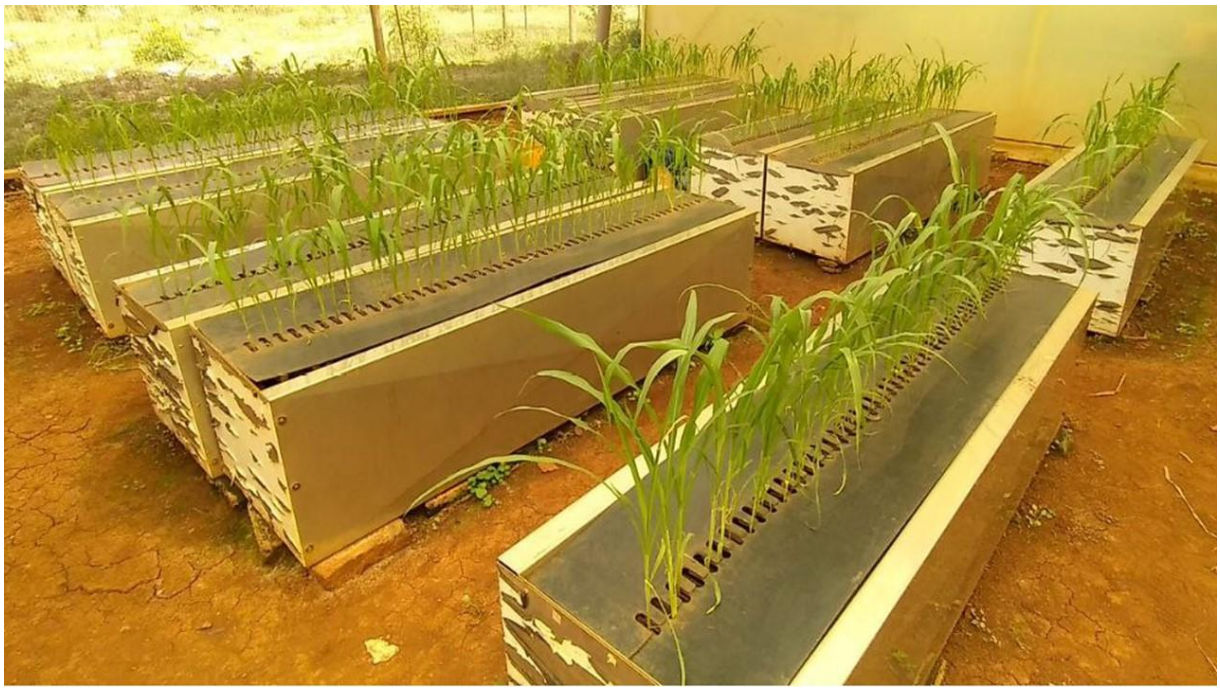


Figure 3.2. Sorghum genotypes grown in a greenhouse.

3.1.4. Data collection

3.1.4.1. Phenotypic traits

Phenotyping of the study materials was performed at the sixth leaf stage, and the first flush of the nodal roots was visible. Data were collected on 15 root and shoot traits *viz*. leaf angle (LAg), shoot length (ShL), leaf area (LAr), shoot diameter (ShD), root length (RL), number of nodal roots (NR), shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), root dry weight (RDW), root hair number (RHN), root angle (RA), stomatal density (StD), root to

shoot ratio (RSR) and stomatal area (SA) (Table 3.1). Scoring App called Field Scorer 4 Android software (https://www.katmandoo.org.au) was used to measure the leaf angle (°). The abaxial surface of the leaf was painted with clear nail polish and allowed to dry for 5 minutes. The nail polish was then removed using forceps and placed on a dry slide, and the area of stomata was determined using a microscope (Model: AE-BK102TR, A&E LAB UK CO., LTD) with a USB digital camera attached to it, with a resolution of 2048 × 1536mm. An elliptical fitting method was used to measure stomatal area (Jayakody et al., 2017). Stomatal density was calculated as the number of stomata per unit area (mm^2) by dividing the mean number of stomata by the area of the field of view at × magnification of x400. Shoot length (cm/plant) was measured from the base of the plant to the leaf ligule at the sixth leaf node using a ruler (Menamo et al., 2023). Stem diameter (mm) was measured using a digital caliper, and leaf area (cm^2) was determined from in situ measurements of leaf length and width, multiplied by a shape factor of 0.69 (Lafarge & Hammer, 2002). The shoot of each plant was removed by cutting the base of the stem, and shoot fresh weight (g) was measured immediately after harvest, while shoot dry weight was recorded after shoot samples were oven dried at 60°C for 72 h using an analytical balance.

After harvesting the shoots, the roots were imaged *in situ* using an imaging box that included two CANON SX610 HS digital cameras and four light boxes on both sides. Free software *openGelPhoto* developed by the University of Queensland (www.activestate.com/activetcl) (Singh et al., 2011; Joshi et al., 2017; Elias et al., 2023; Menamo et al., 2023) provided accurate nodal root angle measurements from the digital photos at a distance of 2 cm from the plant's base. The observed root angle (°C) for each plant was determined by averaging four measurements taken on both (left and right) sides of the root chamber (Singh et al., 2011; Emma S Mace et al., 2012; Joshi et al., 2017).

After extracting the roots from the root chamber and cleaning them to remove soil and surface water, measurements were taken of the number of nodal roots, root length, and root fresh weight. To determine the number of root hairs, a root sample of 2 cm in length was taken from the longest nodal root. The root hairs per plant were counted using a microscope (Model: AE-BK102TR, A&E LAB UK CO., LTD) equipped with a USB digital camera, with a resolution of 2592 × 1944 mm. The roots were then dried at 60°C for 72 hours and weighed using an

analytical balance to determine the root dry weight. The root-to-shoot ratio was determined by dividing the dry weight of roots by the dry weight of shoots. The experimental procedure during data collection is presented in Appendix 8.3.

Table 3.1. Fifteen phenotypic traits recorded for the study

Sno	Traits	Abbreviations	Data recorded from three replicates
1	Leaf angle (°)	LAg	Using a software app
2	Shoot length (cm/plant)	ShL	Using a ruler
3	Leaf area(cm^2)	LAr	Determined from in situ measurements of leaf length and width, multiplied by a shape factor of 0.69
4	Shoot diameter(mm)	ShD	Digital caliper
5	Root length /plant	RL	Using a ruler
6	Number of nodal roots (count)	NR	Counting
7	Shoot fresh weight (g)	SFW	Using analytical balance
8	Shoot dry weight(g)	SDW	Using analytical balance
9	Root fresh weight (g)	RFW	Using analytical balance
10	Root dry weight (g)	RDW	Using analytical balance
11	Root hair number (count)	RHN	Counted using microscope
12	Root angle (°C)	RAg	Using software *openGelPhoto*
13	Stomata density (stomata/mm2)	StD	Calculated as the number of stomata per unit area (mm2)
14	Root to shoot ratio (ratio)	RSR	Determined by dividing the dry weight of roots by the dry weight of shoots.
15	Stomatal area (μm2)	SA	Determined using a microscope and a software app

3.1.5. Data analyses

All statistical evaluations were conducted using version 4.2.2 of the R Statistical Software (R Core Team, 2022). The ANOVA analysis was carried out through a linear mixed model fitted using the REML algorithm, utilizing the lmer function from the lme4 R package v67 (1).1-48 (Bates et al., 2014).

$$\boldsymbol{y = x\tau + zu + e}$$

where y is the data recorded for each trait, τ = the trial's fixed effects, X is the fixed effects design matrix, u is the analysis's random effects (column and row), Z is the random effects design matrix, and e is the residual error effect.

Genetic components were analyzed using the *variability* R package v0.1.0 (Popat et al., 2020). Components of variance, such as environmental (σ^2e), genotypic (σ^2g), phenotypic (σ^2p), the method provided by Burton, (1952) was used to compute the environmental coefficient of variation (ECV), genotypic coefficient of variation (GCV), and phenotypic coefficient of variation (PCV). The Burton & Devane (1953) formula was used to assess the broad sense heritability (*h*2). Additionally, using the method proposed by Johnson et al. (1955), genetic advance (GA) and genetic advance as a percentage of mean (GAM) were computed as follows:

$$\boldsymbol{\sigma^2 e = MSE\ ,}$$

Where MSE= Mean square error.

$$\boldsymbol{\sigma^2 g = \frac{MSG - MSE}{r}},$$

Where: MSG is the genotypic mean square, MSE is the mean square error, and r is the replication number.

$$\boldsymbol{\sigma^2 p = \sigma^2 g + \sigma^2 e,}$$

Where: σ^2g is genotypic variance and σ^2e is environmental variance.

$$\boldsymbol{PCV = \frac{\sqrt{\sigma^2 p}}{\bar{X}} * 100,}$$

Where: σ^2p is phenotypic variance and $\bar{X}$ is grand mean of the trait under study.

$$\boldsymbol{GCV = \frac{\sqrt{\sigma^2 g}}{\bar{X}}},$$

Where: σ^2g is genetic variance and $\bar{X}$ is grand mean of the trait under study.

$$\boldsymbol{ECV = \frac{\sqrt{\sigma^2 e}}{\bar{X}} * 100}\ ,$$

Where: σ^2e is environmental variance and the grand mean for the attribute being studied is denoted by$\bar{X}$.

According to Deshmukh et al. (1986), the values of GCV and PCV were classified into three levels: low, ranging from 0 to 10%, moderate, spanning 11 to 20%, and high, defined as 21% and above.

$$\boldsymbol{h^2 = \frac{\sigma^2 g}{\sigma^2 p} * 100},$$

Where σ²g signifies genotypic variance and σ²p represents phenotypic variance. As outlined by Robinson et al. (1949), heritability percentages are divided into three categories: low, ranging from zero to thirty percent; moderate, spanning thirty-one to sixty percent; and high, starting at sixty-one percent and extending beyond.

$$\boldsymbol{GA = \frac{\sigma^2 g}{\sigma^2 p} * K * \sigma p = h^2 * K * \sigma p},$$

Where: h^2 = heritability coefficient, K = Selection differential standard units which is 2.06 for 5% selection intensity, and σp = phenotypic standard deviation.

$$\boldsymbol{GAM = \frac{GA}{\bar{X}} * 100},$$

Where: GA = Genetic advance and $\bar{X}$= Mean of trait/character.

According to Johnson et al. (1955), GAM is classified into three distinct levels: low, ranging from zero to ten percent; moderate, spanning eleven to twenty percent; and high, exceeding twenty percent.

Pearson's correlation coefficient was computed by the *doebioresearch* R package v0.1.0 (Popat and Banakara, 2020) and plotted using *metan* R package (Olivoto & Lúcio, 2020). The average values for shoot root parameters were estimated by the *dplyr* R package v1.1.2 (Wickham et al., 2023). Principal component analysis (PCA) and cluster analysis were performed using the scaled mean value data because the recorded values had different units of measurement.

To determine which parameters contributed most to the observed variance and to find correlated parameters, principal component analysis, was used. It was executed by *prcomp function* of *factoextra* R package v1.0.7 (Kassambara and Mundt, 2020) and the plot was generated by *ggbiplot* R package (Vu, 2011). Clustering was executed by Ward.D2 algorithm (Murtagh and Legendre, 2014) on the basis of Euclidean distance: $d_{euc}(x, y) = \sqrt{\sum_{i=1}^{n}(x_i - y_i)^2}$, where *x* and *y* are two vectors of length *n* using *hclust* function of the *stats* R package(R Core Team, 2022) and the dendrogram was generated by *ggplot2* R package (Wickham et al., 2016). The appropriate cluster count was identified by *NbClust* R package (Charrad et al., 2014). The intracluster and intercluster distances were estimated by *clv* R package v0.3-2.3 (Nieweglowski, 2023). Box plot analyses were performed for the root traits in order to examine the variance in root attributes among clusters. Mean comparison of root parameters across the

four clusters was conducted using Tukey's (HSD) test, with a significance level set at p less than 0.05.with the *agricolae* R package v1.3-7 (de Mendiburu, 2023).

3.2. Responses of growth and transpiration to increasing soil moisture depletion

3.2.1. Study materials

Forty genotypes from the 158 genotypes used in our prior research was utilized (Table 3.2). The basis for choosing these genotypes was based on their root angle. We selected genotypes with contrasting root angles (wide and narrow root angle) to allow for comparisons between genotypes for drought adaptation under greenhouse conditions.

Table 3.2. Genotypes selected for the experiment

SET-I/ EXP-I					
S no	**Genotype**	**ID #**	**Race**	**Region**	**Root angle**
1	#18(W)	72554	Durra- Caudatum	Amhara	25.07
2	#24(W)	16476	Bicolor	Oromia	24.88
3	#97(W)	70961	Bicolor	Oromia	24
4	#107(W)	210962	Durra- Caudatum	Oromia	23.88
5	#70(W)	69229	Durra- Caudatum	Oromia	23.42
6	#2(W)	16443	Bicolor	Oromia	23.34
7	#91(W)	101358	NA	NA	22.98
8	#121(W)	2001PaweColl#057	Durra-Caudatum	Benishangul Gumuz	22.84
9	#137(W)	69498	Bicolor	SEPR	22.09
10	#141(W)	2014WestColl#22	Durra -Caudatum	Benishangul Gumuz	21.67
11	#142(N)	75009	Caudatum Bicolor	Oromia	11.92
12	#27(N)	71109	Bicolor	Oromia	11.17
13	#155(N)	239193	Durra -Caudatum	Amhara	11.09
14	#118(N)	75007	Bicolor	Oromia	10.09
15	#21(N)	73050	Durra- Bicolor	Amhara	10
16	#51(N)	69354	Durra- Bicolor	Gambela	9.84
17	#149(N)	Ag061	Durra- Bicolor	Benishangul Gumuz	9.63
18	#45(N)	71653	Caudatum-Bicolor	Gambela	9.25
19	#96(N)	16128	Bicolor	Oromia	8.25
20	#30(N)	74071	Guinea -Bicolor	Tigray	7.88

Table 3.2 (continued)

SET-II/ EXP-II					
S no	**Genotype**	**ID #**	**Race**	**Region**	**Root angle**
21	#92(W)	69533	Bicolor	Oromia	25.17
22	#7(W)	210961	Durra- Caudatum	Oromia	23.13
23	#78(W)	100872	NA	NA	22.67
24	#44(W)	71937	Caudatum-Bicolor	Oromia	22.63
25	#104(W)	239219	Durra- Caudatum	Amhara	22.5
26	#133(W)	237304	Durra -Caudatum	Tigray	22.42
27	#127(W)	200199	NA	Oromia	22.09
28	#87(W)	9152	Durra	Oromia	21.67
29	#135(W)	16112	Caudatum-Bicolor	Oromia	21.5
30	#40(W)	73784	Durra- Caudatum	Tigray	21.34
31	#26(N)	73045	Caudatum	Amhara	12.09
32	#13(N)	70960	Caudatum-Bicolor	Oromia	11.82
33	#67(N)	210937	Caudatum	Oromia	11.75
34	#112(N)	IS38412	Caudatum-Bicolor	Amhara	11.67
35	#143(N)	100837	NA	NA	11.67
36	#114(N)	231464	Caudatum	Somali	11.59
37	#98(N)	100040	NA	NA	11.5
38	#120(N)	70998	Durra- Caudatum	Oromia	11.42
39	#25(N)	245060	Bicolor	Oromia	9.63
40	#151(N)	74195	Bicolor	Tigray	7.75

3.2.2. Experimental procedure

In 2023, two separate pot experiments were carried out under greenhouse conditions at Jimma University, College of Agriculture and Veterinary Medicine, Ethiopia (latitude 7° 33′ N; longitude 36°57′ E; altitude 1710 m). The first experiment (Exp 1) took place between mid-March and mid-May, utilizing 20 genotypes—10 with a wide root angle and 10 with a narrow root angle—under a day/night temperature regime of 43°C/10°C. The second experiment (Exp 2) was conducted from early September to late November, involving a different set of genotypes but under slightly different temperature conditions of 44°C/11°C. The study utilized a

randomized complete block design (RCBD) comprising three replications to ensure experimental reliability. For both experiments, plants were planted in 20 L pots. The pots had small holes in the bottom to drain an extra water easily. An Alfisol soil obtained from the university's research farm was used in the experiments. To maintain uniform soil compaction within the pots, the soil was first screened to achieve a particle size below 1 cm and then blended with sand before being added to the pots. Each pot was filled with 10 kilograms of the soil–sand mixture in a 5:3 ratio. For nutrient supplementation, urea and di-ammonium phosphate were incorporated at application rates of 125 and 500 mg per kg, respectively. A supplemental dose of 1.30 g of urea was applied to each plant four weeks post-sowing. Initially, three seeds were planted per pot, but two seedlings were thinned out after two weeks, ensuring only one plant remained in each. The pots were regularly irrigated until plants developed a sufficient number of leaves, approximately six on the main stem, to clearly differentiate transpiration rates. Sorghum plants grown in pots shown in Figure 3.3.

Figure 3.3. Sorghum plants grown in pots

3.2.3. Data collection

Observations were made on ten traits related to growth and transpiration (refer to Table 3.3). The dry-down procedure followed the methodology outlined by Sinclair and Ludlow (1986). On the 45th day following sowing, when all genotypes had developed approximately six fully expanded leaves, each pot was heavily watered between 3:00 and 5:00 PM, then left to release

excess water overnight. On the subsequent day, between 8:00 and 9:00 AM, plastic sheets were laid over the soil surfaces of each pot, followed by the addition of evenly distributed layers of fine and coarse sand on top.

After covering the soil, each pot was weighed (initial pot weight), and daily measurements were taken between 8:00 and 9:00 in the morning. The study incorporated two treatments, well-watered (WW) and water-stressed (WS), as primary factors, with genotypes serving as secondary factors. These were arranged in a randomized complete block design (RCBD) with three replications per genotype. The WW group of each genotype was maintained at approximately 80% of field capacity by replenishing daily water loss, allowing a maximum deficit of 200 grams below field capacity. In contrast, the WS group experienced progressive drought stress by only partially replacing water lost through transpiration. Specifically, any daily water loss exceeding 150 grams was replenished, as previously described by Vadez & Sinclair (2001). Experimental procedures are shown in Figure 3.4.

Daily transpiration for each pot was determined by calculating the difference in water content between consecutive days and adding any water that had been supplemented. To estimate the daily transpiration rate (TR) for each water-stressed plant, the transpiration value of the individual dry-down plant was normalized against the mean transpiration of three corresponding well-watered plants from the same genotype. This normalization helped reduce the impact of daily environmental fluctuations among replicates within each genotype.

$$\text{Transpiration rate (TR)of WS plant} = \frac{\text{Transpiration of WS plant}}{\text{Mean transpiration of WW plants}}$$

Every water-stressed plant was normalized so that the normalized transpiration ratios were centered at 1.0 using the average TR of the first three days of the experiment prior to the onset of water stress. This reduced the differences in plant size across replicates within the genotype and made it simple to compare the genotypes (Kholova et al., 2010).

$$\text{Normalized transpiration ratio(NTR)} = \frac{\text{Daily TR}}{\text{Average TR of first three days}}$$

When the NTR of stressed plants dropped below 0.1, the dry-down tests were over. All plants were subsequently harvested, and the dry mass of their leaves, stems, and roots was measured following a 48-hour oven-drying period at 60 degrees Celsius.

Leaf area was assessed using measurements of length and width, applying the formula: LA = leaf length × width × 0.71, where 0.71 serves as the shape factor (Casadebaig et al., 2008). Following the final harvest, the daily fraction of transpirable soil water (FTSW) was determined. This value reflects the proportion of the remaining volumetric soil moisture available for plant transpiration each day and served as the experimental indicator of drought stress (Ritchie, 1981). The FTSW for any given day, denoted as day n, was computed using the following equation:

$$\text{Fraction of transpirable soil water (FTSW)} = \frac{\text{Pot weight dayn} - \text{Final pot weight}}{\text{Initial pot weight} - \text{Final pot weight}}$$

Table 3.3. Growth and transpiration parameters recorded in the study

No	Traits	Abbreviations	Data collected
1	Shoot length	ShL(cm)	Using tape
2	Shoot dry mass	SDM (g)	Using analytical balance
3	Chlorophyll content	ChlC (SPAD unit)	Using chlorophyll meter
4	Leaf area	LA (cm^2)	Length × width × 0.71, where 0.71 is a shape factor
5	Root dry mass	RDM(g)	Using analytical balance.
6	Root to total dry mass ratio	RTR (g g-1)	Determined by dividing the dry mass of the roots by the dry mass of the whole plant.
7	Initial transpiration	ITr (g plant-1)	Each dry-down plant's transpiration was divided by the average transpiration of three WW plants from the same genotype.
8	Total transpiration	TTr (g plant-1)	The overall weight loss is calculated by deducting the ending weight from the starting weight.
9	Total water extraction	TWE (g plant-1)	The overall weight loss is calculated by deducting the ending weight from the starting weight.
10	Fraction of transpirable soil water	FTSW	Obtained by taking the difference between the pot's weight on a given day and its final weight, then dividing that result by the total amount of transpirable water.

Figure 3.4. Procedures in the dry down experiment. A) Pot weighing; B) Adding water to the pots using a measuring cylinder; C) Shoot harvest; D) Measuring root dry mass

3.2.4. Data analysis

Analysis of variance (ANOVA) was employed to evaluate genotypic variation within each treatment group, utilizing version 4.2.2 of the R software (R Core Team, 2022). The ANOVA model used for the experiment was as follows:

$$\boldsymbol{Y_{ij} = \mu + \tau_i + \beta_j + \epsilon_{ij}}$$

Where:

$\boldsymbol{Y_{ij}}$: The measured response for the *i-th* treatment within the *j-th* block.

$\boldsymbol{\mu}$: The grand average across all observations.

$\boldsymbol{\tau_i}$: The effect attributed to the *i-th* treatment.

$\boldsymbol{\beta_j}$: The effect associated with the *j-th* block

$\boldsymbol{\epsilon_{ij}}$: Denotes the random deviation not accounted for by treatment or block effects.

Pearson's correlation coefficient was performed by the *doebioresearch* R package v0.1.0 (Popat & Banakara, 2020). Mean values for the growth and transpiration response traits were calculated by *dplyr* R package v1.1.2 (Wickham et al., 2023). Heritability in broad sense (h^2) was estimated using the formula of Burton & Devane (1953).

$$h^2 = \frac{\sigma^2 g}{\sigma^2 p} * 100,$$

Where $\sigma^2 g$ = genotypic variance and

$\sigma^2 p$ = phenotypic variance.

Genotypic mean differences were assessed through the least significant difference (LSD) method at a 5% significance threshold, utilizing META-R software (Alvarado et al., 2015). Using the *tidyverse* R package v4.43, a t-test was performed to see if there was a significant difference between the means of the two groups (WW&WS) (Wickham et al., 2019). Regression analysis was conducted using *ggpubr* R packages v0.6.0 (Alboukadel Kassambara, 2018).To assess FTSW threshold levels, normalized transpiration ratio (NTR) values for each plant within a genotype were plotted against their corresponding daily FTSW measurements throughout the experiment. A segmented (split-line) regression analysis was then performed using Genstat version 18 (VSN, 2015), following the approach outlined by (Ray & Sinclair, 1998). This method allowed for the identification of a threshold point, the break in the fitted regression line, marking a significant change in slope where NTR began to decline.

3.3. Multi-locus Genome wide association study

3.3.1. Study materials

This study included 129 different genotypes of sorghum (Table 3.4). Different geographic regions of Ethiopia were represented among the genotypes. SEPR (8), Somali (2), Tigray (15), Dire Dawa city administration (2), Gambela (13), Oromia (43), Amhara (26), Benshangul Gumuz (13), Afar (2), and from an unidentified region (5). The Melkassa Agricultural Research Centre (MARC) provided us with the genotypes, which were first gathered by the Ethiopian Biodiversity Institute (EBI).

Table 3.4. Sorghum genotypes used in the genome- wide association study

S.no	Origin	Entry no	Sample size
1	Afar	#90, #144	**2**
2	Amhara	#1, #6, #8, #9,#18, #22, #26, #36, #38, #49, #54, #55, #62, #66, #81, #86, #95, #100, #104, #109, #110, #112, #123, #131, #147, #155	**26**
3	Benishangul Gumuz	#11, #12, #42, #61, #83, #94, #99, #119, #121, #122, #136, #141, #149	**13**
4	Dire Dawa City Adm	#125, #126	**2**
5	Gambela	#4, #15, #17, #29, #35, #37, #45, #51, #56, #74, #128, #145, #148	**13**
6	Oromia	#2, #3, #5, #7, #14, #24, #25, #27, #32, #41, #44, #47, #48, #50, #52, #53, #57, #63, #69, #70, #72, #75, #76, #80, #82, #85, #87, #96, #97, #106, #107, #113, #117, #118, #120, #127, #130, #132, #135, #139, #140, #142, #152	**43**
7	Southwest Ethiopia Peoples' Region (SEPR)	#19, #23, #71, #115, #124, #137, #146, #150	**8**
8	Somali	#89, #114	**2**
9	Tigray	#10, #20, #30, #39, #40, #58, #59, #60, #65, #79, #84, #101, #133, #138, #151	**15**
10	Not available	#16, #33, #46, #68, #108	**5**
	Total		**129**

3.3.2. DNA extraction and genotyping

Genomic DNA was isolated using a CTAB-based extraction method adapted for 96-well plate processing, following the protocol outlined by Mace et al., (2003). Genotyping-by-sequencing (GBS) was conducted with the *ApeKI* restriction enzyme, which targets the recognition site G|CWGC (Elshire et al., 2011). Library preparation for sequencing was performed according to the manufacturer's instructions and carried out on the Illumina HiSeq 2500 platform. SNP identification was completed using the TASSEL GBS v5 pipeline (Glaubitz et al., 2014), aligning reads to the *Sorghum bicolor* reference genome version 3.1.1 (McCormick et al., 2018).

3.3.3. Genotype data

Genotypic information from 129 entries was obtained from a publicly available dataset hosted at the Purdue University Research Repository (https://doi.org/10.4231/PYQV-AT79). An

initial pool of 334,521 SNPs was derived from these genotypes. To ensure data quality, SNPs with a minor allele frequency (MAF) below 0.05 and those with over 30% missing values, across markers and genotypes with over 30% missing values were excluded using TASSEL version 5.0 (Glaubitz et al., 2014). This filtering process retained 127,804 SNP markers spanning all 10 chromosomes for downstream analysis. Missing genotype calls were subsequently imputed using Beagle version 5.0 (Browning et al., 2018).

3.3.4. Genotypic data analysis

3.3.4.1. Population structure and linkage disequilibrium analysis

SNP density was visualized by SRplot (Tang et al., 2023). Population structure was examined using the R package "LEA" by estimating membership coefficients (Frichot & François, 2015). A cross-entropy plot was used to assess various values of K, helping to identify the optimal number of ancestral populations or the most appropriate replicate for a given K. The optimal K value was determined by applying the entropy-based "elbow" method as described by Frichot and François (2015). Linkage disequilibrium (LD) was performed using TASSEL (Glaubitz et al., 2014), where LD values were derived through pairwise comparisons of the filtered SNP markers, based on squared allele frequency correlation coefficients (r^2).

3.3.4.2.Multi-Locus genome wide association analysis

A multi-locus genome-wide association study (ML-GWAS) was performed using the "mrMLM.GUI" tool in R (R Core Team, 2022). The analysis incorporated six models: Multi-locus random-SNP-effect MLM (mrMLM) (Wang et al., 2016), Factored spectrally transformed multi-locus random-SNP-effect MLM (FASTmrMLM) (Tamba & Zhang, 2018), Factored spectrally transformed multi-locus random-SNP-effect EMMA (FASTmrEMMA)(Wen et al., 2018), Polygenic-background-control-based least angle regression plus empirical Bayes (pLARmEB) (Zhang et al., 2017), Iterative sure independence screening EM-Bayesian LASSO (ISIS EM-BLASSO) (Tamba et al., 2017), and Polygenic-background-control-based Kruskal–Wallis test plus empirical Bayes (pKWmEB) (Ren et al., 2018). Marker–trait associations were explored based on phenotypic data and a filtered dataset of 127,804 SNP markers. The study applied default parameter settings across all models. Significant associations with quantitative trait nucleotides (QTNs) were identified using a LOD

score cutoff of 3.0 or more, following criteria outlined by Tamba et al. (2017) and Wondimu et al. (2023). QTNs were considered robust if detected by three or more ML-GWAS models (Wondimu et al., 2023; Elias et al., 2024). The resulting –log10 (P) values were visualized using Manhattan and Q-Q plots generated through the mrMLM.GUI tool (Wen et al., 2020).

3.3.4.3. Candidate gene analysis

To pinpoint genomic regions potentially harboring candidate genes, the average linkage disequilibrium (LD) decay was examined, focusing on instances where adjacent SNP markers exhibited strong LD ($r^2 > 0.1$). Candidate gene discovery was conducted through the Biomart tools (Smedley et al., 2009) hosted on the Phytozome portal (Goodstein et al., 2012), using a window of approximately 14.4 kb around the genomic locations of significant QTNs. All genes located within these associated regions and annotated with known or predicted functions were retrieved from the latest version (v5.0) of the Sorghum bicolor reference genome (McCormick et al., 2018), available on Phytozome (https://phytozome.jgi.doe.gov).

3.4. Assessment of genetic diversity

3.4.1. Plant materials

The same set of sorghum genotypes (n = 129) utilized in the ML-GWAS was also employed for the genetic diversity analysis (Table 3.4). These genotypes included genotypes obtained from various geographical regions across Ethiopia. To minimize bias due to small sample sizes, genotypes from regions with less than eight samples were merged with genotypes of adjacent regions. Thus, two genotypes each from Afar, Dire Dawa city administration/ Somali, along with five genotypes from unknown regions, were merged with Tigray, Oromia, and Southwest Ethiopia Peoples' Region (SEPR) regions, respectively. As a result of this reclassification, the original ten regions were consolidated into six: Amhara (26), Benishangul Gumuz (13), Gambela (13), Oromia (47), Tigray (17), and Southwest Ethiopia Peoples' Region (SEPR) (13).

3.4.2. Data analysis

The initial set of 334,521 SNPs was filtered to remove SNPs with a MAF of less than 0.05 and those with missing data for the sorghum genotypes were excluded using TASSEL 5.0 (Glaubitz et al., 2014). After applying these quality control filters, 8,698 SNP markers spanning all 10 chromosomes were retained for the evaluation of genetic variation among the sorghum

genotypes. Then the SNP data were changed into a binary format, where 0 indicated absence and 1 indicated presence, utilizing the *dplyr* R package v1.1.2 (Wickham et al., 2023). This transformation resulted in a binary matrix with 8,698 columns representing SNP loci and 129 rows corresponding to genotypes. Key genetic parameters, including Gene Diversity(GD),Observed Heterozygosity (Ho), Polymorphic Information Content (PIC), Minor Allele Frequency (MAF), and Major Allele Frequency (MaF), were computed by the *diveRsity* R package's divBasic function (Keenan et al., 2013). The Dice dissimilarity coefficient (Dice, 1945) as: $d_{ij} = \frac{b+c}{2a+(b+C)}$, where a = is the number of alleles present in both genotypes; b = is the number of alleles present only in genotype *i*; c = is the number of alleles present only in genotype *j* and the Neighbor Joining tree with 1000 bootstraps, and Principal Coordinate Analysis were performed using the DARwin 6 software package v5.0.158) (Perrier & Jacquemoud-Collet, 2006).

Analysis of molecular variance (Excoffier et al., 1992) and the fixation index (Fst) used for population differentiation with 999 permutations were estimated utilizing GenAlEx 6.5 software (Peakall & Smouse, 2006). Gene flow (Nm) among populations was estimated using the formula, as described by (Wright, 1965). $Nm = \frac{(1-\mathrm{Fst})}{4\mathrm{Fst}}$, where fixation index (Fst) was estimated according to Weir & Cockerham (1984), $Fst = \frac{AP}{TOT}$, where AP as the variance among populations and TOT as total genetic variations. Fst values are interpreted as follows: values greater than 0.25 indicate high genetic differentiation, those ranging from 0.15 to 0.25 reflect moderate differentiation, and values below 0.05 suggest low levels of population structure (Wright, 1978).

CHAPTER FOUR

4. RESULTS

4.1. Phenotypic traits variation in Ethiopian sorghum genotypes

4.1.1. Analysis of variance

Highly significant differences ($p < 0.001$) were detected among the 158 genotypes for all traits assessed, as shown in Table 4.1. The distribution of trait values approximated normality (Figure 4.1), supporting the quantitative nature of these traits. The coefficient of variation (CV) ranged from 5.06% for root fresh weight to 14.2% for root angle, indicating varying degrees of trait variability

Table 4.1. Fixed and random effects with significant values among sorghum genotypes evaluated

Traits	**Mean squares**				**Error**	**CV%**	**p-value**
	Fixed effect	**Random effect**					
	Genotypes	**Replication**	**Column**	**Row**			
LAg (°)	27.97***	0.44(*)	0.5	0.49	2.19	7.22%	<0.001
ShL (cm)	13.29***	-0.02(ns)	0.36	0.00	1.54	10.92%	<0.001
LAr (cm^2)	27.02***	0.07(ns)	0.00	0.00	3.12	10.86%	<0.001
ShD(mm)	2.46***	-0.002(ns)	0.00	0.00	0.20	9.99%	<0.001
RL (cm)	46.25***	-0.12(ns)	0.45	0.00	3.40	7.13%	<0.001
NR (count)	5.08***	-0.04(ns)	0.09	0.00	0.46	8.77%	<0.001
SFW(g)	2.72***	0.003(ns)	0.00	0.00	0.31	10.88%	<0.001
SDW(g)	0.93***	0.0002(ns)	0.008	0.00	0.06	6.09%	<0.001
RFW(g)	1.59***	-0.0006(ns)	0.00	0.01	0.08	5.06%	<0.001
RDW(g)	0.58***	0.0005(ns)	0.007	0.00	0.05	7.59%	<0.001
RHN (count)	31.84***	-0.27 (ns)	0.32	0.00	2.17	6.85%	<0.001
RAg (°)	16.08***	0.09(ns)	0.23	0.55	2.03	14.2%	<0.001
StD(stomata/mm^2)	162.31***	0.98(ns)	0.00	0.00	12.02	7.28%	<0.001
RSR (ratio)	0.62***	-0.0007(ns)	0.004	0.00	0.05	8.24%	<0.001
SA (mm^2)	7783***	-63.79(ns)	83.02	0.00	900.30	13.49%	<0.001

*, **, *** Significance levels are indicated at $p < 0.05$, 0.01, and 0.001, respectively. "ns" denotes traits with no statistically significant differences. Trait abbreviations used include: LAg – leaf angle; ShL – shoot length; LAr – leaf area; ShD – shoot diameter; RL – root length; NR – number of roots; SFW – shoot fresh weight; SDW – shoot dry weight; RFW – root fresh weight; RDW – root dry weight; RHN – root hair number; RA – root angle; StD – stomatal density; RSR – root-to-shoot ratio; SA – stomatal area; C.V% – coefficient of variation.

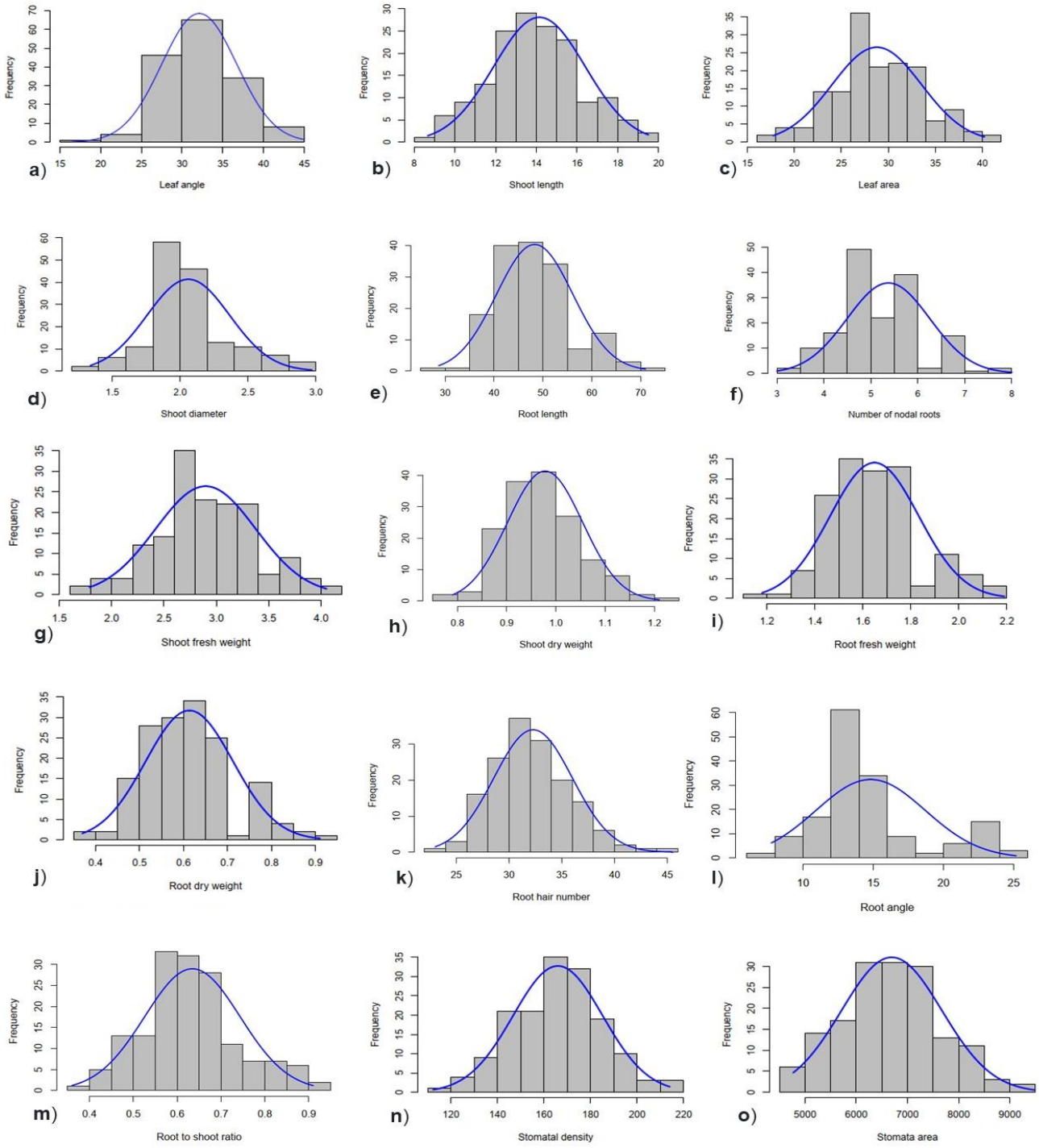


Figure 4.1. Frequency distribution graphs for drought-responsive traits: (A) Leaf angle (B) Shoot length (C) Leaf area (D) Shoot diameter (E) Root length (F) Number of nodal roots (G) Shoot fresh weight (H) Shoot dry weight (I) Root fresh weight (J) Root dry weight (K) Root hair number (L) Root angle (M) Root to shoot ratio (N) Stomatal density (O) Stomata area.

4.1.2. Mean performance of genotypes

Substantial variation in both root and shoot traits was observed among the genotypes, as illustrated in Table 4.2. Nodal root angles ranged from 7.75° (genotype #151) to 25.17° (#92), with an average of 14.81°. Root lengths varied between 28.67 cm and 71 cm, yielding a mean of 48.32 cm. The number of roots per plant spanned from three to eight, averaging 5.37. Root fresh weight ranged between 1.18 g and 2.19 g, with a mean of 1.65 g, while root dry weight values fell between 0.37 g and 0.91 g, averaging 0.61 g. Root hair counts fluctuated from 23 to 46, with a mean value of 32.28. The root-to-shoot ratio ranged from 0.36 to 0.99, with an average of 0.64. As for shoot characteristics, shoot lengths extended from 8.67 cm to 19.5 cm, averaging 14.15 cm, and shoot diameters ranged from 1.34 mm to 2.97 mm, with a mean of 2.06 mm. Shoot fresh weight values varied from 1.79 g to 4.05 g, with a mean of 2.89 g, and dry weights ranged from 0.79 g to 1.65 g, averaging 0.98 g. Mean trait values recorded for leaf area, leaf angle, stomatal density, and stomatal area were 28.67 cm², 32.11°, 166 stomata/mm², and 6692.45 μm², respectively.

Table 4.2. Mean performance, genetic variance components, heritability and genetic advance as a percentage of mean for 15 root and shoot traits of 158 Ethiopian sorghum genotypes.

S.No	Traits	Range	Mean	SEm	$\sigma^2 p$	$\sigma^2 g$	$\sigma^2 e$	PCV	GCV	ECV	h^2%	GA	GAM
1	LAg (°)	16.5 -45	32.11	1.15	18.16	12.89	7.16	13.29	11.20	7.16	70.97	6.23	19.43
2	ShL (cm)	8.67-19.5	14.15	0.77	5.25	2.86	2.39	16.25	11.99	10.96	54.52	2.57	18.25
3	LAr (cm^2)	17.76-40.22	28.76	1.56	22.89	13.19	9.70	16.69	12.67	10.86	57.62	5.68	19.81
4	ShD(mm)	1.34-2.97	2.06	0.10	0.09	0.05	0.04	15.05	11.23	10.03	55.65	0.35	17.25
5	RL (cm)	28.67-71	48.32	1.71	49.38	37.65	11.73	14.62	12.77	7.12	76.25	11.04	22.96
6	NR (count)	3-8	5.37	0.23	0.67	0.46	0.22	15.39	12.65	8.76	67.56	1.14	21.42
7	SFW(g)	1.79-4.05	2.89	0.16	0.23	0.13	0.1	16.68	12.63	10.89	57.34	0.57	19.70
8	SDW(g)	0.79-1.20	0.98	0.03	0.007	0.003	0.004	8.35	5.72	6.12	46.97	0.08	8.07
9	RFW(g)	1.18-2.19	1.65	0.04	0.03	0.02	0.007	10.20	8.85	5.06	75.27	0.26	15.81
10	RDW(g)	0.37-0.91	0.61	0.02	0.008	0.006	0.002	14.82	12.76	7.59	74.07	0.14	22.62
11	RHN (count)	23-46	32.28	1.08	12.70	7.97	4.74	11.07	8.78	6.77	62.72	4.60	14.32
12	RAg (°)	7.75-25.17	14.81	1.05	14.08	9.68	4.4	25.23	20.92	14.10	68.76	5.31	35.73
13	StD(st/mm^2)	112-214	166	5.99	362.56	218.82	143.74	11.51	8.94	7.25	60.36	23.67	14.31
14	RSR (ratio)	0.36-0.91	0.63	0.03	0.01	0.007	0.002	15.94	13.62	8.25	73.00	0.15	23.97
15	SA (μm^2)	4769.21-9475.68	6692.45	451.3	1253540	438806.9	814733	16.71	9.88	13.47	35.01	807	12.05

Legend: SEm: refers to the standard error of the mean. The symbols σ^2_p, σ^2g, and σ^2_e represent phenotypic, genotypic, and environmental variances, respectively. **PCV, GCV**, and **ECV** indicate the phenotypic, genotypic, and environmental coefficients of variation. Broad-sense heritability is denoted by h^2 (%), while **GA** stands for genetic advance, and **GAM** represents the genetic advance expressed as a percentage of the trait mean.

4.1.3. Genetic parameters for phenotypic traits

The phenotypic coefficient of variation (PCV) ranged from 8.34% for shoot dry weight to a peak of 25.23% for root angle, as outlined in Table 4.2. Root angle also showed the highest genotypic coefficient of variation (GCV) at 20.92%, while the lowest GCV was observed in shoot dry weight at 5.72%. Similarly, genetic advance as a percentage of the mean (GAM) spanned from 8.07% for shoot dry weight up to 35.73% for root angle. Notably, root angle exhibited elevated values for both GCV and GAM. Heritability estimates varied from 35.51% (stomatal area) to 76.25% (root length), with an overall mean of 62.40%. Moderate heritability ($30\% \leq h^2 \leq 60\%$) was seen in traits such as stomatal area (SA), shoot dry weight (SDW), shoot length (ShL), shoot diameter (ShD), shoot fresh weight (SFW), and leaf area (LAr), while high heritability ($h^2 > 60\%$) was observed for traits like root length (RL), root fresh weight (RFW), root dry weight (RDW), root-to-shoot ratio (RSR), leaf angle (LAg), root angle (RAg), number of roots (NR), root hair number (RHN), and stomatal density (StD).

4.1.4. Relationships between studied traits

Phenotypic associations among the 15 traits of the 158 sorghum genotypes are presented in Figure 4.2. Nearly all root-related traits exhibited strong and statistically significant positive correlations with one another ($r = 0.76–0.99$, $p < 0.001$), with the exception of root angle, which did not follow this trend. Similarly, shoot traits also showed significant and positive associations ($r = 0.27–0.98$, $p < 0.001$), although leaf angle (LAg) and stomatal area (SA) had no significant correlations with several other traits. Shoot dry weight (SDW) showed a weak but significant positive relationship with certain root traits, including root hair number (RHN; $r = 0.10$, $p < 0.05$), root angle (RAg; $r = 0.10$, $p < 0.05$), and root dry weight (RDW; $r = 0.11$, $p < 0.05$). Conversely, SDW had a negative correlation with the root-to-shoot ratio (RSR; $r = -0.38$, $p < 0.001$). RSR, in turn, displayed highly significant negative correlations with shoot fresh weight (SFW), leaf area (LAr), shoot length (ShL), and stomatal density (StD), with correlation coefficients of -0.43, -0.43, -0.42, and -0.40, respectively ($p < 0.001$ for all). However, RAg showed non-significant (ns) and poor correlations with all traits.

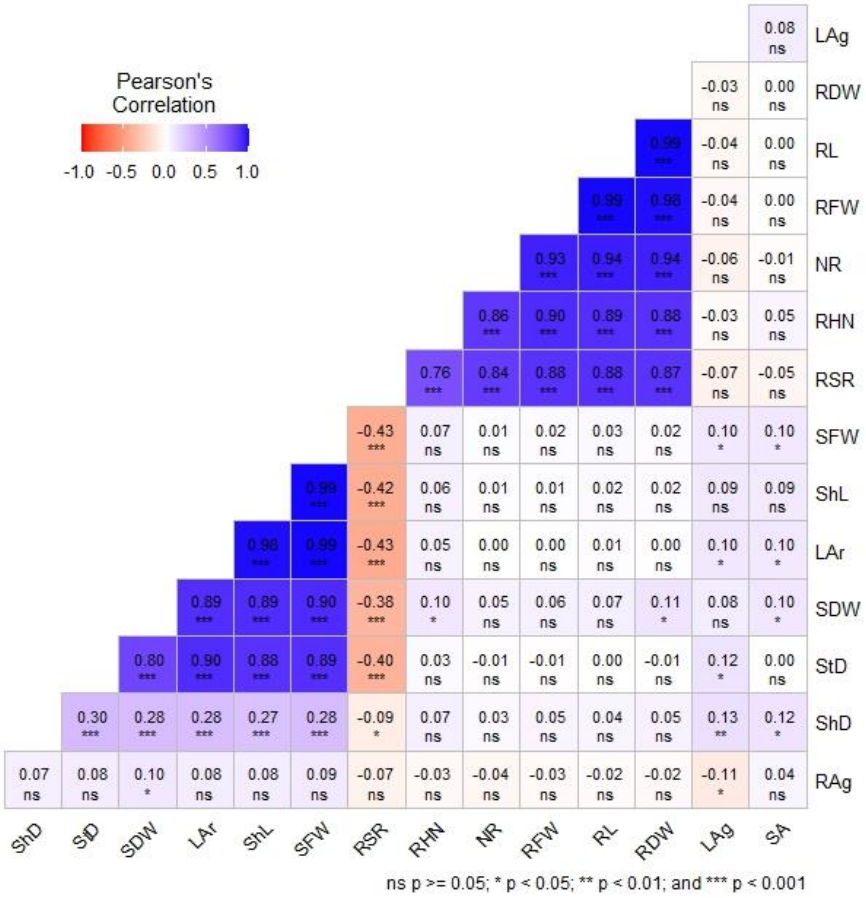


Figure 4.2. Pearson correlation matrix for 15 root and shoot traits measured in 158 sorghum genotypes. The strength of trait associations are visually represented by color gradients, with purple indicates positive associations, while red denotes negative ones. Trait abbreviations: LAg – leaf angle; ShL – shoot length; LAr – leaf area; ShD – shoot diameter; RL – root length; NR – number of roots; SFW – shoot fresh weight; SDW – shoot dry weight; RFW – root fresh weight; RDW – root dry weight; RHN – root hair number; RA – root angle; StD – stomatal density; RSR – root-to-shoot ratio; SA – stomatal area.

4.1.5. Cluster analysis and distances

The 158 genotypes were divided into four genetic groups using cluster analysis based on phenotypic features (Appendix 8.1), and the regions of origin and botanical races of sorghum genotypes grouped under the four different clusters are given in Table 4.3. Most of the assessed genotypes 52 (32.91%) were allocated to Cluster IV, consisting of 21, 3, 7, 4, 2, 5, 1, 1, and 8 genotypes from Oromia, Amhara, Benishangul Gumuz, Gambela, SEPR, Tigray, Somali/Afar, and from unknown regions, respectively. This cluster comprised of genotypes with minimum values for leaf angle (30.52°), shoot length (12.23 cm), leaf area (24.65 cm^2), shoot diameter (1.97 mm), root length (44.15 cm), number of nodal roots (4.91), shoot fresh weight (2.49g), shoot dry weight (0.92g), root fresh weight (1.55g), root dry weight (0.56g), root hair number (29.95) and stomatal density (149.54stomata/mm2) and with higher root angle (15.78°) compared to other clusters (Table 4.4). Moreover, the cluster consisted of 14, 3, 9, 5, 8, 2, 1, and 10 genotypes from Bicolor, Caudatum, Caudatum-bicolor, Durra, Durra- caudatum, Guinea-bicolor, Guinea-caudatum and unknown races, respectively (Table 4.3).

Cluster III included 51 genotypes [(16 from Oromia), Amhara (11), Benishangul Gumuz (3), Gambela/SEPR/Tigray (4), Somali (1), and Unknown regions (8)] and consisted of genotypes with a wide leaf angle (34.07°) and narrow root angle (13.66°) relative to genotypes in other clusters. Genotype #151, with the narrowest root angle (7.75°), was placed under this cluster. The cluster consisted of 12, 10, 5, 1, 2, 8, 1, 1, and 11 genotypes from Bicolor, Caudatum, Caudatum- bicolor, Durra, Durra-bicolor, Durra-caudatum, Guinea-caudatum, Kafir-caudatum, and unidentified races, respectively (Table 4.3).

Cluster I with 38 genotypes [(9 from Oromia/Amhara), Benishangul Gumuz/Gambela (2),Tigray (5), SEPR/Dire Dawa city administration/Afar (1) and NA (8)] consisted of genotypes with maximum cluster mean values for shoot length (16.94 cm), leaf area (34.66 cm^2), shoot diameter (2.2 mm), shoot fresh weight (3.5 g), shoot dry weight (1.07 g), stomatal density (187.29 stomata/mm2) and stomatal area (7075.78 μm^2), whereas it had a minimum value for root to shoot ratio (0.55)(Table 4.4). Moreover, the cluster consisted of 9, 6, 2, 1, 4, 8, and 7 genotypes from Bicolor, Caudatum, Caudatum-bicolor, Durra/kafir-caudatum, Durra-bicolor, Durra-caudatum, and unidentified races, respectively (Table 4.3).

In comparison to genotypes in other clusters, the genotypes in Cluster II (n = 17) had high values of root length (63.12 cm), number of nodal roots (7), root fresh weight (2g), root dry weight (0.81g), number of root hairs (37.58), and root-to-shoot ratio (0.84). This cluster had the minimum values for stomatal area (6549.04μm^2) (Table 4.4). Cluster II comprised of genotypes mostly from Oromia and Amhara regions accounting for (4, 23.52%), each, Gambela (3, 17.64%), unknown regions (2, 11.76%) each, and Benishangul Gumuz/Tigray/SEPR/Dire Dawa city Administration (1, 5.88%). Cluster II composed of races from Bicolor (7, 41.17%), Caudatum- bicolor and unknown races (3, 17.65%) each, Caudatum/Durra/Durra bicolor/Durra-caudatum constituting of (1, 5.88%, each) (Table 4.3).

Table 4.3. Grouping of 158 sorghum genotypes into four different clusters along with their region of origin and botanical race.

Cluster	No of genotypes	Region	Entry number	Race	Entry number
I	**38**	Oromia (9)	#41,#76,#85,#118,#48,#117, #130,#70, #107	Bicolor 9)	#100, #147, #126, #35,#41,#76,#85, #118,#138
		Amhara (9)	#100, #147, #26, #123, #81, #49, #18, #54, #104	caudatum (6)	#144, #26, #123,#48,#117,#58
		Benishangul Gumuz (2)	#42,#61	Caudatum-bicolor (2)	#81, #46
		Gambela (2)	#35,#51	Durra (1)	#49
		Dire Dawa city administration (1)	#126	Durra-bicolor (4)	#42, #61,#51,#130
		SEPR (1)	#146	Durra-caudatum (8)	#18,#54,#104,#70,#107,#10,#39, #133,
		Tigray (5)	#138,#58,#10,#39,#133	Guinea-bicolor (0)	-
		Somali (0)	-	Guinea-caudatum (0)	-
		Afar (1)	#144	Kafir-caudatum (1)	#146
		NA (8)	#46, #64, #91, #102, #154, #156, #157,#158	NA (7)	#64,#91,#102,#154,#156,#157,#158
II	**17**	Oromia (4)	#3,#75,#120,#142	Bicolor (7)	#62,#86,#15,#75,#137,#60,#125
		Amhara (4)	#21,#55,#62,#86	Caudatum (1)	#83
		Benishangul Gumuz (1)	#83	Caudatum-bicolor (3)	#56,#145,#142
		Gambela (3)	#15, #56, #145	Durra (1)	#3
		Dire Dawa city administration (1)	#125	Durra-bicolor (1)	#21
		SEPR (1)	#137	Durra-caudatum (1)	#120
		Tigray (1)	#60	Guinea-bicolor (0)	-
		Somali (0)	-	Guinea-caudatum (0)	-
		Afar (0)	-	Kafir-caudatum (0)	-
		NA (2)	#77, #129	NA (3)	#55,#77,#129
III	**51**	Oromia (16)	#13,#32,#47,#50,#52,#57,#63 ,#67,#69, #73, #80, #96,#106,#116, #127,#140	Bicolor (12)	#36,#66,#32,#47,#50,#57,#63,#69,#96, #71,#124,#151

Table 4.3 (continued)

		Amhara (11)	#1, #6, #9, #36, #38, #66, #109, #110, #112, #131, #155	Caudatum (10)	#1,#6,#9,#109,#67,#80,#116,#114,#65,#79
		Benishangul Gumuz(3)	#99, #136, #149	Caudatum-bicolor (5)	#38,#112,#131,#13,#23
		Gambela (4)	#4,#17,#37,#74	Durra (1)	#110
		Dire Dawa city administration (0)	-	Durra-bicolor (2)	#149, #17
		SEPR (4)	#19, #23,#71,#124	Durra-caudatum (8)	#155, #99, #136,#4,#74,#52,#106,#140
		Tigray (4)	#59, #65,#79,#151	Guinea-bicolor (0)	-
		Somali (1)	#114	Guinea-caudatum (1)	#19
		Afar (0)	-	Kafir-caudatum (1)	#16
		NA (8)	#16,#28,#34,#78,#93,#103,#111,#134	NA (11)	#37,#28,#34,#78,#93,#103,#111, #134,#73,#12,#59
IV	**52**	Oromia (21)	#2,#5,#7,#14,#24,#25,#27,#31,#44,#53,#72,#82,#87,#92,#97,#105,#113,#132,#135,#139,#152	Bicolor (14)	#2, #5, #14, #20, #24, #25, #27, #72, #82, #84, #97,#108,#115,#132
		Amhara (3)	#8, #22, #95	Caudatum (3)	#31, #90, #94
		Benishangul Gumuz(7)	#11, #12, #94, #119, #121, #122, #141	Caudatum-bicolor (9)	#8,#22,#44,#45,#53,#101,#105,#135,#150
		Gambela(4)	#29, #45, #128, #148	Durra (5)	#33,#87,#89,#95,#152
		Dire Dawa city administration (0)	-	Durra-bicolor (0)	-
		SEPR (2)	#115,#150	Durra-caudatum (8)	#7,#11,#12,#40,#119,#121,#139,#141
		Tigray (5)	#20, #30, #40, #84,#101	Guinea-bicolor (2)	#29,#30
		Somali (1)	#89	Guinea-caudatum (1)	#128
		Afar (1)	#90	Kafir-caudatum (0)	-
		NA (8)	#33,#43,#68,#88,#98,#108, #143,#153	NA (10)	#43,#68,#88,#92,#98,#113,#122,#143,#148,#153

Table 4.4. Cluster mean values with standard error (the highest with **bold** and the lowest with *italic*) of 15 different traits of 158 genotypes.

Traits	Clust-I (38) *	Clust-II (17)	Clust-III (51)	Clust-IV (52)
Leaf angle (°)	32.2±0.85	30.94±0.76	**34.07±0.52**	*30.52±0.65*
Shoot length (cm)	**16.94±0.2**	13.88±0.46	14.14±0.14	*12.23±0.23*
Leaf area (cm^2)	**34.66±0.43**	28.13±0.94	28.78±0.3	*24.65±0.47*
Shoot diameter (mm)	**2.2±0.07**	2.04±0.06	2.07±0.04	*1.97±0.04*
Root length (cm)	46.52±1.29	**63.12±0.94**	48.98±0.54	*44.15±0.78*
Number of nodal roots (count)	5.17±0.16	**7±0.11**	5.48±0.06	*4.91±0.09*
Shoot fresh weight g)	**3.5±0.05**	2.83±0.10	2.9±0.03	*2.49±0.05*
Shoot dry weight (g)	**1.07±0.01**	0.97±0.02	0.98±0.01	*0.92±0.01*
Root fresh weight (g)	1.61±0.04	**1.99±0.03**	1.67±0.01	*1.55±0.02*
Root dry weight (g)	0.59±0.02	**0.81±0.02**	0.63±0.01	*0.56±0.01*
Root hair number (count)	32.86±0.69	**37.58±0.86**	32.46±0.26	*29.95±0.37*
Root angle (°)	15.25±0.65	14.38±0.74	*13.66±0.38*	**15.78±0.66**
Stomatal density (stomata/mm^2)	**187.29±1.88**	164.22±3.91	167.35±1.54	*149.54±2.09*
Root to shoot ratio (ratio)	*0.55±0.02*	**0.84±0.02**	0.64±0.01	0.62±0.01
Stomatal area (μm^2)	**7075.78±138.37**	*6549.04±235.22*	6583.74±132.68	6565.82±145.87

* Number of genotypes in each cluster is shown in parenthesis.

Table 4.5 presents both inter- and intra-cluster distances. Inter-cluster distances ranged from 10.69 to 13.28, with the greatest expanse observed between Clusters II and III (13.50), followed by Clusters I and IV (13.28). The smallest inter-cluster distance was between Clusters II and IV (10.68), closely followed by Clusters I and II (11.06). Intra-cluster distances varied between 7.17 and 9.34, where Cluster IV showed the highest internal variation (9.34), followed by Cluster III (8.69). Cluster I had the lowest intra-cluster distance (7.17), with Cluster II next at 8.22.

Table 4.5. Intra cluster and inter cluster distances.

Cluster	Clust-I	Clust-II	Clust-III	Clust-IV
Clust-I	7.17	11.07	11.57	13.28
Clust-II		8.22	13.50	10.69
Clust-III			8.69	13.17
Clust-IV				9.34

4.1.6. Principal component analysis

Principal component analysis (PCA) was performed using data from 15 root and shoot traits, as detailed in Table 4.6. These traits were organized into a 15-variable matrix for analysis. Based on the criterion of eigenvalues greater than 1, four principal components (PCs) collectively explained 87.20% of the total variation in the dataset. The first and second components (PC1 and PC2) alone contributed 38.10% and 34.10%, respectively, accounting for a combined 72.20% of the total variability. PC1, PC2, PC3, and PC4 had eigenvalues of 5.71, 5.11, 1.19, and 1.06, respectively. The traits that contributed significantly to PC1 were RL, RFW, RDW, NR, RHN, and RSR. In PC2, ShL, LAr, SFW, SDW, and StD contributed to most of the variation. PC3 was associated with LAg, whereas the high contribution to the variation in PC4 was attributed to RAg and SA. The sorghum genotypes (#86), (#42), (#142), (#49), (#115), (#130), and (#125) contributed the most to both Dim-1 and Dim-2, whereas the genotypes (#93), (#16), and (#69) contributed the least (Figure 4.3).

Table 4.6. Principal components analysis (PCA) of root and shoot parameters among sorghum genotypes, along with the contributions (loadings) of each parameter to the respective components.

Traits	**PC-1**	**PC-2**	**PC-3**	**PC-4**
LAg	0.032	0.058	**-0.704**	0.151
ShL	0.057	**0.431**	0.067	0.061
LAr	0.057	**0.433**	0.056	0.064
ShD	-0.006	0.185	-0.379	-0.342
RL	**-0.411**	0.075	0.014	-0.001
NR	**-0.407**	0.069	0.022	0.018
SFW	0.055	**0.433**	0.065	0.051
SDW	0.035	**0.425**	0.063	0.039
RFW	**-0.411**	0.074	0.003	0.008
RDW	**-0.410**	0.075	0.008	0.004
RHN	**-0.393**	0.101	-0.024	0.010
RAg	0.023	0.050	0.418	**-0.690**
StD	0.054	**0.401**	0.058	0.134
RSR	**-0.400**	-0.120	-0.012	-0.018
SA	0.001	0.106	-0.408	**-0.595**
Eigenvalue	**5.7158**	**5.1114**	**1.1931**	**1.0668**
Difference	**0.6044**	**3.9183**	**0.1263**	**0.2537**
Variance percent	**38.10**	**34.10**	**8.0**	**7.1**
Cum.Var percent	**38.10**	**72.2**	**80.10**	**87.20**

Abbreviations: LAg (leaf angle), ShL (shoot length), LAr (leaf area), ShD (shoot diameter), RL (root length), NR (number of roots) SFW (shoot fresh weight), SDW (shoot dry weight), RFW (root fresh weight), RDW (root dry weight), RHN (root hair number), RA (root angle), StD (stomatal density), RSR(root to shoot ratio), SA(stomatal area). PC=Principal component

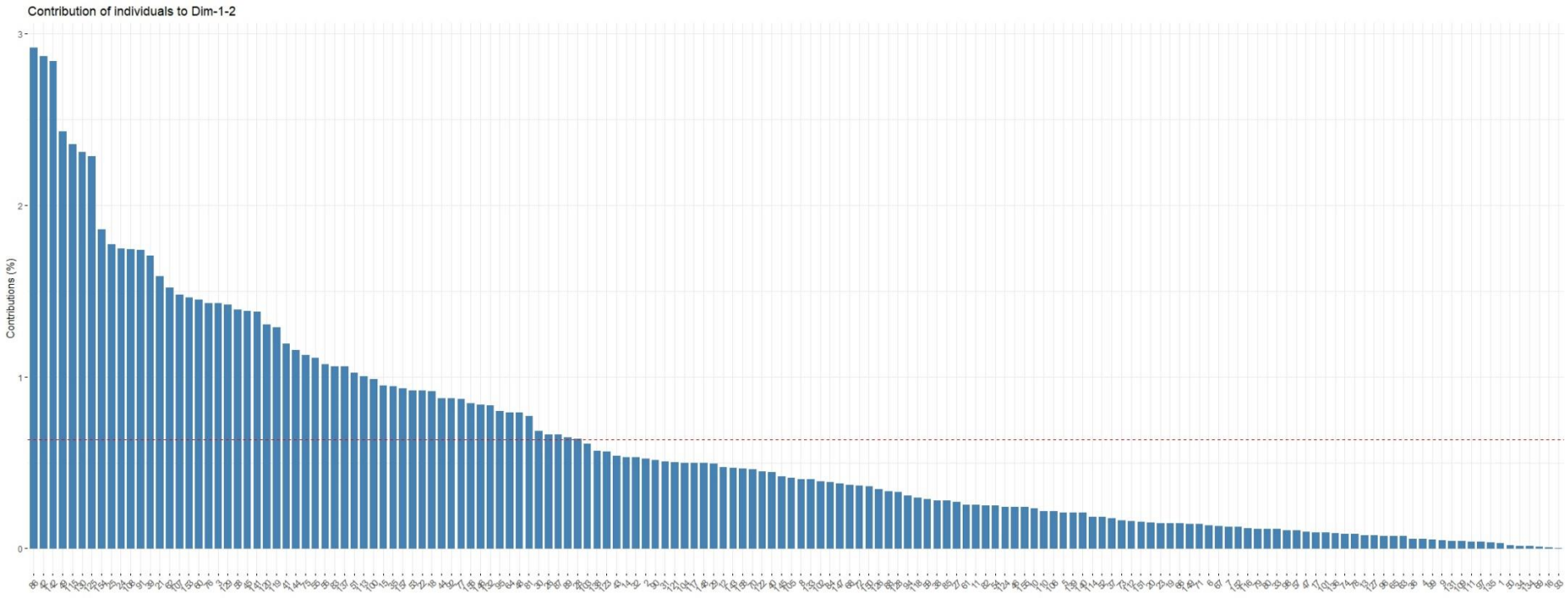


Figure 4.3. Contribution of 158 sorghum genotypes to Dim-1 and Dim-2.

The second principal component analysis that considered root variables showed that the first two PCs explained 95.7% of the entire variability, with PC-1 and PC-2 accounting for 81.4% and 14.3% of the total, respectively. In PC1, RL, NR, RFW, RDW, RHN, and RSR made significant contributions. However, in PC2, most of the disparity was ascribed to RAg (Figure 4.4).

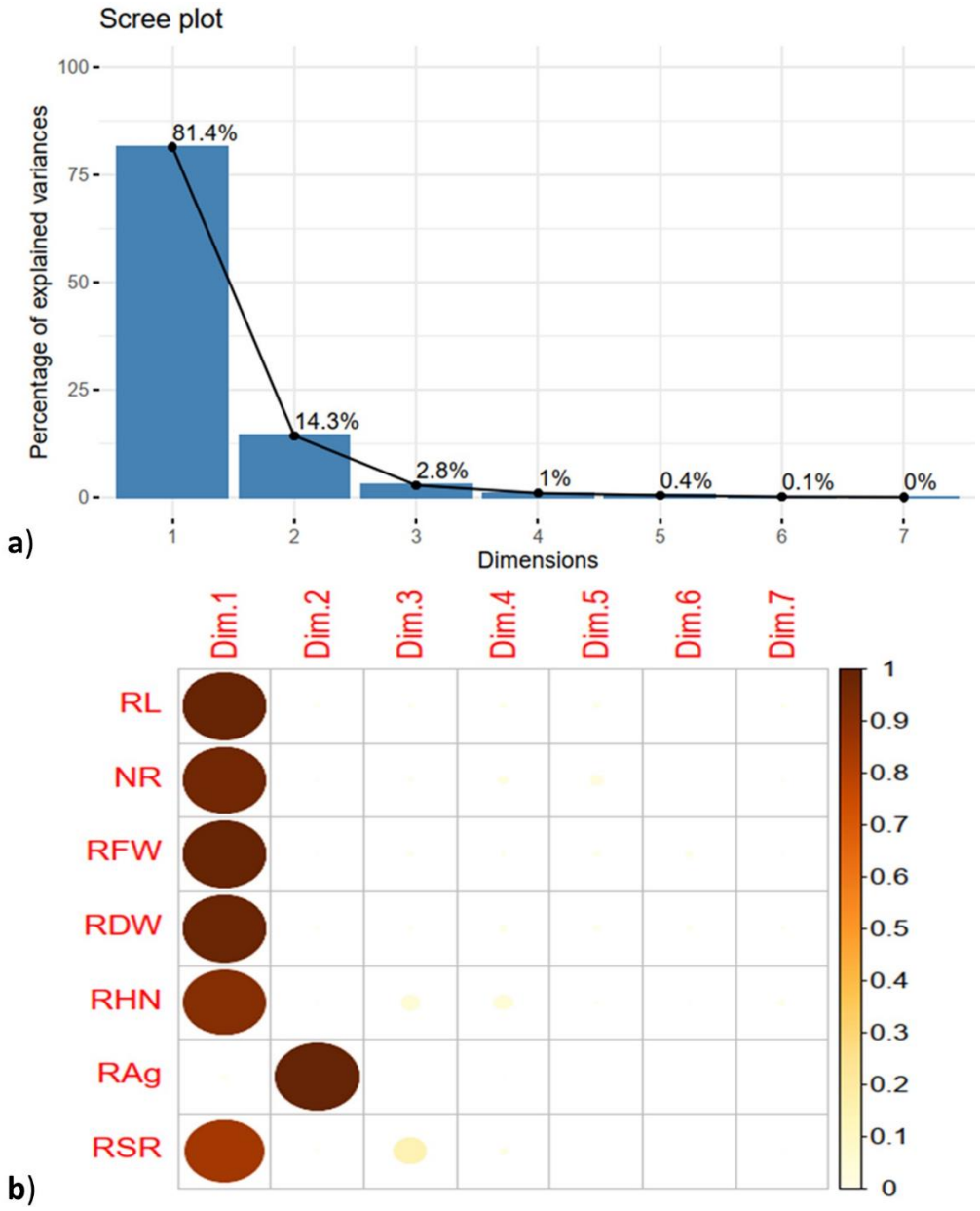
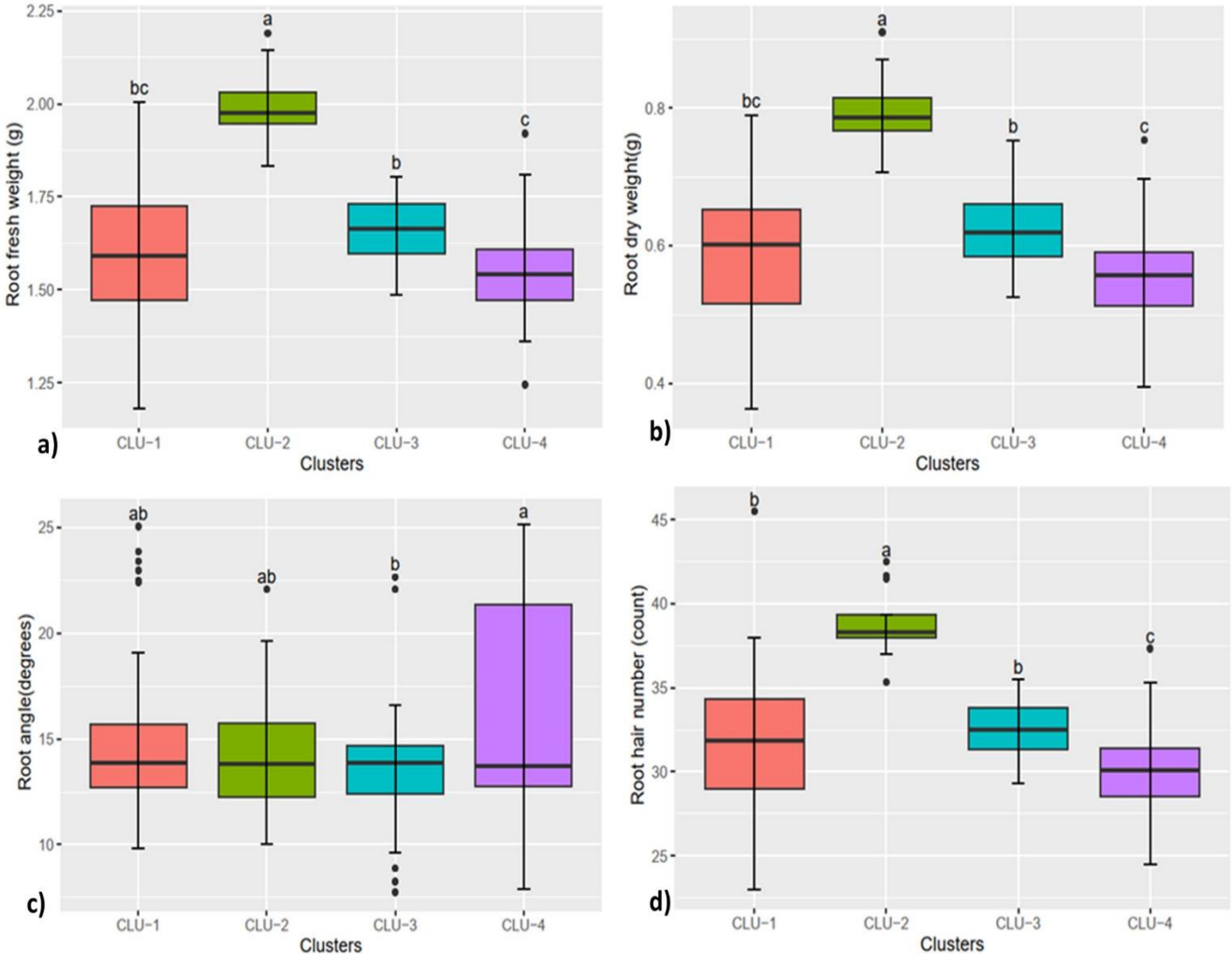


Figure 4.4. a) Scree plots showing percentage of explained variance by each PCs. b) Plots showing contribution of the variables to the first two PCs by the 7 root traits. The color and size of a circle reflects the value of the contributing variables.

Means comparisons among clusters across the root traits at 95% confidence level (Tukey's (HSD) test) are shown by letters. The cluster influence was substantial for all the root traits ($P < 0.05$). The pair comparisons conducted at a 95% confidence level using Tukey's (HSD) post-hoc test, showed that clusters having the similar letter are not considered significantly diverse for that specific trait, while clusters with different letters exhibit statistically significant differences in their means (Figure 4.5).



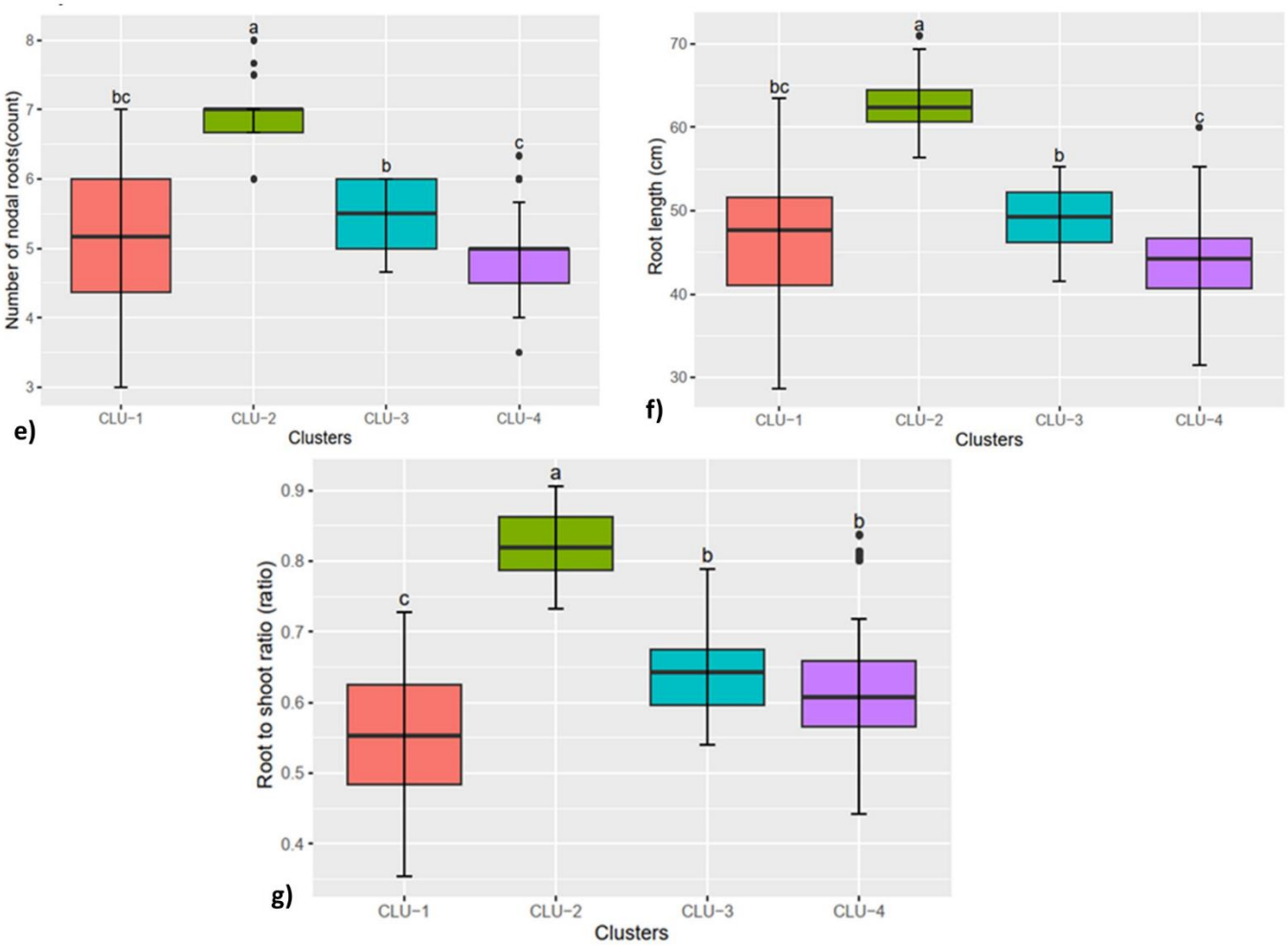


Figure 4.5. Mean comparison between the four clusters: (A) Root fresh weight (b) Root dry weight (c) Root angle (d) Root hair number (e) Number of nodal roots (f) Root length, and (g) Root-to-shoot ratio. Letters above boxplots indicate significant differences between clusters based on a one-way ANOVA and *Tukey's HSD* post hoc test ($p < 0.05$). CLU-1 to CLU- 4 represent cluster 1 to cluster 4, respectively.

The PCA biplot graphically illustrates how the various traits and genotypes are related in relation to the first two PCs (Figure 4.6). The expanses between genotypes on the biplot represent their dissimilarities. When the variable vectors align in the same direction and have narrower angles, it suggests a high correlation between the traits/variables. For example, shoot traits like shoot dry weight (SDW), Shoot fresh weight (SFW), Shoot length (ShL), and Leaf area (LAr) were positively correlated and pointed in the same direction on the PC plane. Similarly, root traits such as RDW, RFW, RHN, and RL also pointed in the same direction, indicating their strong association.

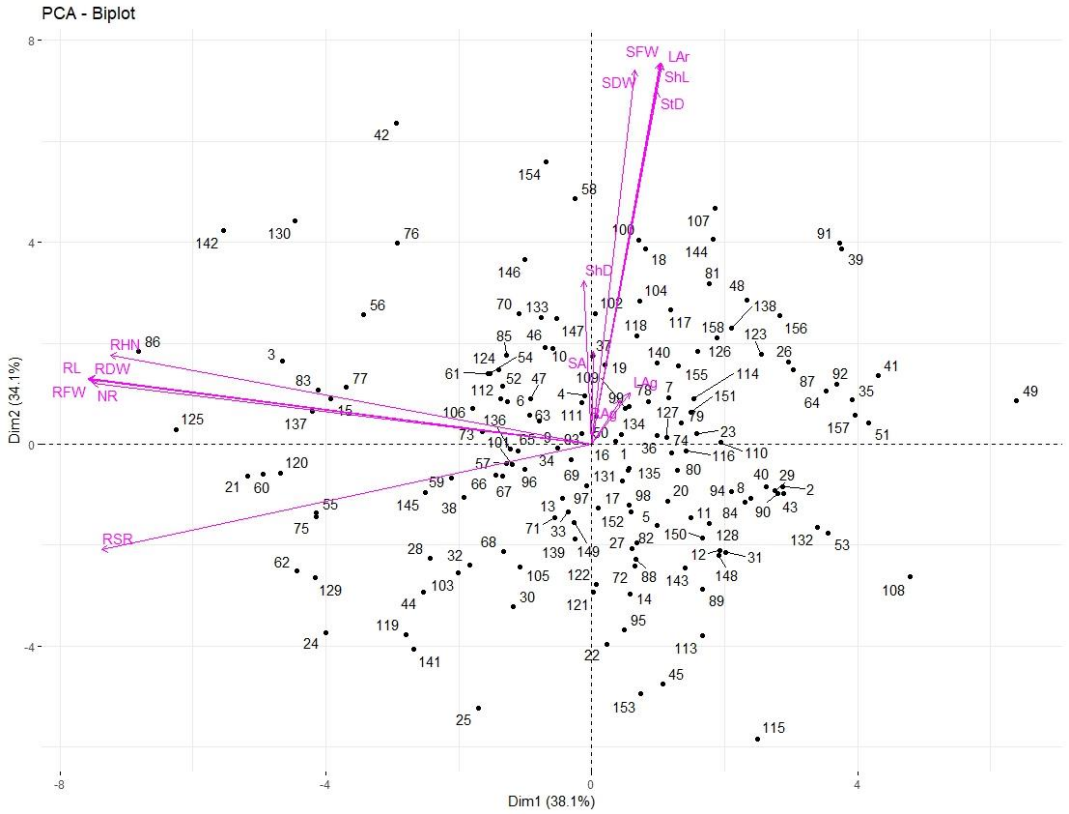


Figure 4.6. PCA biplot illustrating the pattern of 158 sorghum genotypes based on the variation in 15 root and shoot trait. The arrow lengths represent the degree of variability for each trait, while the cosine of the angles between arrows reflects their pairwise correlations. Genotypes plotted near the direction of a given variable exhibit higher trait values, whereas those positioned opposite to a variable show lower values. (For the accession number of sorghum genotypes, refer to Table 3.1).

Principal component analysis (PCA) incorporating 15 phenotypic traits categorized the genotypes into four well-defined groups (Figure 4.7). Cluster II contained genotypes demonstrating superior performance in root-related characteristics, including root biomass (both fresh and dry weights), root hair count, nodal root count, and root-to-shoot biomass allocation. In contrast, Cluster I grouped genotypes exhibiting maximal values for shoot-related parameters: shoot biomass (fresh and dry weights), stem length, stomatal traits (density and area), and foliar area. Cluster III included two distinct genotype sets: those displaying minimal root angles (#151 [7.75°], #96 [8.25°], and #66 [8.87°]) and those with maximal leaf angle (#34 [42.34°], #69 [41.83°], and #37 [41.50°]). The fourth cluster comprised genotypes with generally inferior trait performance, except for their exceptional root angles, particularly genotypes #92 (25.17°), #24 (24.87°), and #97 (24°), which showed the most pronounced values. Notably, the PCA clustering pattern showed no apparent correlation with the geographical origins of the genotypes (Figure 4.8).

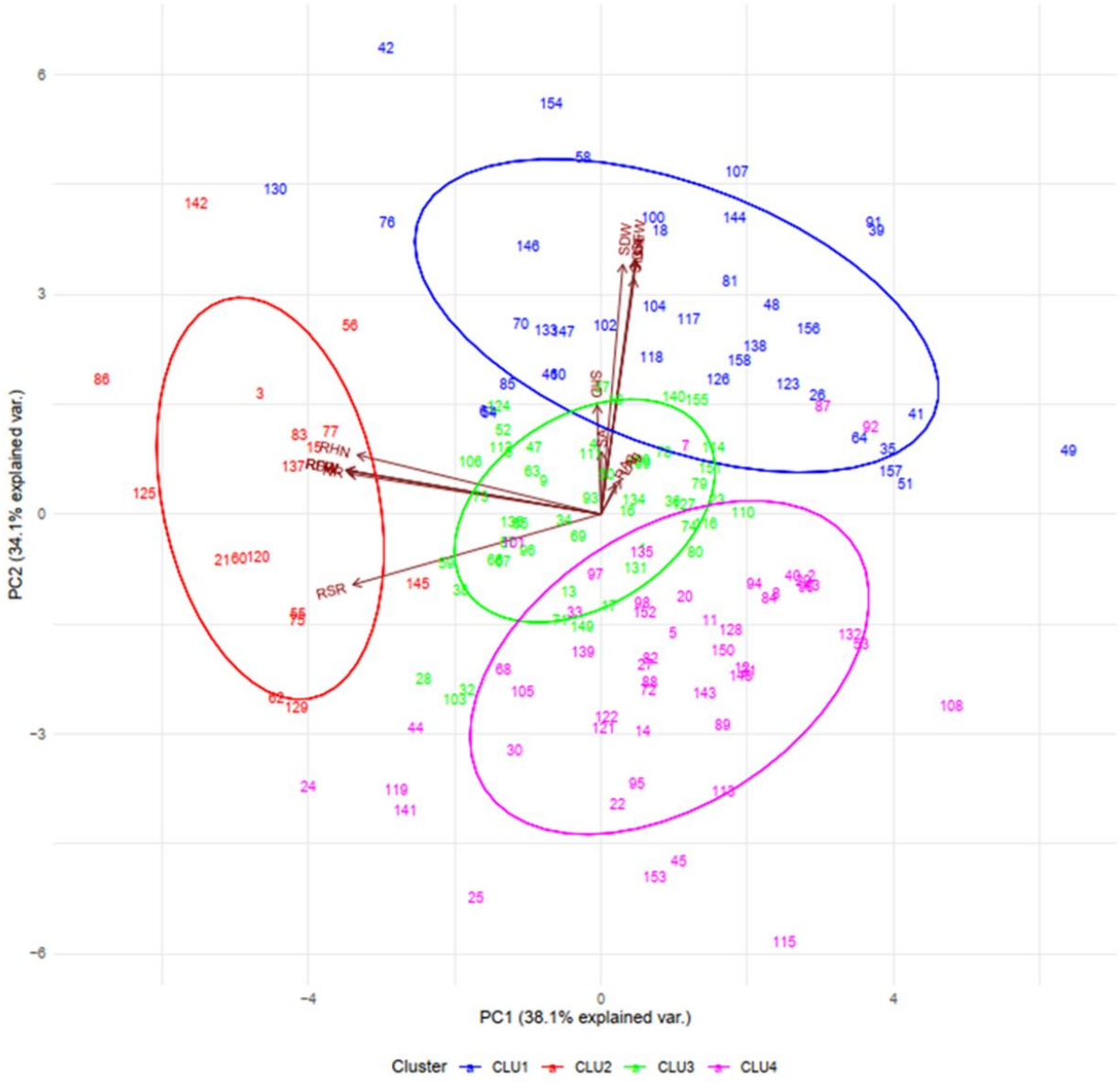


Figure 4.7. Principal component analysis (PCA) was conducted on 158 sorghum accessions using 15 morphological traits related to root and shoot architecture. The biplot visualization displays principal component 1 (PC1) along the horizontal axis, representing 38.10% of the total phenotypic variation, while principal component 2 (PC2) on the vertical axis accounts for 34.10% of the observed variation. Color-coded data points correspond to distinct genetic clusters identified among the sorghum accessions (specific genotype identification numbers are provided in Table 3.1).

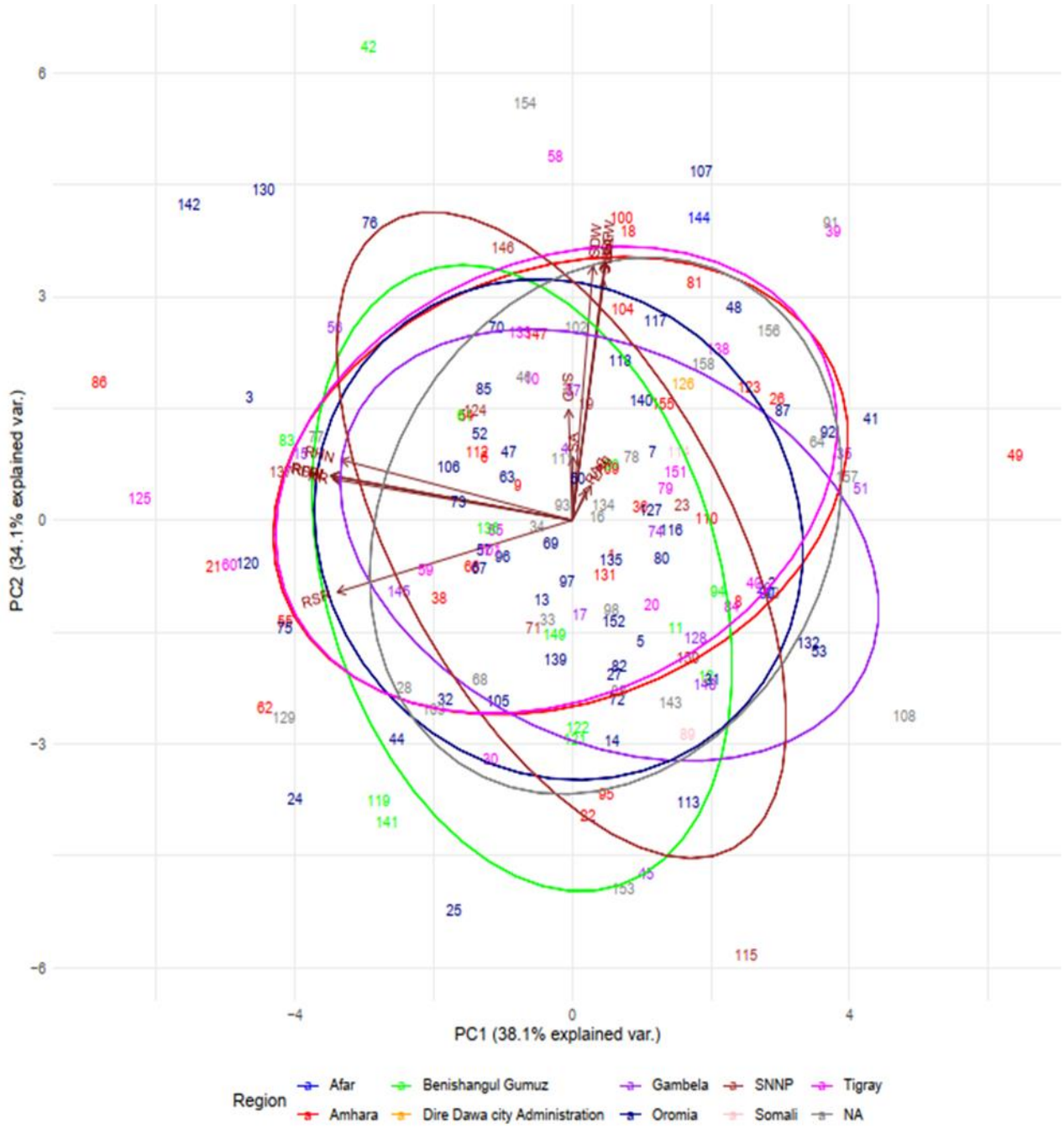


Figure 4.8. PCA showing genetic relationship among sorghum genotypes belonging to nine regions: NA: refers to unknown region. The first principal component (PC1), displayed along the horizontal axis, captured 38.10% of the observed variation in the dataset. The second principal component (PC2), represented on the vertical axis, accounted for 34.10% of the total variance. In the resulting plot, color differentiation among data points corresponds to geographical origins of the 158 sorghum accessions analyzed. (Specific genotype identification numbers are provided in Table 3.1).

4.2. Changes in growth and transpiration to progressive soil water depletion

4.2.1. ANOVA assessment for plant growth and transpiration parameters

ANOVA results showed statistically significant variation ($p < 0.05$) among genotypes across all measured traits under both well-watered and water-deficit conditions. This confirms substantial genetic diversity within the sorghum population, suggesting strong potential for selective breeding. Heritability values ranged from 26% for chlorophyll content to 97% for FTSW under water stress conditions in experiment 1, and from 34% for shoot length to 99% for total water extraction in experiment 2. In comparison to the well-watered condition, most growth and transpiration response traits showed a reduction in mean values under water-deficit conditions, except for the root to total dry mass ratio and initial transpiration, which exhibited increased values (Table 4.7).

Table 4.7. Genotypic variability for growth and transpiration response traits of sorghum genotypes evaluated in conditions of adequate watering and imposed water stress during the first and second experimental trials.

	ShL (cm)	**SDM (g)**	**RDM (g)**	**RTR (ratio)**	**ChlC (SPAD unit)**	**LA (cm^2)**	**ITr (g $plant^{-1}$)**	**TTr (g $plant^{-1}$)**	**TWE (g $plant^{-1}$)**
Well-watered condition (Exp1)									
Mean	108.31	59.92	18.65	0.24	51.12	511.51	111.42	5266.33	5193.83
Min	70	35.21	10.13	0.17	38.39	345.94	40	3160	3200
Max	160	75.61	35.17	0.36	69.54	816.08	200	9780	9580
$LSD_{0.05}$	20.52	5.42	4.97	0.05	9.67	117.37	32.48	908.43	960.77
Significance	***	***	***	***	**	**	***	***	***
h^2	0.76	0.90	0.66	0.58	0.34	0.39	0.74	0.88	0.86
Water stress condition (Exp1)									
	ShL (cm)	**SDM (g)**	**RDM (g)**	**RTR (ratio)**	**ChlC (SPAD unit)**	**ITr (g $plant^{-1}$)**	**TTr (g $plant^{-1}$)**	**TWE (g $plant^{-1}$)**	**FTSW**
Mean	93.72	30.64	14.17	0.33	45.55	217.83	3430.67	1375	0.39
Min	69	22.24	12.10	0.26	32.75	140	2760	840	0.25
Max	118	39.86	17.09	0.38	58.73	280	3866.67	1790	0.65
$LSD_{0.05}$	10.49	3.50	1.80	0.03	7.80	31.69	403.63	96.46	0.03
Significance	***	***	***	***	*	***	***	***	***
h^2	0.54	0.71	0.65	0.56	0.26	0.66	0.52	0.95	0.97

Table 4.7 (continued)

	ShL (cm)	SDM (g)	RDM (g)	RTR (ratio)	ChlC (SPAD unit)	LA (cm^2)	ITr (g $plant^{-1}$)	TTr (g $plant^{-1}$)	TWE (g $plant^{-1}$)
Well-watered (Exp 2)									
Mean	135.42	55.42	17.48	0.25	53.72	541.06	125.67	5758.33	5578.33
Min	100	41.18	10.19	0.14	47.34	338.19	40	2620	2420
Max	195	76.13	30.58	0.40	59.06	751.79	280	8420	8220
$LSD_{0.05}$	22.16	7.93	5.44	0.05	3.36	92.79	54	1112.08	1163.76
Significance	***	***	***	***	***	***	***	***	***
h^2	0.59	0.76	0.46	0.62	0.51	0.60	0.50	0.79	0.77
Water stress (Exp2)									
	ShL (cm)	**SDM (g)**	**RDM (g)**	**RTR (ratio)**	**ChlC (SPAD unit)**	**ITr (g/ plant)**	**TTr (g /plant)**	**TWE (g/plant)**	**FTSW**
Mean	115.38	37.52	15.48	0.30	46.35	206.17	4150.17	1823	0.34
Min	90	25.56	10.51	0.24	36.21	140	3506.67	900	0.19
Max	135	48.32	19.81	0.41	62.52	280	4793.34	2840	0.71
$LSD_{0.05}$	13.51	4.25	3.97	0.05	6.95	44.99	411.14	89.25	0.03
Significance	**	***	***	***	**	**	***	***	***
h^2	0.34	0.76	0.65	0.66	0.35	0.42	0.58	0.99	0.98

Statistical significance levels are denoted as follows: *P < 0.05, **P < 0.01, and ***P < 0.001; ns indicates non-significant results. $LSD_{0.05}$ = least significance difference, H^2=heritability. Symbol: Shoot length (ShL), shoot and root dry mass (SDM & RDM), root- to- total dry mass ratio (RTR), chlorophyll content (ChlC), initial transpiration (ITr), total transpiration (TTr), total water extracted (TWE), leaf area (LA), and fraction of transpirable soil water (FTSW).

4.2.2. Mean performance of genotypes

In the first experiment, under well-watered condition, Genotype #107 (W) showed the highest shoot length and the greatest shoot dry mass (155cm and 73.35g, respectively), whereas #30 (N) and #45 (N) had the shortest shoot length (75 cm) and the smallest shoot dry mass (35.29g), respectively. The root dry mass ranged from 13.46g #30 (N) to 33.7g #24 (W), while the root to total dry mass ratio ranged from 0.2 (#142 (N)) to 0.32 (#24 (W)), and (#45 (N)), each. Chlorophyll content ranged from 41.21 SPAD unit (#45 (N)) to 61.74 SPAD unit (#141 (W)). Genotype #107 (W) showed the largest leaf area (656.86cm^2), whereas genotype #24 (W) showed the smallest leaf area (396.28cm^2). Genotype #18 (W) showed the highest value for initial transpiration (170g $plant^{-1}$), whereas #21 (N) and #2 (W) had the lowest values for initial transpiration (60 g $plant^{-1}$ and 61.67g $plant^{-1}$, respectively). Genotype #97(W) had the greatest values for total transpiration and total water extracted (8600g $plant^{-1}$, and 8583.34g $plant^{-1}$, respectively), whereas #155 (N) and #142 (N) showed the smallest values for total transpiration and total water extracted (3523.34g/plant and 3623.34 g/ plant, respectively) (Table 4.8).

Under water stress conditions, #96 (N) showed the highest shoot length (113.34 cm), whereas #70 (W) showed the shortest one (82.34 cm). #30 (N) exhibited the highest shoot dry mass, measuring 39.86g, whereas #121 (W) had the lowest, measuring 22.24g. The root dry mass ranged between 12.10 g for #141 (W) and 17.09 for #96 (N). The root to total dry mass ratio ranged between 0.26 for #142 (N) and 0.38 for #70 (W). #91 (W) had the greatest values for chlorophyll content (56.22 SPAD unit), whereas #155 (N) showed the least value (41.37 SPAD unit). Genotypes #142(N) and #118 (N) had the highest value for initial transpiration (260g plant^{-1}), whereas genotype #24 (W) had the least value for initial transpiration (153.34g plant^{-1}). Genotype #30 (N) showed the greatest value for total transpiration (3866.67plant^{-1}), whereas #137 (W) showed the smallest values for total transpiration (2760g plant^{-1}). Genotype #45 (N) showed the greatest value for total water extracted (1776.67g plant-1), whereas #107 (W) showed the smallest value (933.34g plant-1) (Table 4.8).

Table 4.8. Comparative mean performance metrics of sorghum accessions under differential water regimes (WW: well-watered; WS: water-stress) in Experiment 1, quantifying: (i) shoot morphology [ShL: shoot length; SDM: shoot dry mass]; (ii) root architecture [RDM: root dry mass; RTR: root-to-total biomass ratio]; (iii) photosynthetic parameters [ChlC: chlorophyll content; LA: leaf area]; (iv) transpiration dynamics [ITr: initial transpiration; TTr: total transpiration]; and (v) soil water utilization [TWE: total water extracted; FTSW: fraction of transpirable soil water].

Exp1(first set of genotypes)									
Under well-watered (WW) condition									
Genotype	**ShL (cm)**	**SDM (g)**	**RDM (g)**	**RTR (ratio)**	**ChlC (SPAD unit)**	**LA (cm^2)**	**ITr (g/plant)**	**TTr (g/ plant)**	**TWE (g/plant)**
#18(W)	130	63.24	19.54	0.25	53.97	546.64	170	7756.67	7556.67
#24(W)	116.67	73.09	33.7	0.32	47.17	396.28	80	3830	3630
#97(W)	92.5	67.24	19.4	0.23	57.36	571.62	153.34	8600	8583.34
#107(W)	155	73.35	18.57	0.21	54.43	656.86	166.67	8080	7880
#70(W)	80	65.35	22.99	0.26	53.81	435.37	86.67	6236.67	6046.67
#2(W)	116	63.63	20.58	0.24	48.59	416.37	61.67	4873.34	4673.34
#91(W)	100.34	53.22	14.39	0.22	46.12	458.82	113.34	5613.34	5453.34
#121(W)	88	54.38	21.07	0.28	48.73	492.32	136.67	4386.67	4186.67
#137(W)	125	62.93	16.66	0.22	50.19	477.12	73.34	4360	4493.34
#141(W)	112.67	68.35	20.21	0.23	61.74	580.6	113.34	7013.34	6813.34
#142(N)	115	61.11	14.77	0.2	47.75	483.38	143.34	3823.34	3623.34
#27(N)	122.34	52.81	16.67	0.25	44.59	597.4	106.67	4880	4866.67
#155(N)	90	72.31	23.25	0.25	58.02	487.62	130	3523.34	3990
#118(N)	87.34	60.16	16.54	0.22	51.24	416.34	73.34	5466.67	5266.67
#21(N)	93	43.26	18.08	0.3	52.64	564.66	60	3980	4113.34
#51(N)	138	70.63	18.24	0.22	59.61	576.77	153.34	4860	4660
#149(N)	91.67	54.68	14.02	0.21	46.96	545.96	86.67	4140	4273.34
#45(N)	87.67	35.29	16.21	0.32	41.21	527.42	120	4253.34	4053.34
#96(N)	150	53.74	14.75	0.23	46.56	540.44	120	5906.67	6040
#30(N)	75	49.68	13.46	0.22	51.85	458.32	80	3743.34	3673.34

W: Wide, N: Narrow, Results show the average of three replicate plants per genotype

Table 4.8 (continued)

Exp1(first set of genotypes)									
Under water-stress (WS) condition									
Genotype	**ShL (cm)**	**SDM (g)**	**RDM (g)**	**RTR (ratio)**	**ChlC (SPAD unit)**	**ITr(g/ plant)**	**TTr(g/ plant)**	**TWE(g/ plant)**	**FTSW**
#18(W)	89.34	27.33	12.73	0.33	45.16	250	3486.67	1426.67	0.36
#24(W)	89.34	26.82	14.65	0.36	42.34	153.34	3413.34	1133.34	0.36
#97(W)	102.67	32.81	13.65	0.3	47.67	226.67	3370	1206.67	0.31
#107(W)	90.67	26.25	13.8	0.35	42.03	220	2931.67	933.34	0.35
#70(W)	82.34	24.65	14.52	0.38	43.11	220	3243.34	1113.34	0.37
#2(W)	97	30.49	12.73	0.3	45.36	226.67	3373.34	1040	0.33
#91(W)	100	32.89	15.79	0.33	56.22	180	3293.34	1133.34	0.45
#121(W)	86.34	22.24	12.05	0.36	41.94	206.67	3056.67	1240	0.42
#137(W)	89	26.55	12.96	0.34	42.88	173.34	2760	1113.34	0.49
#141(W)	90	27.61	12.1	0.31	45.38	220	3516.67	1233.34	0.38
#142(N)	87.34	36.62	12.83	0.26	41.93	260	3140	1346.67	0.37
#27(N)	97	30.57	12.93	0.3	47	206.67	3446.67	1446.67	0.34
#155(N)	85.34	34.26	13.97	0.29	41.37	226.67	3543.34	1426.67	0.35
#118(N)	105.67	33.82	15.8	0.32	51.36	260	3580	1510	0.35
#21(N)	88	26.64	14.45	0.36	45.04	240	3820	1640	0.37
#51(N)	101.34	32.14	13.65	0.3	51.68	220	3626.67	1626.67	0.24
#149(N)	90	31.24	15.14	0.33	44.33	233.34	3685	1773.34	0.35
#45(N)	97.34	30.47	16.05	0.35	49.01	226.67	3746.67	1776.67	0.35
#96(N)	113.34	39.61	17.09	0.31	43.42	173.34	3713.34	1753.34	0.26
#30(N)	92.34	39.86	16.53	0.3	43.83	233.34	3866.67	1626.67	0.26

W: Wide, N: Narrow, Results show the average of three replicate plants per genotype

In the second experiment, under well-watered condition, Genotype #87(W) and Genotype #127 (W) showed the highest shoot length and shoot dry mass (173.34cm and 72.57g, respectively), whereas genotype #67(N) had the shortest shoot length and the lowest shoot dry mass (116cm and 42.59g, respectively). Genotype #98 (N) had the highest root architecture [RDM: root dry mass and RTR: root-to-total biomass ratio](29.44g and 0.38, respectively), whereas #127 (W) showed the lowest root architecture [RDM: root dry mass and RTR: root-to-total biomass ratio] (13.3g and 0.16, respectively). Genotype #151 (N) had the greatest values for chlorophyll content (58.27 SPAD unit), whereas #25 (N) showed the least value (47.26 SPAD unit). Genotype #98 (N) showed the largest leaf area (682.57 cm^2), whereas genotype #151 (N) showed the smallest (354.83cm^2). Genotype #78 (W) showed the greatest value for initial

transpiration (213.34g), whereas #114 (N) and #92 (W) had the least value for initial transpiration (66.67g plant-1 and 60g plant^{-1}, respectively). Genotype #67(N) showed the greatest values for total transpiration and total water extracted (7560 g/plant and 7360 g/plant, respectively), whereas #120(N) showed the smallest values for total transpiration and total water extracted (3110g plant^{-1} and 3076.67g plant^{-1}, respectively) (Table 4.9).

Under water stress conditions, Genotype #78 (W) showed the highest shoot length (126.67cm), whereas #98(N) showed the smallest value (100cm). Genotype #151 (N) exhibited the highest shoot dry mass, measuring 48.32g, whereas #44 (W) had the lowest, measuring 25.56g. The root dry mass ranged between 10.51 g for #127 (W) and 19.81 for #151 (N). The root to total dry mass ratio ranged between 0.24 for #133 (W) and #127 (W) and 0.41 for #44 (W). Genotype #78 (W) had the greatest values for chlorophyll content (53.58 SPAD unit), whereas #44 (W) showed the least value (40.26 SPAD unit). Genotype #7 (W) and #133 (W) showed the greatest value for initial transpiration (253.34 g plant^{-1}, each), whereas #87(W) had the least value for initial transpiration (160 g plant^{-1}). Genotype #151 (N) showed the greatest values for total transpiration (4793.34g plant^{-1}), whereas genotype #7 (W) showed the smallest values for total transpiration (3506.67g plant^{-1}). Genotype #151 (N) showed the greatest value for total water extracted (2826.67g plant-1), whereas #44 (W) and #135 (W) showed the smallest value (1020 g plant-1) (Table 4.9).

Table 4.9. Comparative mean performance metrics of sorghum accessions under differential water regimes (WW: well-watered; WS: water-stress) in Experiment 2, quantifying: (i) shoot morphology [ShL: shoot length; SDM: shoot dry mass]; (ii) root architecture [RDM: root dry mass; RTR: root-to-total biomass ratio]; (iii) photosynthetic parameters [ChlC: chlorophyll content; LA: leaf area]; (iv) transpiration dynamics [ITr: initial transpiration; TTr: total transpiration]; and (v) soil water utilization [TWE: total water extracted; FTSW: fraction of transpirable soil water].

Exp2 (second set of genotypes)									
Under well-watered (WW) condition									
Genotype	**ShL (cm)**	**SDM (g)**	**RDM (g)**	**RTR (ratio)**	**ChlC (SPAD unit)**	**LA (cm^2)**	**ITr (g plant-1)**	**TTr (g plant-1)**	**TWE**
#92(W)	110.67	60	18.51	0.25	50.8	602.52	60	5100	4566.67
#7(W)	170	64.15	14.79	0.2	55.63	442.85	113.34	6213.34	6013.34
#78(W)	161.67	63.69	18.96	0.24	54.15	569.76	213.34	6800	6600
#44(W)	131.34	71.61	17.6	0.21	57.22	493.93	100	6736.67	6536.67
#104(W)	135.34	62.53	15.94	0.21	53.04	667.72	106.67	7046.67	6846.67
#133(W)	124	57	16.74	0.24	53.81	480.86	140	6546.67	6680
#127(W)	140.34	72.57	13.3	0.16	52.97	521.95	146.67	6493.34	6293.34
#87(W)	173.34	62.23	20.55	0.26	54.24	497.69	110	6330	6130
#135(W)	120	59.44	18.39	0.24	50.61	569.91	93.34	6440	6106.67
#40(W)	126.67	53.29	16.18	0.24	56.5	563.03	120	6120	5920
#26(N)	141.67	48.18	13.28	0.22	52.5	544.49	100	4573.34	4706.67
#13(N)	131.67	57.62	19.52	0.27	56.3	582.48	146.67	6806.67	6606.67
#67(N)	116	42.59	20.8	0.33	50.85	518.53	160	7560	7360
#112(N)	131.67	47.64	16.95	0.27	53.44	573.16	133.34	6806.67	6606.67
#143(N)	153	46.28	14.16	0.25	52.15	575.45	186.67	6646.67	6446.67
#114(N)	125.67	48.2	16.01	0.26	55.53	467.34	66.67	3406.67	3206.67
#98(N)	119	50.14	29.44	0.38	52.79	682.57	166.67	3556.67	3390
#120(N)	145	50.95	18.51	0.27	54.62	505.56	130	3110	3076.67
#25(N)	116.34	46.02	14.25	0.25	49.17	606.62	120	5246.67	5046.67
#151(N)	135	44.39	15.83	0.27	58.27	354.83	100	3626.67	3426.67

W: Wide, N: Narrow, Results show the average of three replicate plants per genotype

Table 4.9 **(**continued)

Exp2(second set of genotypes)									
Under water stress (WS) condition									
Genotype	**ShL (cm)**	**SDM (g)**	**RDM (g)**	**RTR (ratio)**	**ChlC (SPAD unit)**	**ITr (g/ plant)**	**TTr (g/ plant)**	**TWE (g/ plant)**	**FTSW**
#92(W)	104.34	30.19	12.23	0.29	42.78	200	4240	1160	0.3
#7(W)	116	32.02	12	0.28	45.21	253.34	3506.67	1193.34	0.35
#78(W)	126.67	45.58	15.99	0.26	53.58	240	4190	1260	0.24
#44(W)	117.34	25.56	17.39	0.41	40.26	193.34	3826.67	1020	0.36
#104(W)	120	30.38	12.93	0.3	42.99	170	3573.34	1073.34	0.34
#133(W)	108.67	35.68	11.04	0.24	48.73	253.34	4000	1166.67	0.32
#127(W)	109.67	34.36	10.51	0.24	46.25	220	4026.67	1133.34	0.28
#87(W)	119.67	37.52	12.38	0.25	48.27	160	3826.67	1060	0.46
#135(W)	122	37.95	16.54	0.31	47.87	213.34	3833.34	1020	0.29
#40(W)	125	39.85	14.42	0.27	50.9	240	4050	1153.34	0.29
#26(N)	119.67	37.47	15.83	0.3	50.68	180	3990	2150	0.29
#13(N)	120	38.28	15.09	0.29	40.71	193.34	4236.67	2196.67	0.33
#67(N)	109	35.82	19.53	0.36	48.88	226.67	4500	2480	0.24
#112(N)	111.67	36.9	14.08	0.28	47.89	193.34	3786.67	2520	0.32
#143(N)	125	38.37	18.74	0.33	40.8	180	3896.67	2540	0.27
#114(N)	115	44.6	15.64	0.26	47.03	180	4603.34	2413.34	0.25
#98(N)	100	39.11	17.36	0.31	41.28	220	4660	2620	0.27
#120(N)	113	37.48	18.69	0.34	46.72	213.34	4676.67	2753.34	0.29
#25(N)	105	45.01	19.43	0.31	44.75	173.34	4786.67	2720	0.2
#151(N)	120	48.32	19.81	0.3	51.6	220	4793.34	2826.67	0.23

W: Wide, N: Narrow, Results show the average of three replicate plants per genotype

4.2.3. Growth and transpiration attributes of genotypes under contrasting moisture regimes

In both trials, the sorghum genotypes exhibited a reduction in biomass production under water stress conditions. In Exp 1, shoot dry mass experienced the most significant reduction (48.84% of well-watered), while in Exp 2, the reduction was 32.33% of well-watered. Root dry mass was reduced by 24.08% of well-watered in Exp 1 and 11.50% of well-watered in Exp 2 (Table 4.10).

Under well-watered conditions, the wide-root angle genotypes displayed significantly ($p < 0.01$) higher mean shoot dry mass relative to the narrow-root angle genotypes in both

experiments (Table 4.10). The average root dry mass proved significantly greater ($p < 0.05$) among genotypes with wide root angles when measured against those with narrow angles, but only in Experiment 1. Conversely, when exposed to water stress, there were notable statistical differences in both shoot and root dry mass between the wide and narrow-root angle genotypes, with the narrow-root angle genotypes showing higher values.

Table 4.10. Comparative analysis of growth and water-use characteristics across sorghum genotypes under contrasting water regimes in two experimental runs, including: shoot biomass (SDM), root bio mass (RDM), root biomass allocation ratio (RTR), and total transpiration (TTr) values

	Experiment-1				**Experiment-2**			
Traits	**WW**	**WS**	***t-test***	**%**	**WW**	**WS**	***t-test***	**%**
SDM (g)	59.92	30.64	***	48.84%	55.43	37.52	**	32.33%
RDM (g)	18.66	14.17	**	24.08%	17.49	15.48	*	11.50%
RTR (gg-1)	0.24	0.33	***	37.50%	0.25	0.30	**	20%
TTr (g)	5266.34	3430.67	***	34.84%	5758.34	4150.17	***	27.85%

ns = Not significant ($P > 0.05$),* = Significant at $P < 0.05$,** = Significant at $P < 0.01$, *** = Significant at $P < 0.001$ (All comparisons made between well-watered (WW) and water-stressed (WS) conditions), % denotes percentage values

Furthermore, under well-watered conditions, the average total transpiration was markedly ($p < 0.05$) lesser in narrow-root angle accessions compared to wide-root angle genotypes in both experiments. However, under water stress conditions, there were substantial changes ($p < 0.05$) in average total transpiration among narrow-root angle & wide-root angle genotypes, with the narrow-root angle genotypes exhibiting higher mean values. Furthermore, under water stress conditions, the narrow-root angle genotypes demonstrated significantly higher mean total water extraction ($p < 0.05$) & significantly lower average FTSW measurements ($p < 0.05$) compared to the wide-root angle genotypes in both experiments, as shown in Table 4.11.

Table 4.11. Comparative analysis of shoot biomass (SDM), root biomass (RDM), cumulative transpiration (TTr), soil water extraction (TWE), and transpirable water fraction (FTSW) between genotypes exhibiting contrasting root architectures (wide vs. narrow angles) under contrasting moisture regimes (well-watered vs. water-stressed conditions) across two experimental trials

SI No	Trait	Root angle	Exp1		Exp2	
			WW	WS	WW	WS
			Mean	Mean	Mean	Mean
1	SDM	Wide	64.5 ± 6.75 [a]	27.8 ±3.40 [b]	62.7 ± 5.95 [a]	34.9 ± 5.71 [a]
		Narrow	55.4 ± 11.4 [b]	33.5 ± 4.22 [a]	48.2 ± 4.15 [b]	40.1 ± 4.24 [b]
2	RDM	Wide	20.7 ± 5.15 [a]	13.5 ± 1.21 [a]	17.1 ± 2.14 [a]	13.5 ± 2.40 [b]
		Narrow	16.6 ± 2.84 [b]	14.8 ± 1.51[b]	17.9 ± 4.94 [a]	17.4 ± 2.10 [a]
3	TTr	Wide	6075 ± 1750 [a]	3245 ± 250 [b]	6383 ± 530 [a]	3907 ± 241 [b]
		Narrow	4458 ± 790 [b]	3617 ± 210 [a]	5134 ± 1698 [b]	4393 ± 383 [a]
4	TWE	Wide	5932 ± 1715[a]	1157 ± 132[b]	6169 ± 643 [a]	1124 ± 78 [b]
		Narrow	4456 ± 761[b]	1593 ± 154[a]	4987 ± 1663 [a]	2522 ± 224 [a]
5	FTSW	Wide	-	0.38 ± 0.06 [a]	-	0.32 ± 0.06 [a]
		Narrow	-	0.32 ± 0.05 [b]	-	0.27 ± 0.04 [b]

Within each experimental trial, mean values sharing common alphabetical superscripts indicat e no significant difference ($p > 0.05$) among treatments according to Tukey's honestly signific - ant difference test.

4.2.4. FTSW Threshold

The present study identified significant genotypic variations ($p < 0.001$) among sorghum genotypes for their FTSW to progressive soil drying. Table 4.12 presents genotype-specific responses of normalized transpiration ratio (NTR) to declining soil moisture availability (FTSW) across both experimental trials under progressive drought conditions. In Experiment 1, transpiration began to decline at FTSW values ranging from 0.24 for the narrow root angle genotype #51 (N) to 0.49 for the wide root angle genotype #137 (W), while in Exp 2, transpiration decline was observed at FTSW levels between 0.20 for the narrow root angle genotypes #25 (N), while wide root angle genotypes #87 (W) had the highest FTSW thresholds at 0.46, respectively.

Table 4.12. Critical soil water depletion (FTSW) threshold at which transpiration reduction initiates under progressive drought stress in sorghum accessions with divergent root architectures (wide vs. narrow angles) across both experimental trials.

EXP1				EXP2			
Genotype	**Root angle**	**FTSW threshold**	**95%CI**	**Genotype**	**Root angle**	**FTSW threshold**	**95%CI**
#18(W)	25.07	0.36 ± 0.13	0.69-0.71	#92	25.17	0.30 ± 0.09	0.37-0.58
#24(W)	24.88	0.36 ± 0.17	0.54-0.64	#7	23.13	0.35 ± 0.08	0.37-0.65
#97(W)	24	0.31 ± 0.06	0.20-0.45	#78	22.67	0.24 ± 0.08	0.27-0.44
#107(W)	23.88	0.35 ± 0.11	0.51-0.68	#44	22.63	0.36 ± 0.16	0.23-0.73
#70(W)	23.42	0.37 ± 0.05	0.15-0.49	#104	22.5	0.34 ± 0.05	0.55-0.78
#2(W)	23.34	0.33 ± 0.05	0.15-0.47	#133	22.42	0.32 ± 0.09	0.34-0.54
#91(W)	22.98	0.45 ± 0.04	0.30-0.56	#127	22.09	0.28 ± 0.08	0.35-0.45
#121(W)	22.84	0.42 ± 0.05	0.17-0.56	#87	21.67	0.46 ± 0.09	0.32-0.82
#137(W)	22.09	0.49 ± 0.05	0.38-0.82	#135	21.5	0.29 ± 0.15	0.34-0.55
#141(W)	21.67	0.38 ± 0.08	0.24-0.56	#40	21.34	0.29 ± 0.08	0.18-0.37
#142(N)	11.92	0.37 ± 0.09	0.36-0.42	#26	12.09	0.29 ± 0.05	0.38-0.90
#27(N)	11.17	0.34 ± 0.02	0.43-0.56	#13	11.82	0.33 ± 0.05	0.32-0.74
#155(N)	11.09	0.35 ± 0.03	0.24-0.44	#67	11.75	0.24 ± 0.11	0.53-0.66
#118(N)	10.09	0.35 ± 0.26	0.22-0.72	#112	11.67	0.32 ± 0.11	0.52-0.69
#21(N)	10	0.37 ± 0.14	0.34-0.42	#143	11.67	0.27 ± 0.10	0.31-0.65
#51(N)	9.84	0.24 ± 0.04	0.26-0.31	#114	11.59	0.25 ± 0.04	0.67-0.88
#149(N)	9.63	0.35 ± 0.08	0.17-0.61	#98	11.5	0.27 ± 0.07	0.15-0.54
#45(N)	9.25	0.35 ± 0.05	0.12-0.36	#120	11.42	0.29 ± 0.08	0.32-0.45
#96(N)	8.25	0.26 ± 0.03	0.46- 0.71	#25	9.63	0.20 ± 0.13	0.31-0.72
#30(N)	7.88	0.26 ± 0.05	0.09-0.92	#151	7.75	0.23 ± 0.06	0.29-0.89
Mean W.		0.38 ± 0.06		Mean W.		0.32 ± 0.06	
Mean N.		0.32 ± 0.05		Mean N.		0.26 ±0.04	

W: Wide, S: Narrow, FTSW: Fractional of Transpirable Soil Water, CI: Confidence intervals

4.2.5. Traits association

In the first experiment, under well-watered condition shoot length demonstrated a statistically significant positive association with leaf area under moisture stress condition (r = 0.46*) (Figure 4.9a), whereas under water stress condition shoot length showed positive correlation with shoot dry mass (r = 0.57**), root dry mass (r = 0.49*), and chlorophyll content (r = 0.58**), while the association between shoot length and FTSW was negative (r = - 0.47*) (Figure 4.9b). In Exp 2, shoot length had no significant correlation with all the traits both under well-watered and water stress condition (Figure 4.9 c & d). In the first tria1, shoot dry biomass demonstrated

a statistically significant positive association with root dry biomass regardless of the soil water condition (r = 0.55* and 0.57*, respectively) (Figure 4.9a & b), however, in Exp 2, this relationship was only present when the plants were under water stress condition (r = 0.45*) (Figure 4.9d). Shoot dry mass was also positively correlated with total transpiration rate (r = 0.48*), total water extraction (r = 0.49*) and negatively correlated with root to total dry mass ratio (r = - 0.74***) and FTSW (r = - 0.57*), under water stress condition (Figure 4.9b). In Exp2, under well-watered condition, shoot dry biomass also demonstrated a statistically significant positive association with chlorophyll content (r = 0.64**) and demonstrated a statistically significant negative association with root to total dry mass ratio (r = - 0.63**) (Figure 4.9c), but it was positively correlated with chlorophyll content (r = 0.53*), total transpiration (r=0.63*), and total water extracted (r = 0.55*) under water stress condition (Figure 4.9d).

Root dry biomass was positively correlated with root to total dry biomass ratio (r = 0.58**) under well-watered condition (Figure 4.9a), however, in Exp 2, this correlation was positive (r = 0.81*** and 0.68***), regardless of soil moisture status (Figure 4.9c & d). In Exp 1, root dry mass demonstrated a statistically significant positive association was also positively correlated with total transpiration (r = 55*) and total water extracted (r = 0.52*) under water stress conditions (Figure 4.9b). Root dry mass demonstrated a statistically significant negative association with FTSW (r = - 0.58**) and positively associated with total transpiration (r = 62**) and total water extracted (r = 0.71***) under water stress conditions (Figure 4.9d). In Exp1, under well-watered condition, leaf area was positively linked to initial transpiration (r = 0.57**), total transpiration (r = 0.47*) and total water extracted (r = 0.49*). Similarly, initial transpiration was positively associated with total transpiration (r = 0.52*) and total water extracted (r = 0.52*) (Figure 4.9a). Total transpiration was positively interrelated with total water extracted (r = 0.70*** to 0.99***) regardless of the soil water condition in both experiments (Figure 4.9a-d). In both experiments, under water stress condition, total transpiration showed negative relationships with FTSW with coefficients r = - 0.65 and r = - 0.68, respectively. Moreover, total water extraction demonstrated a negative correlation with FTSW, with association coefficients of r = - 0.50* and r = - 0.56*, correspondingly (Figure 4.9 b & d).

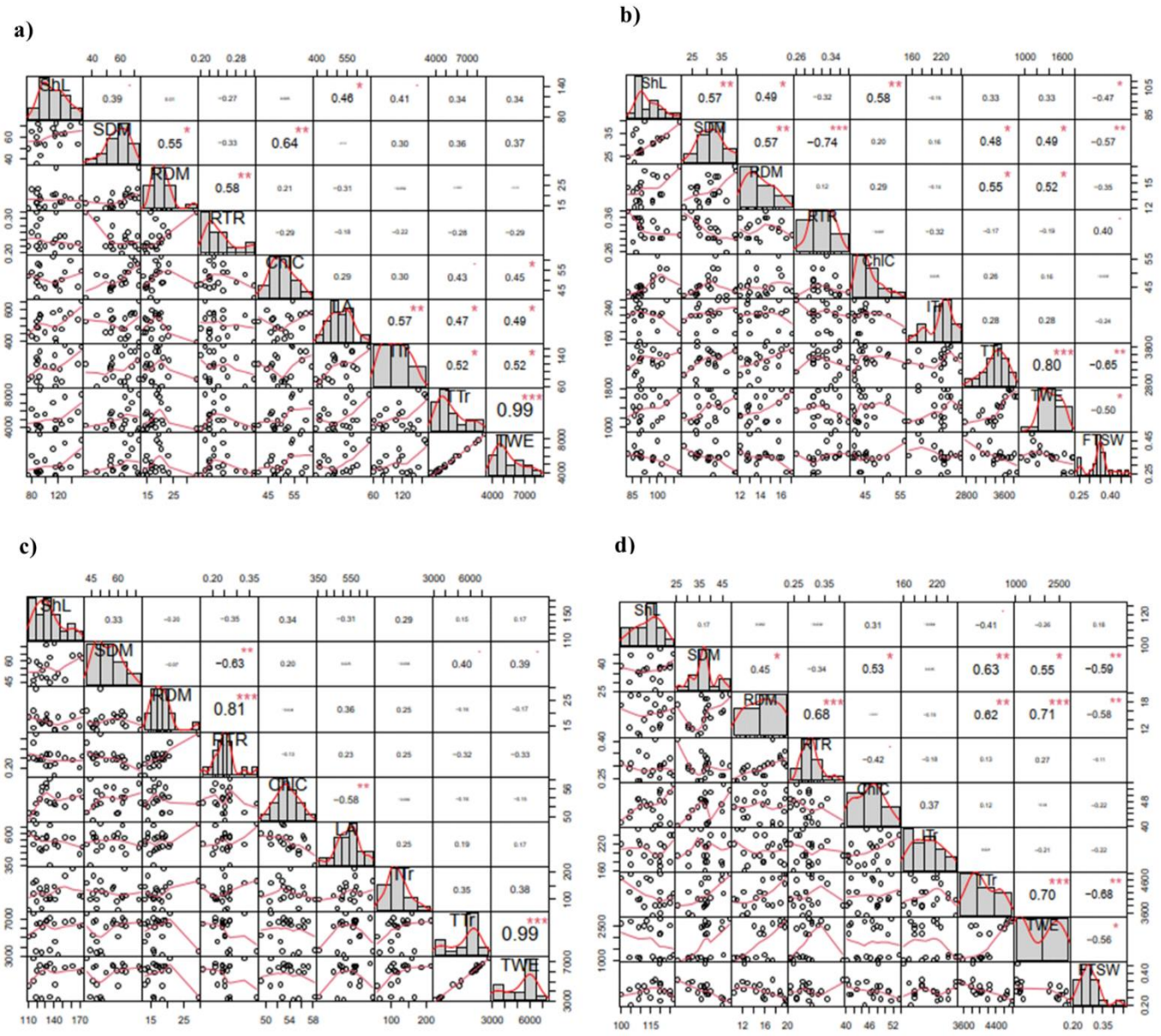


Figure 4.9. Pairwise trait correlations (Pearson's r) across moisture regimes: (a) well-watered (WW) and (b) water-stressed (WS) conditions in Experiment 1; (c) WW and (d) WS conditions in Experiment 2. Significance levels: ns (P > 0.05), * (P < 0.05), ** (P < 0.01), *** (P < 0.001). Measured traits: Shoot length (ShL), shoot dry mass (SDM), root dry mass (RDM), root:total mass ratio (RTR), chlorophyll content (ChlC), initial transpiration (ITr), total transpiration (TTr), water extraction (TWE), leaf area (LA), and transpirable soil water fraction (FTSW).

4.2.6. Regression analysis between growth and transpiration attributes

Regression analysis revealed a significant link between biomass accumulations (RDM & SDM) and and total transpiration (TTr) under water stress conditions, with R^2 values of 0.32 ($p < 0.01$)

and 0.30 ($p < 0.05$) for experiment 1, and R^2 values of 0.20 ($p < 0.05$) and 0.38 ($p < 0.01$) for experiment 2, respectively (Figure 4.10). Moreover, initial transpiration also proved a substantial relationship with FTSW threshold in water deficient conditions, with R^2 values of 0.32; at $p < 0.01$ and 0.56 at $p < 0.001$ in trial 1 and 2, respectively (Figure 4.11).

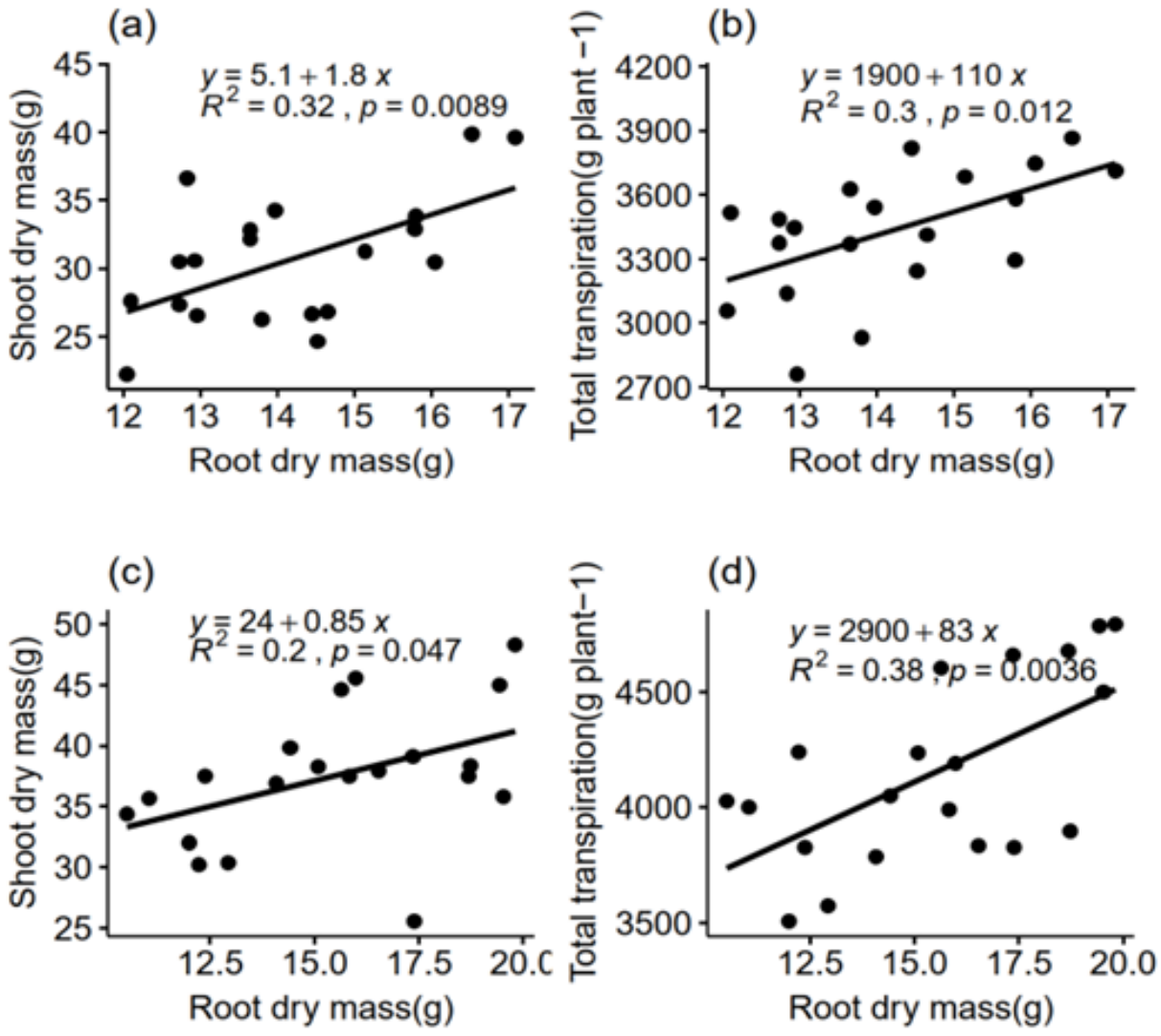


Figure 4.10. Regression analysis between root and shoot biomass and total transpiration (g plant^{-1}), for twenty sorghum genotypes in water deficient conditions (a&b)in trial1, and (c&d) in trial 2. ns at $p > 0.05$, while significant at $p < 0.05$.

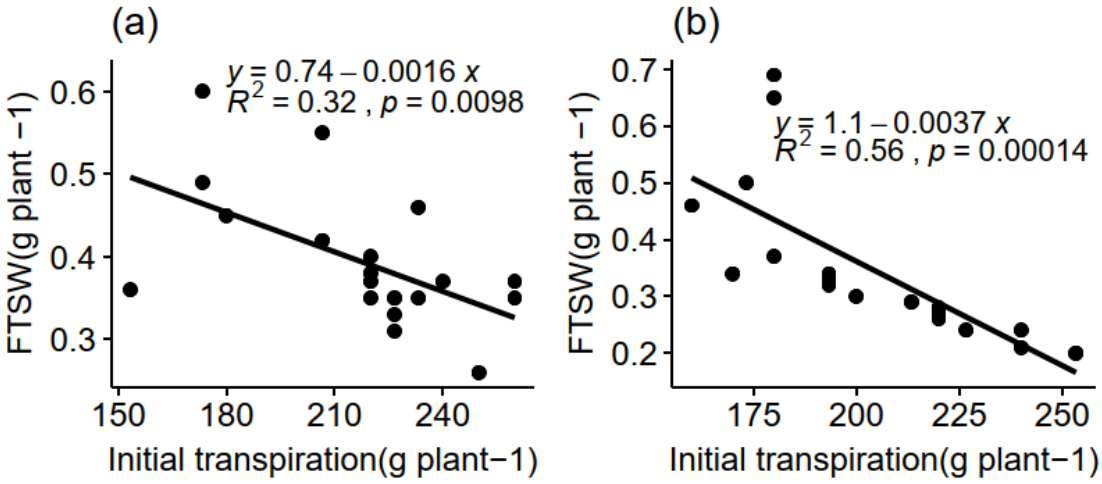


Figure 4.11. Regression analysis between initial transpiration (gplant-1) and FTSW, for twenty performance of sorghum accessions under drought stress conditions: (a) Experiment 1 and (b) Experiment 2. Statistical significance thresholds: ns = P > 0.05; * = P < 0.05.

4.3. Multi Locus-Genome wide association analysis

4.3.1. Single Nucleotide Polymorphism markers, population stratification and linkage disequilibrium

Genome-wide SNP distribution analysis revealed 127,804 high-confidence polymorphic sites distributed across the 10 sorghum chromosomes. Chromosomal mapping showed the highest marker densities on chromosome 4 (24,470 SNPs), followed by chromosome 1 (16,547 SNPs) and chromosome 2 (14,909 SNPs) (Figure 4.12). Concentration of SNP markers within 1Mb window size across ten chromosomes. Colored bars represent SNP counts within the 1Mb intervals across the sorghum chromosomes as shown in Figure 4.13. The color gradient is as follows; The twelve colors in the figure, ordered by increasing intensity and corresponding SNP count within a 1Mb window size, are: white (0 SNP), lightest green (1 SNP), medium light green (34 SNPs), medium green (67 SNPs), medium dark green (100 SNPs), light yellow (133SNPs), medium yellow (166 SNPs), dark yellow to light orange (199SNPs), light red (232 SNPs), medium red (265 SNPs), dark red (298SNPs), and very dark red (>= 300SNPs). This gradient visually represents the density of SNPs across the chromosomes, with white indicating the lowest and very dark red indicating the highest SNP counts within the specified window size. The chromosomal heatmap effectively illustrates variations in SNP density across the sorghum genome, with color intensity corresponding to marker concentration levels. The scale

ranges from white (0 SNPs) to deep red (≥300 SNPs) per 1 Mb genomic interval, revealing non-uniform distribution patterns (Figure 4.13). Comparative analysis identified chromosomes 2 and 3 as having markedly elevated SNP densities relative to chromosomes 1, 5, and 8. These observed disparities likely reflect underlying variations in chromosomal structure and meiotic recombination frequencies.

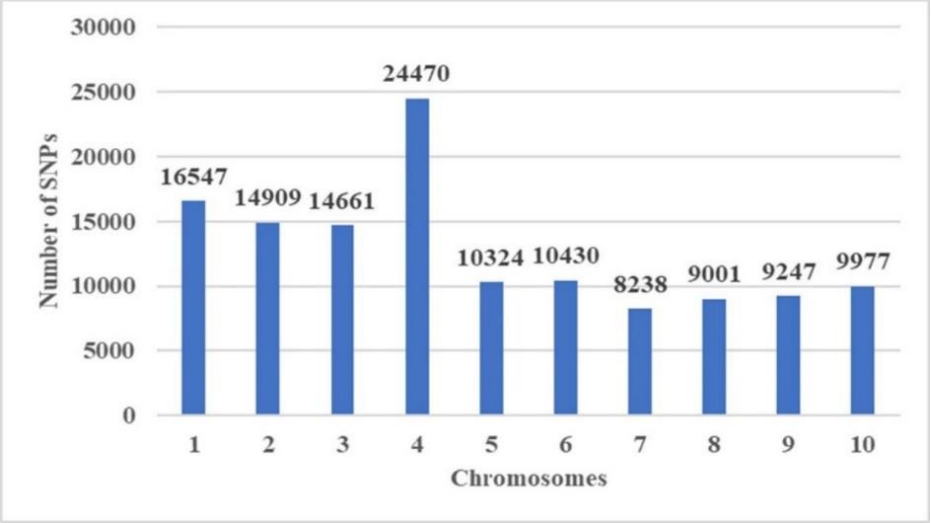


Figure 4.12. Frequency of SNPs identified across ten sorghum chromosomes

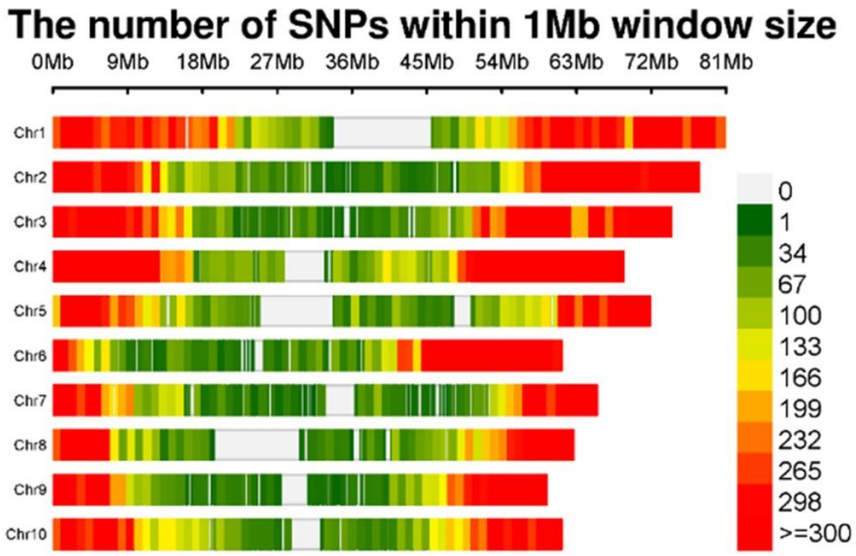


Figure 4.13. Density of SNP markers with in 1Mb window size across ten chromosomes. Colored bars are SNP counts in 1Mb intervals (using SRplot).

Genetic clustering analysis using the K method with entropy-based optimization revealed five distinct subpopulations (K=5) among the 129 sorghum genotypes, designated as SP1 through SP5 (Figure 4.14). The optimal number of clusters was determined by identifying the inflection point in the entropy criterion (Figure 4.15). The subpopulations varied in size, with SP3 being the most predominant (59 genotypes, 45.7% of total), followed by SP2 (33 genotypes) and SP5 (17 genotypes), while SP1 contained 13 genotypes and SP4 comprised 7 genotypes (Appendix 8.5).

Geographic distribution patterns were evident among the subpopulations. SP1 included genotypes primarily from Gambela (6) and Amhara (4), with additional representatives from SEPR (2) and one of unknown origin. SP2 was dominated by accessions from Oromia (19), along with genotypes from Tigray (5), SEPR (4), Amhara (2), and single accessions from Dire Dawa, Gambela, and unknown regions. The largest subpopulation, SP3, contained genotypes from multiple regions including Amhara (17), Oromia (16), Tigray (9), Gambela (6), Benishangul Gumuz/unknown regions (3), SEPR/Somali (2), and Afar (1). SP4 was composed of genotypes from Amhara/Oromia (2) and Tigray/SEPR/Benishangul Gumuz (1), while SP5 consisted mainly of accessions from Benishangul Gumuz (9) and Oromia (6), with one each from Dire Dawa and Amhara. Within each subpopulation, genotypes could be further classified as either pure or admixed types based on their genetic profiles.

Genotypes with membership proportions (Q-value) $\geq 0.60\%$ were considered pure and those $< 60\%$ were considered admixtures (Agre et al., 2019). The genetic architecture of the five subpopulations exhibited distinct patterns of purity and admixture. SP3 demonstrated the highest proportion of pure genotypes (81.3%), followed by SP5 (88.2%) and SP2 (75.7%), while SP4 showed predominantly admixed composition (71.4%). SP1 presented a more balanced distribution with 53.8% pure and 46.2% admixed genotypes. Across all subpopulations combined, pure types constituted 75.1% of genotypes compared to 24.9% admixed individuals (Figure 4.14). Genome-wide analysis of linkage disequilibrium revealed a decay distance of approximately 14.4 kilobases when using an r^2 threshold of 0.1 (Figure 4.16).

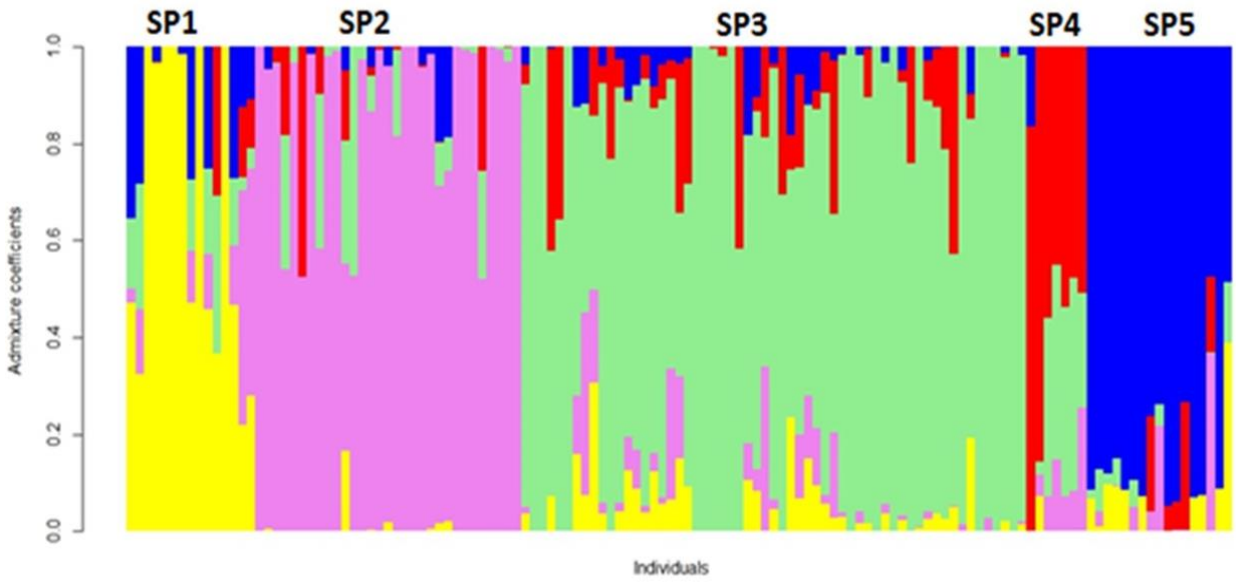


Figure 4.14. The ancestry composition plot illustrates the genetic stratification of 129 sorghum genotypes analyzed using 127,804 SNP markers at K=5 clusters. The vertical axis quantifies admixture proportions, while each vertical bar corresponds to an individual genotype. Color segmentation within each bar represents the probabilistic assignment to one of the five distinct subpopulations (SP1-SP5), as denoted in the ancestry matrix. Subpopulation designations are annotated above the graphical representation, with the varying color patterns demonstrating the degree of genetic admixture among the accessions.(SP1= yellow, SP2= purple, SP3= light green, SP4 = red, and SP5 =blue).

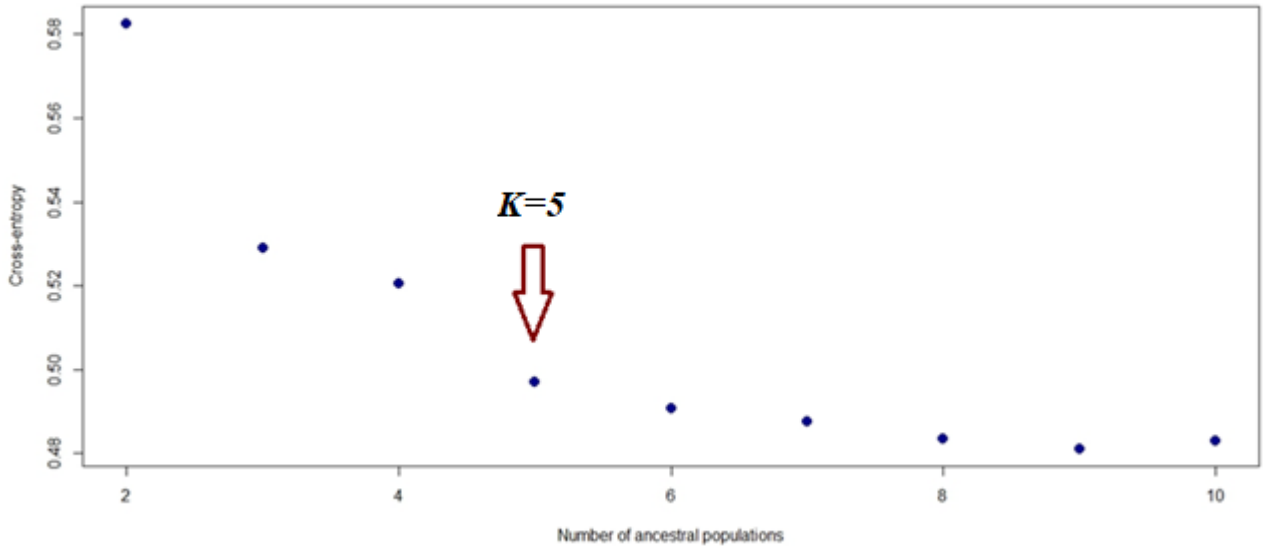


Figure 4.15.The maximum *K* determined by LEA package (***K=5***), which divided the full population into five subgroups.

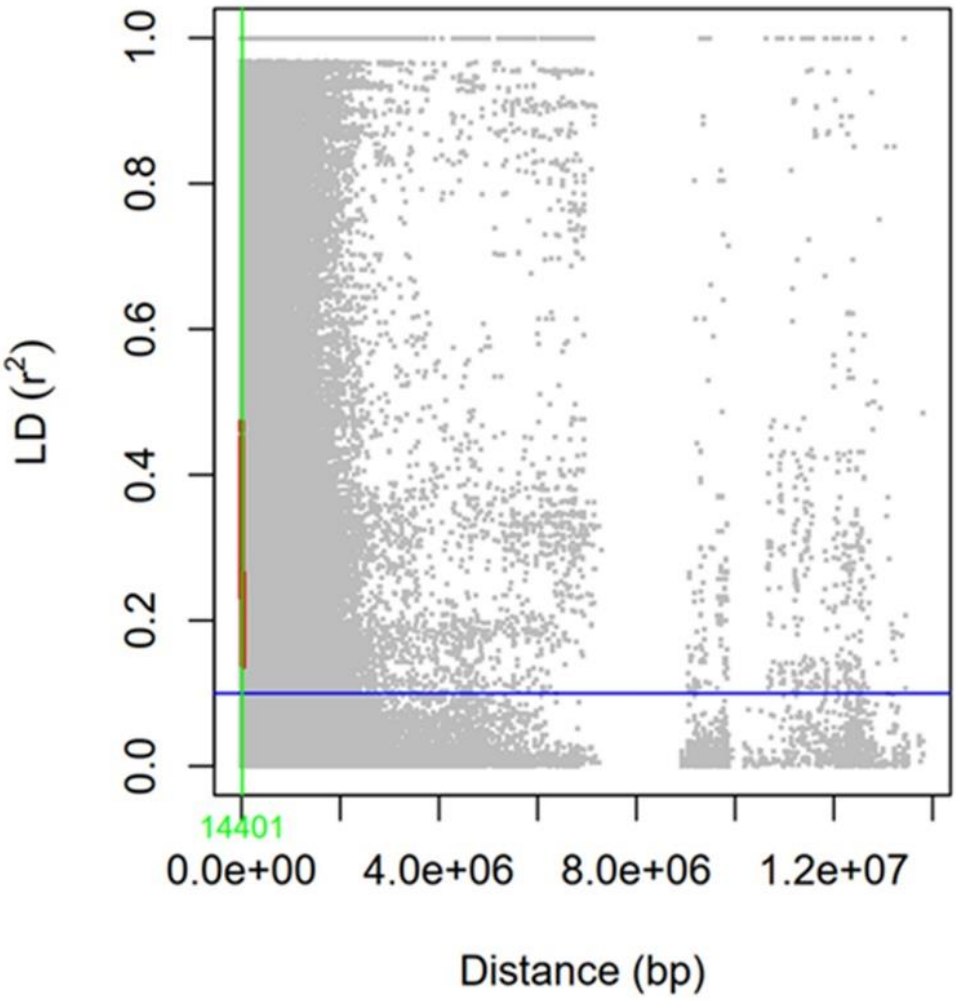


Figure 4.16. Genome-wide linkage disequilibrium (LD) decay pattern visualized through pairwise marker r^2 values. A horizontal reference line (blue) indicates the half-decay threshold (r^2=0.01), while the vertical line (green) marks the average LD decay distance of 14,401 base pairs across the entire genome.

4.3.2. Identification of significant Quantitative Trait Nucleotides (QTNs) for thirteen Traits

Genome-wide association analysis identified 388 significant QTNs associated with thirteen key phenotypic traits, using a LOD score cutoff of ≥3.0 (equivalent to -log10 (10^{-3})). These trait-associated markers were distributed across all ten sorghum chromosomes, with complete details provided in Appendix 8.6. Comparative evaluation of six multi-locus GWAS approaches demonstrated varying detection efficiencies: the mrMLM method proved most effective, detecting 190 QTNs, followed by FASTmrMLM with 97 associations, while pKWmEB failed

to identify any significant QTNs. Chromosomal distribution analysis revealed chromosome 3 as having the highest marker density (60 QTNs), preceding chromosomes 1 (52 QTNs) and 4 (48 QTNs). The detected QTNs exhibited LOD scores ranging from 3 to 62, with explained phenotypic variance (r^2) varying between 6.91×10^{-6} and 27.3 (Table 4.13).

Table 4.13. Summary of five multi-locus GWAS models used for QTN detection

Models	**QTN effect**	**LOD score**	**r^2%**	**QTNs**
mrMLM	-515 -456	3 - 62	0.007 - 27.3	190
FASTmrMLM	-752 - 392	3 - 43	0.0003 - 16.9	97
FASTmrEMMA	-564 - 7.4	3- 5	0.000000131 -9.85	9
pLARmEB	-648 - 329	3 -39	0.00000691- 14.42	64
ISIS EM-BLASSO	-436 - 368	3 - 16	0.00000885-24.97	28
Total	**-752 - 456**	**3- 62**	**0.000000131- 27.3**	388

Note: r^2 (%) is the proportion of total phenotypic variation explained by each QTN.

Abbreviations: (i) FASTmrEMMA (spectral-transformed random-SNP-effect mixed model),(ii) FASTmrMLM (optimized multi-locus MLM with spectral transformation),(iii) ISIS EM-BLASSO (iterative Bayesian LASSO with EM screening),(iv) pLARmEB (polygenic-adjusted least angle regression with empirical Bayes), and(v) mrMLM (multi-locus random-SNP-effect MLM).

Genome-wide association analysis identified 49 stable QTNs associated with thirteen phenotypic traits, with genomic positions detailed in Table 4.14 and Figure 4.17. These quantitative trait nucleotides were distributed across all sorghum chromosomes, exhibiting variable distribution patterns. Chromosome 1 contained the highest number of associations (10 QTNs influencing eight traits), while chromosomes 5 and 8 showed the fewest significant associations. Among the detected QTNs, 36 were identified by three different multi-locus GWAS methods, and 12 were detected by four methods. A particularly robust association was observed for QTN S1_6012717 on chromosome 1, which was consistently identified by five independent analytical approaches and showed association with root-to-shoot ratio (LOD scores ranging from 3.50 to 8.70, explaining 1.41-6.98% of phenotypic variance).

The percentage of phenotypic variance explained (PVE) by these stable QTNs varied considerably across traits. For leaf angle (LAg), four QTNs accounted for 5.72-14.81% of phenotypic variation, while three QTNs influenced leaf area (LAr), explaining 3.44-9.49% of variation. Root-related traits showed particularly wide ranges, with five QTNs for root angle (RAg) explaining 0.60-15.37% of variance, nine QTNs for root dry weight (RDW) contributing 0.00-24.97%, and five QTNs for root length (RL) accounting for 2.08-17.42% of phenotypic variation. Shoot characteristics were similarly influenced, with five QTNs for shoot diameter (ShD) explaining 0.46-27.30% of variance and five QTNs for shoot length (ShL) contributing 2.78-14.42%.

The analysis also revealed ten pleiotropic QTNs that each influenced between two and four phenotypic traits. Three specific QTNs (S4_13304368, S4_60963038, and S6_45176048) on chromosomes 4 and 6 were associated with both root length and root dry weight, explaining 2.9-12.33% of phenotypic variance for these traits. Two additional QTNs (S1_28914756 and S3_62564964) on chromosomes 1 and 3 showed association with both root dry weight and root-to-shoot ratio, though with more modest effects (0.002-1.67% PVE). A particularly noteworthy pleiotropic QTN (S1_6012717) on chromosome 1 influenced four traits simultaneously (root length, root-to-shoot ratio, nodal root number, and root dry weight), accounting for 0.27-9.54% of their phenotypic variance. Three additional loci (S1_68144065, S6_49860654, and S6_6049929) on chromosomes 1 and 6 affected both shoot length and leaf area (2.78-10.38% PVE), while another QTN (S8_2624370) on chromosome 8 influenced both shoot length and stomatal density (3.08-7.02% PVE). Importantly, none of the significant markers demonstrated pleiotropic effects spanning both shoot and root traits, suggesting distinct genetic architectures. This comprehensive analysis reveals both trait-specific and pleiotropic genetic factors underlying sorghum's phenotypic variation, with particular chromosomes (especially chromosome 1) harboring multiple significant associations. The identification of these stable QTNs across multiple analytical methods strengthens their potential value for marker-assisted breeding programs

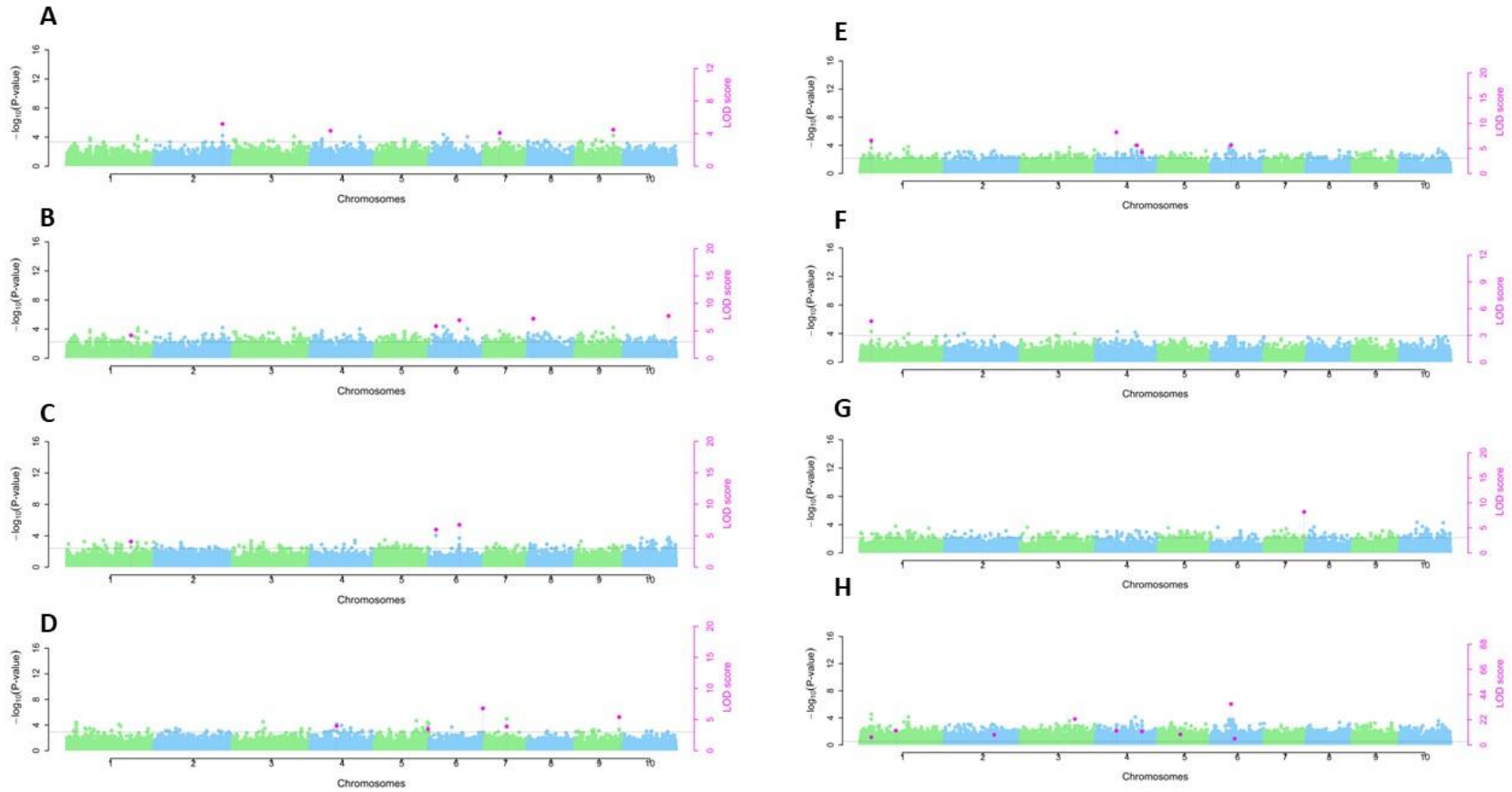
Table 4.14. Forty-nine reliably identified QTNs influencing thirteen key traits

Trait	QTN	Chr	Position	r^2%	LOD score	Method
LAg	S2-72741053	2	72,741,053bp	10.34-13.09	4.09-5.19	2,4,5
LAg	S4-12354434	4	12,354,434bp	5.74-11.29	4.35-5.99	2,4,5
LAg	S7-11107766	7	11,107,766bp	5.72-14.81	3.37-4.16	1,2,3,5
LAg	S9-55485561	9	55,485,561bp	6.57-9.34	4.48-5.72	2,4,5
ShL	S1-68144065	1	68,144,065bp	2.78-7.11	4.05-8.68	2,4,5
ShL	S10-56681073	10	56,681,073bp	9.12-14.42	4.32-11.06	1,2,4,5
ShL	S6-49860654	6	49,860,654bp	5.64-10.38	4.65-10.33	1,2,4,5
ShL	S6-6049929	6	6,049,929bp	7.21-9.12	3.91-7.34	2,4,5
ShL	S8-2624370	8	2,624,370bp	4.81-6.88	5.05-10.82	2,4,5
LAr	S1-68144065	1	68,144,065bp	3.44-4.02	3.10-6.00	2,4,5
LAr	S6-49860654	6	49,860,654bp	4.74-9.27	5.76-9.26	2,4,5
LAr	S6-6049929	6	6,049,929bp	4.79-9.49	3.07-8.14	2,4,5
ShD	S4-40232458	4	40,232,458bp	2.42-10.37	3.32-4.08	2,4,5
ShD	S5-71649865	5	71,649,865bp	0.46-4.89	3.08-3.55	2,3,4
ShD	S6-60594858	6	60,594,858bp	1.01-7.67	4.44-9.01	2,4,5
ShD	S7-44897619	7	44,897,619bp	5.27-27.30	3.48-8.88	1,2,4
ShD	S9-57701415	9	57,701,415bp	3.77-15.99	4.02-6.24	1,2,4,5
RL	S1-6012717	1	6,012,717bp	3.87-8.83	3.99-6.71	2,3,4
RL	S4-13304368	4	13,304,368bp	4.83-15.95	4.21-13.63	1,4,5
RL	S4-57625836	4	57,625,836bp	2.08-6.95	3.10-12.30	1,2,4,5
RL	S4-60963038	4	60,963,038bp	7.73-17.42	3.99-7.31	2,4,5
RL	S6-45176048	6	45,176,048bp	2.59-6.02	3.82-16.28	1,2,4,5
NR	S1-6012717	1	6,012,717bp	2.07-9.54	3.98-4.59	2,3,4
SDW	S7-64332829	7	64,332,829bp	1.55-5.09	3.09-15.25	1,2,4
RDW	S1-28914756	1	28,914,756bp	0.002-0.72	10.61-20.96	1,2,4
RDW	S1-6012717	1	6,012,717bp	0.27-7.21	4.50-26.12	2,4,5
RDW	S2-64672108	2	64,672,108bp	0.006-1.47	8.18-45.33	1,2,4
RDW	S3-62564964	3	62,564,964bp	0.17-1.52	6.67-61.71	1,2,4
RDW	S4-13304368	4	13,304,368bp	0.62-4.69	3.17-22.46	1,2,4,5
RDW	S4-60963038	4	60,963,038bp	0.00-24.97	4.14-22.29	2,3,4,5
RDW	S5-19538615	5	19,538,615bp	0.0002-1.17	4.24-32.17	1,2,4
RDW	S6-45176048	6	45,176,048bp	1.61-4.92	4.62-42.99	1,2,4,5
RDW	S6-47428336	6	47,428,336bp	0.08-4.21	3.20-13.83	2,4,5
RHN	S10-53619913	10	53,619,913bp	6.06-10.14	3.00-4.93	1,2,5
RHN	S9-56346446	9	56,346,446bp	4.62-6.56	3.68-5.13	1,4,5
RAg	S1-53254066	1	53,254,066bp	3.71-15.37	4.93-9.39	1,2,4,5
RAg	S3-18779995	3	18,779,995bp	2.73-12.57	3.35-3.72	1,2,4
RAg	S4-56610955	4	56,610,955bp	0.76-7.98	4.01-6.64	1,2,4,5
RAg	S6-2724950	6	2,724,950bp	0.60-3.77	3.35-3.83	2,4,5

Table 4.14 (continued)

RAg	S9-48742385	9	48,742,385bp	2.28-9.03	3.71-7.42	1,2,4,5
StD	S10-56910652	10	56,910,652bp	4.97-8.57	5.44-6.67	2,4,5
StD	S8-2624370	8	2,624,370bp	3.08-7.02	3.80-5.99	1,4,5
RSR	S1-28914756	1	28,914,756bp	0.000012-1.64	3.03-9.55	1,4,5
RSR	S1-6012717	1	6,012,717bp	1.4-7	3.50-8.70	1,2,3,4,5
RSR	S2-10753898	2	10,753,898bp	5 -9.5	3.09-10.04	2,3,5
RSR	S3-62564964	3	62,564,964bp	0.02-1.7	5.43-6.43	1,4,5
RSR	S4-817571	4	817,571bp	2.5-3.8	5.88-15.86	1,4,5
RSR	S9-55975700	9	55,975,700bp	0.03-1.8	3.20-4.68	2,4,5
SA	S1-72242744	1	72,242,744bp	3.8-7.7	4.65-5.20	2,4,5

Methods 1-5 represent: mrMLM, FASTmrMLM, FASTmrEMMA, pLARmEB, and ISIS EM-BLASSO, respectively. Abbreviations: Leaf angle (LAg), shoot length (ShL), leaf area (LAr), shoot diameter (ShD), root length (RL), number of nodal roots (NR), shoot dry weight (SDW), root dry weight (RDW), root hair number (RHN), root angle (RAg), stomatal density (StD), root to shoot ratio (RSR), and stomatal area (SA). The coefficient of determination (r^2), expressed as a percentage, quantifies the percentage of total phenotypic variability accounted for by individual quantitative trait nucleotides.


A
B
C
D
E
F
G
H
−log10(P-value)
LOD score
Chromosomes

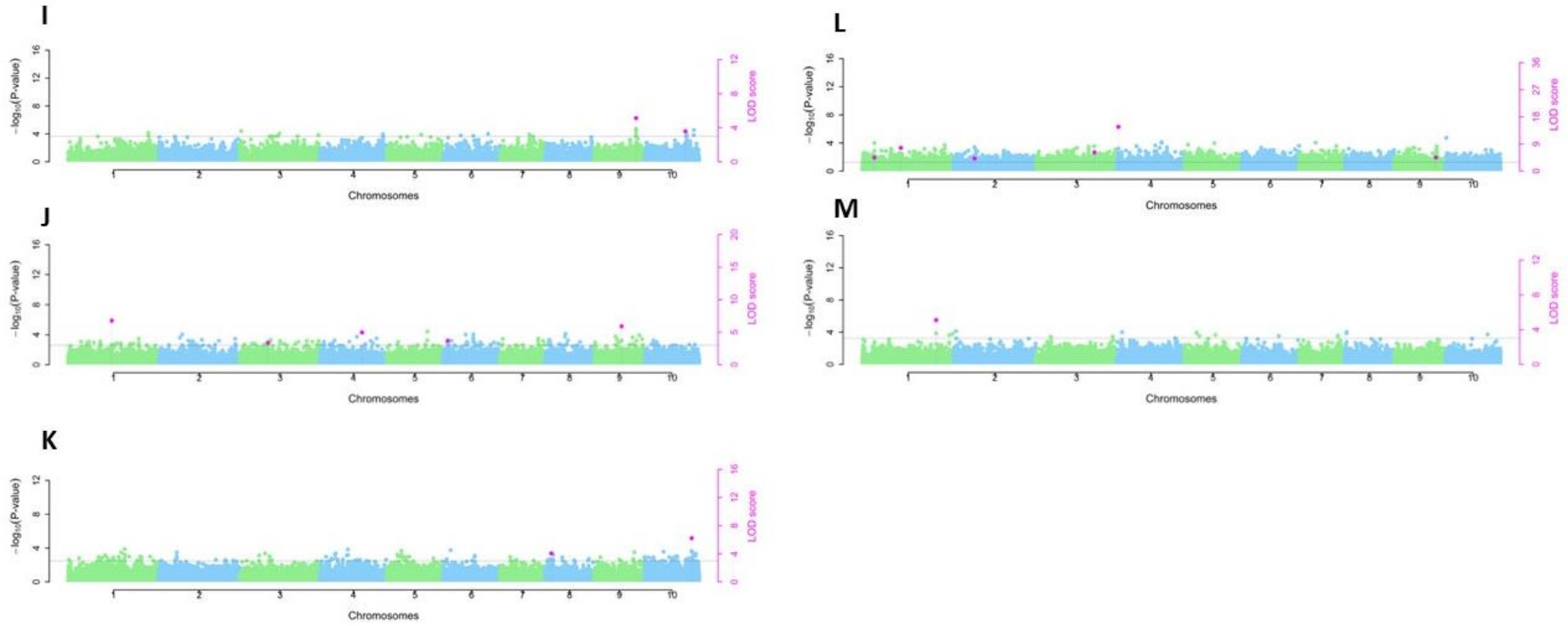


Figure 4.17. Manhattan plots showing significant QTNs associated with 13 different traits using ML-GWAS method. (A) leaf angle, (B) shoot length (C) leaf area (D) shoot diameter (E) root length (F) number of nodal roots (G) shoot dry weight (H) root dry weight (I) root hair number (J) root angle (K) stomatal density (L) root to shoot ratio (M) stomatal area. The horizonatal line and the pink colored dots represent a LOD score threshold and significnat QTNs, respectively.

4.3.3. Candidate gene discovery and functional analysis

To elucidate the genetic architecture underlying root and shoot development, potential candidate genes were identified within a 14.4 kilobase genomic window flanking stable QTNs, consistent with the linkage disequilibrium decay pattern observed in our analysis. Gene discovery was prioritized for traits demonstrating strong genomic associations, as supported by quantile-quantile plots showing close alignment between observed and theoretical p-value distributions (Appendix 8.4). Ten traits were selected for in-depth investigation, including leaf inclination (LAg), shoot biomass (SDW), root elongation (RL), belowground biomass accumulation (RDW), nodal root count (NR), root hair count (RHN), root-to-shoot allocation ratio (RSR), stomatal density (StD), root growth angle (RAg), and stomatal aperture size (SA). Among the 49 high-confidence QTNs identified, 26 co-localized with putative functional genes regulating shoot and root morphology. These candidate genes were distributed across multiple genomic regions, with several showing potential roles in modulating plant architecture and stress responses.

The functional characterization revealed that these genes encode diverse protein classes, including transcription factors, transporters, and enzymes involved in hormone signaling pathways. Particularly noteworthy were genes associated with auxin transport and response, which appeared frequently among the candidates linked to root architecture traits such as root angle and branching patterns. Comprehensive annotations of these candidate genes and their associated QTNs are provided in Appendix 8.7.

4.3.3.1.Leaf angle

Whole-genome analysis identified nine distinct genomic loci showing significant associations with leaf angle variation. Within these regions, two candidate genes (Sobic.004G116200 and Sobic.009G159700) were predicted to encode proteins currently lacking functional characterization. The remaining seven genes exhibited homology to known protein domains, including Sobic.002G349100, which encodes a putative pyridoxamine 5'-phosphate oxidase involved in vitamin B6 metabolism. Adjacent genes Sobic.002G349200 and Sobic.002G349600 were annotated as containing ubiquitin-related domains, potentially participating in protein degradation pathways.

Further examination revealed Sobic.002G349300 as encoding an arabinogalactan peptide 12-related protein. The genomic region also contained Sobic.002G349500, predicted to encode a threonine-specific protein kinase that may function in phosphorylation-dependent signaling cascades. Additionally, two genes located on chromosome 9 (Sobic.009G159500 and Sobic.009G159600) were identified as encoding putative gins complex components and a plant-specific Rop nucleotide exchanger (prone) protein, respectively.

4.3.3.2. Root length

The analysis identified nine candidate genes significantly associated with root length variation. The SNP S1_6012717 showed association with four adjacent genes: Sobic.001G078100 encoding molybdopterin cofactor sulfurase (mosc), Sobic.001G077800 containing a MYB-like DNA-binding domain, Sobic.001G077900 encoding a predicted membrane protein (sharing similarity with Os03g0766000 protein), and Sobic.001G078000 representing a putative uncharacterized protein.

Further examination revealed that SNP S4_13304368 was linked to two candidate genes: Sobic.004G120200, which encodes sf31-sentrin/sumo-specific protease, and Sobic.004G120233, corresponding to a putative uncharacterized protein. Another significant locus, S4_60963038, was associated with Sobic.004G236500, known to encode SF82-glutaredoxin proteins.

Additionally, SNP S6_45176048 demonstrated association with two functionally distinct genes: Sobic.006G075100, encoding a glycosyltransferase family protein, and Sobic.006G075200, producing an ALUMINUM-ACTIVATED MALATE TRANSPORTER (ALMT) family protein.

4.3.3.3. Shoot dry weight

The Analysis revealed a significant quantitative trait nucleotide (*S7_64332829*) located on chromosome 7, showing association with five adjacent candidate genes: Sobic.007G175600, Sobic.007G175702, Sobic.007G175800, Sobic.007G175900, and Sobic.007G176000. The first two genes (Sobic.007G175600 and Sobic.007G175702)

encode putative auxin-responsive family proteins, while the remaining three genes correspond to distinct protein classes: a K+/H+-antiporter, a ring zinc finger family protein, and a mate efflux family protein.

4.3.3.4. Root dry weight

Genomic analysis identified multiple quantitative trait nucleotides (QTNs) with distinct genetic associations. The QTN S1_28914756 was linked to Sobic.001G254700, while S2_64672108 showed associations with four candidate genes: Sobic.002G245100, Sobic.002G245200, Sobic.002G245300, and Sobic.002G245400. The QTN S3_62564964 co-localized with Sobic.003G226100 and Sobic.003G226200, and S6_47428336 was associated with Sobic.006G090500 and Sobic.006G090601. Three QTNs (S1_28914756, S1_6012717, and S3_62564964) exhibited pleiotropic effects on root-to-shoot ratio, while four additional QTNs (S1_6012717, S4_13304368, S4_60963038, and S6_45176048) influenced root length.

4.3.3.5. Number of nodal roots

Genomic analysis identified a significant quantitative trait nucleotide (S1_6012717) located on chromosome 1, showing association with four adjacent candidate genes: Sobic.001G078100, Sobic.001G077800, Sobic.001G077900, and Sobic.001G078000. These genes were annotated as encoding distinct protein classes, including molybdopterin cofactor sulfurase mosc, myb-like dna-binding domain, a membrane protein (sharing similarity with Os03g0766000 protein), and a putative uncharacterized protein.

4.3.3.6. Root hair number

Genomic analysis revealed two significant quantitative trait nucleotides associated with root hair number, located at 53.6 megabases (Mb) on chromosome 10 and 56.3 Mb on chromosome 9. This investigation identified five candidate genes showing significant associations with root hair development. Among these, Sobic.009G170100 was annotated as encoding a putative uncharacterized protein, while four additional genes on chromosome 10 (Sobic.010G181000, Sobic.010G181100, Sobic.010G181150, and Sobic.010G181200) were found to encode distinct protein classes, including ppr-repeat protein-like, monooxygenase, threonine-specific protein kinase, and far1 dna binding domain (sharing similarity with Os06g0597800 protein), respectively.

4.3.3.7. Root angle

Analysis identified three candidate genes significantly associated with root angle quantitative trait nucleotides: Sobic.004G189500, Sobic.004G189600, and Sobic.009G112400. These genes were annotated as encoding distinct protein classes, including a putative uncharacterized protein (Sobic.004G189500), phosphopantothenoyl cysteine synthetase (Sobic.004G189600), and f-box domain protein (Sobic.009G112400).

4.3.3.8. Stomatal density

Genomic analysis identified four candidate genes associated with stomatal density quantitative trait nucleotides. Among these, Sobic.010G209450 was found to lack a functionally defined domain, while the remaining three genes (Sobic.010G209501, Sobic.008G028600, and Sobic.008G028700) encoded distinct protein classes, including glycosyltransferase-like domain, wrky dna-binding domain, and set domain protein, respectively.

4.3.3.9.Root-to-shoot ratio

The QTN S4_817571 was found to co-localize with five candidate genes on chromosome 4: Sobic.004G009100 (encoding fam32a protein), Sobic.004G008700 (knottin), Sobic.004G008800 and Sobic.004G008900 (both encoding acid phosphatase-like proteins), along with Sobic.004G008900 (putative uncharacterized protein). Additionally, QTN S9_55975700 showed association with two genes on chromosome 9: Sobic.009G165800 (bud31 homolog 2) and Sobic.009G165900 (putative uncharacterized protein). In contrast, the QTN S2_10753898 demonstrated no detectable association with any candidate genes in the analyzed genomic region.

4.3.3.10. Stomatal area

The analysis identified a quantitative trait nucleotide (S1_72242744) on chromosome 1, showing association with the candidate gene Sobic.001G395100. This gene was annotated as encoding a dead-box atp-dependent RNA helicase 24 protein.

4.4. Analysis of genetic variation

4.4.1. SNP discovery

A total of 334,521 SNPs loci were obtained from 129 sorghum genotypes. Following quality control measures that removed single nucleotide polymorphisms with minor allele frequencies below 5% and those containing missing data across genotypes, a final set of 8,698 high-quality SNPs distributed across all ten sorghum chromosomes was obtained for analysis. These genetic variants were further classified based on their underlying nucleotide substitution patterns, distinguishing between transition-type mutations (adenine-guanine or cytosine-thymine interchanges) and transversion-type mutations (all other possible base substitutions). Evaluation of these substitution patterns revealed a transition-to-transversion ratio of 1.1, corresponding to 4,557 transition polymorphisms versus 4,141 transversion polymorphisms, as detailed in Table 4.15.

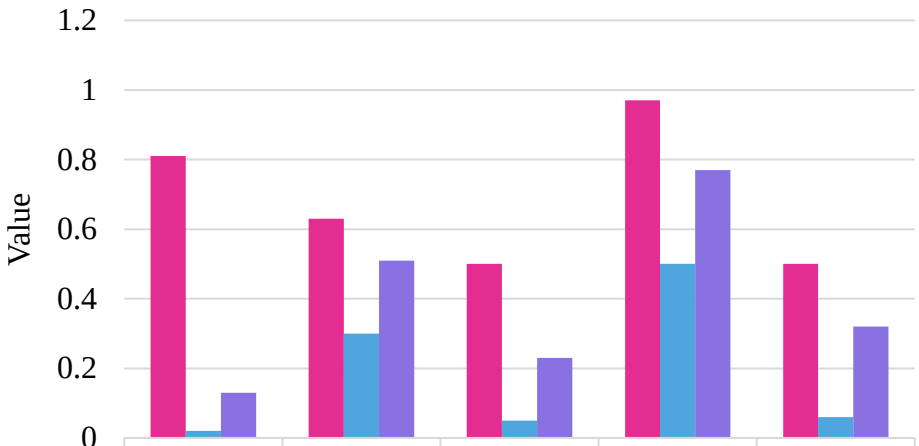
Table 4.15. Transition and transversion of SNPs

	Transition		**Transversion**			
	A /G	**C/T**	**A /C**	**A /T**	**G/C**	**G/T**
Number of allelic sites	2,306	2,251	946	639	1,648	908
% of allelic sites	26.51	25.88	10.88	7.35	18.95	10.44
Total	4,557		4,141			
Percentage	52.39		47.61			

4.4.2. Genetic diversity indices

The 8,698 high-quality SNP markers demonstrated substantial genetic variation, as illustrated in Figure 4.18. Analysis revealed gene diversity (GD) estimates spanning 0.30 to 0.63, with a mean GD of 0.51 across all markers. Heterozygosity measurements (Ho) exhibited considerable variation, ranging from 0.02 to 0.81 with an average of 0.13. Minor allele frequency (MAF) distribution showed values between 0.05 and 0.50, averaging 0.23 across the dataset. Detailed examination of MAF distribution indicated that 46.67% of markers displayed MAF values below 0.2, while 45.52% exceeded this threshold. Notably, 7.81% of markers exhibited balanced allele frequencies (MAF = 0.5), as depicted in Figure 4.19. Major allele frequencies ranged from 0.50 to 0.97, with a mean of 0.77. Polymorphism information content (PIC) values varied from 0.06 to 0.50, averaging 0.32 across all markers. The PIC value distribution revealed

that only 2.94% of markers showed limited informativeness (PIC < 0.10), while the majority (53%) exceeded the average PIC value of 0.32. Intermediate PIC ranges were well represented: 22.89% (0.11-0.20), 19.37% (0.21-0.30), 22.11% (0.31-0.40), and 32.68% (0.41-0.50), as shown in Figure 4.20. These results demonstrate the markers' substantial utility for genetic analyses, with most exhibiting moderate to high polymorphism levels.



	Ho	GD	MAF	MaF	PIC
Max	0.81	0.63	0.5	0.97	0.5
Min	0.02	0.3	0.05	0.5	0.06
Mean	0.13	0.51	0.23	0.77	0.32

Figure 4.18. Descriptive statistics—including mean, minimum (Min), and maximum (Max) values-for five key genomic diversity parameters: observed heterozygosity (Ho), gene diversity (GD), minor allele frequency (MAF), major allele frequency (MaF), and polymorphic information content (PIC). The mean, minimum (Min), and maximum (Max) values for Ho: Observed heterozygosity; GD: genetic diversity; MAF: Minor allele frequency, MaF: Major allele frequency, and PIC: Polymorphic information content for 8,698 SNP loci.

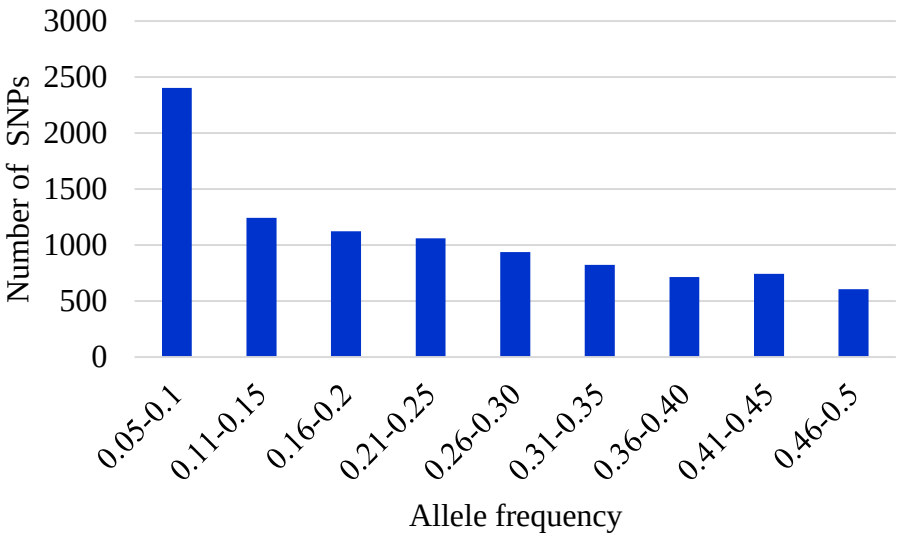


Figure 4.19. Distribution of minor allele frequencies across 129 sorghum accessions

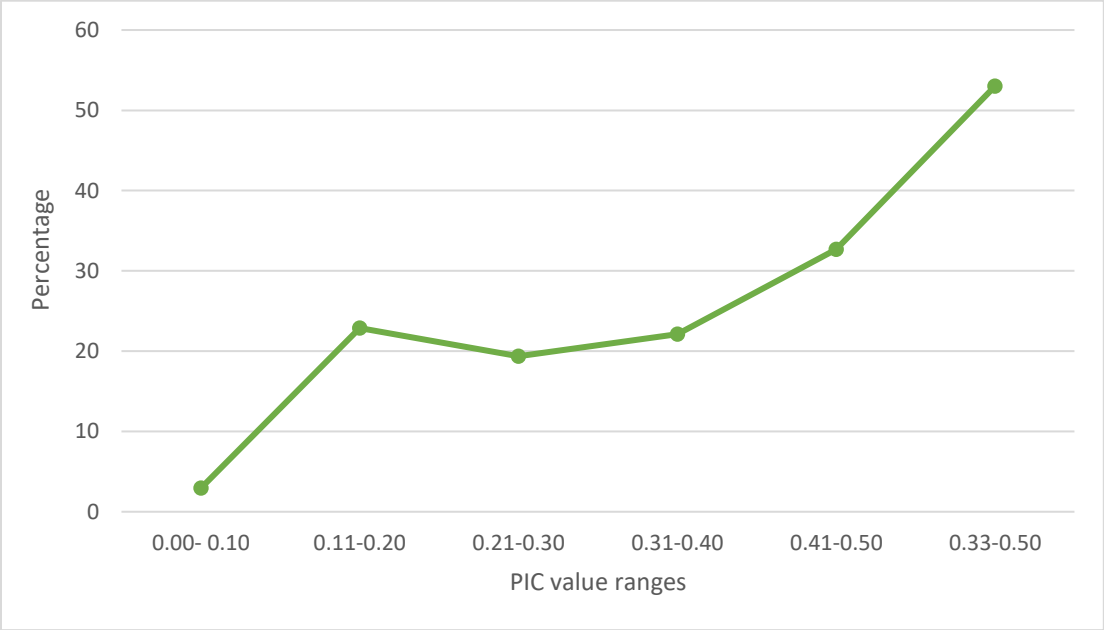


Figure 4.20. Polymorphic Information Content (PIC) values for single nucleotide polymorphisms employed in population genetic analysis.

4.4.3. Genetic divergence between pairs of genotypes

The degree of genetic differentiation between genotypes is categorized into four levels based on pairwise genetic distance measurements: low divergence (values below 0.1), moderate divergence (ranging from 0.101 to 0.200), high divergence (falling between 0.201 and 0.300),

and very high divergence (values exceeding 0.300) (Botstein et al., 1980). The 129 sorghum genotypes showed high genetic variability ranging from 0.13 (between genotype #47 and genotype #123) to 0.95 (genotype #49 and genotype #13), with a mean of 0.69 and 55% of pairs of genotypes were above the average mean suggesting highly differentiated genetic backgrounds. Frequency distribution of pairwise genetic dissimilarty calculated from 8698 SNPs across the 129 sorghum genotypes is shown in Figure 4.21.

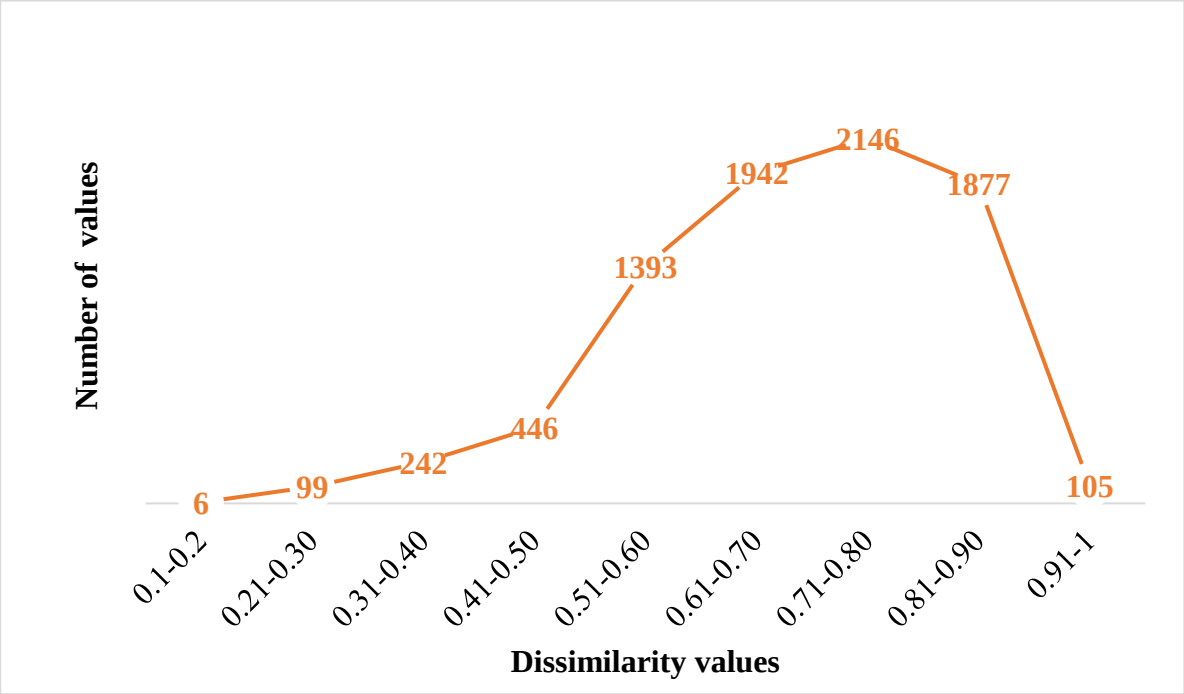


Figure 4.21. Summary of pair wise dissimilarity values between 129 sorghum genotypes

4.4.4. Population differentiation among regional and structure sorghum populations

The population differentiation among regional and structure groups (sub-population) of sorghum was analyzed (Table 4.16; Table 4.17). The smallest value (0.015) was detected amongst the accessions of Oromia and Benishangul Gumuz regions, while the highest value (1.00) was detected between sorghum populations from the Tigray and Gambela regions (Table). Incontrast, for sub-populationmgroups, the lowest value (0.003) was identified amongst sub-populations 2 and sub-populations 3, whereas the highest value (1.00) occurred between sub-populations 1 and 4.

Table 4.16. Population differentiation values between regional sorghum populations

Region	Amhara	Ben. Gumuz	Gambela	Oromia	SEPR	Tigray
Amhara	0.000	0.111	0.555	0.021	0.275	0.505
Ben. Gumuz		0.000	0.348	0.015	0.417	0.080
Gambela			0.000	0.422	0.729	1.000
Oromia				0.000	0.147	0.07
SEPR					0.000	0.161
Tigray						0.000

Table 4.17. Population differentiation values between sorghum sub-populations

Sub-pop	Sub pop 1	Sub pop 2	Sub pop 3	Sub pop 4	Sub pop 5
Sub pop 1	0.000	0.080	0.117	1.000	0.632
Sub pop 2		0.000	0.003	0.233	0.060
Sub pop 3			0.000	0.429	0.074
Sub pop 4				0.000	0.564
Sub pop 5					0.000

4.4.5. Cluster analysis

The dissimilarity matrix, derived from genetic data, served as the basis for the construction of a neighbor-joining (NJ) dendrogram, employed to elucidate the evolutionary interrelationships among the 129 genotypes. The neighbor-joining dendrogram organized the germplasm into three primary phylogenetic clusters, visually distinguished by distinct coloration in Figure 4.22. Among these groupings, Cluster II emerged as the most extensive assemblage, containing 62 accessions representing 48.1% of the total samples. This dominant cluster exhibited substantial geographic heterogeneity, incorporating genotypes from multiple regions, 24 from Oromia, 9 from Gambela, 8 from Benishangul Gumuz, 7 from Amhara, 5 from Tigray, 4 from SEPR, and 2 each from Dire Dawa/NA and Afar. Cluster III contained 34 genotypes, primarily from Amhara (13) and Oromia (10), with smaller contributions from Gambela/Tigray (3), Benishangul Gumuz/Somali (2), and an unknown region (1). Cluster I held 33 genotypes, with a mix of Oromia (9), Tigray (7), Amhara (6), Benishangul Gumuz/SEPR (3), Gambela/NA (2),

and Afar (1). Cluster II, the largest cluster, contained the majority of Oromia genotypes, while Cluster III had the most Amhara genotypes. Notably, geographic origin did not correlate with genetic clustering patterns, as accessions from identical regions appeared dispersed throughout multiple phylogenetic groups.

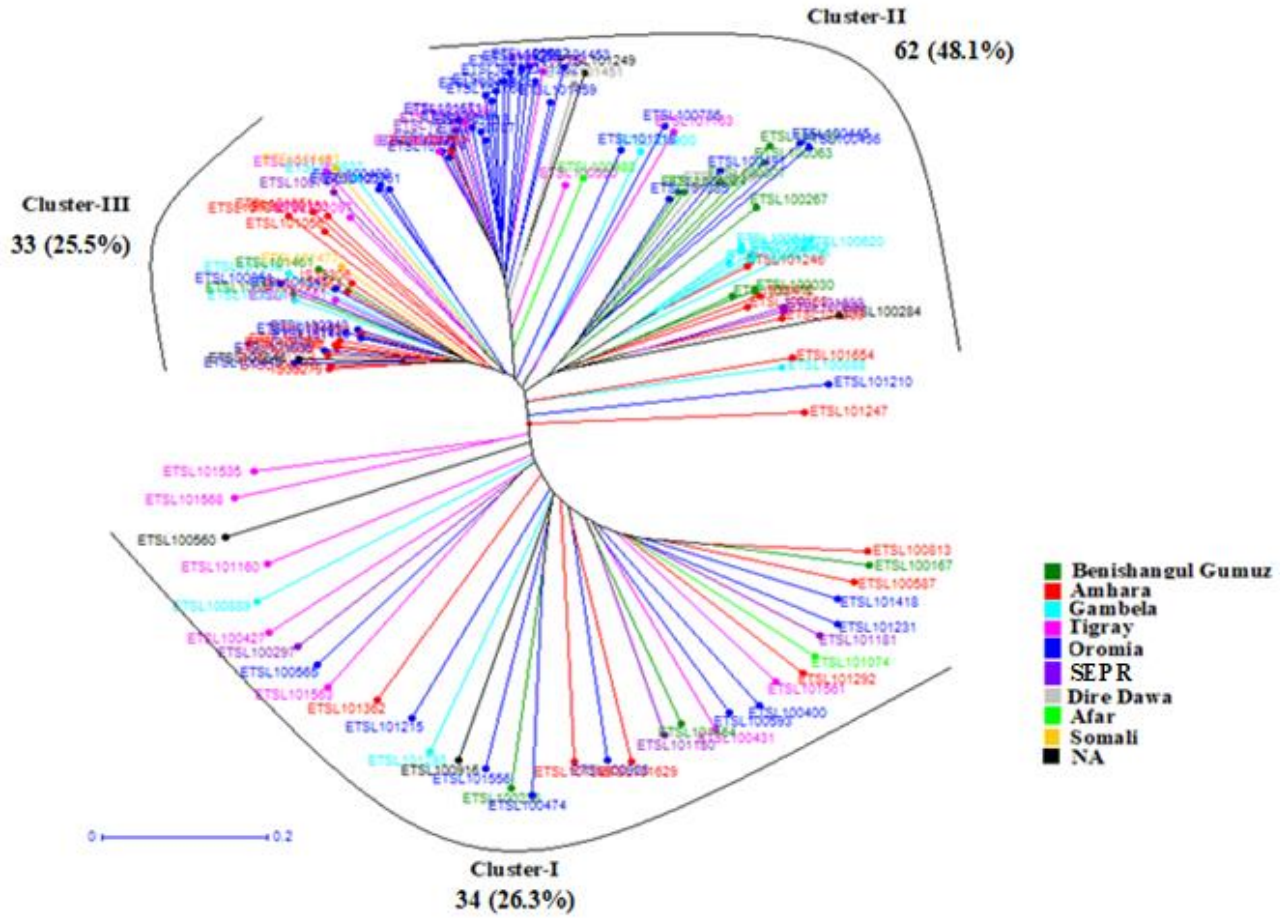


Figure 4.22. Neighbor joining dendrogram of 129 sorghum genotypes generated based on Dice genetic distance (Dice, 1945). Identical coloration of genotypes indicates shared geographic origin

4.4.6. Principal Coordinate Analysis

To assess population structure and genetic affinities among sorghum accessions, we performed Principal Coordinate Analysis (PCoA), with results visualized in Figure 4.23. The initial two principal coordinates collectively accounted for 32.79% of the observed genomic variation, comprising 22.33% (Coordinate 1) and 10.46% (Coordinate 2) of the total explained variance.

Genotype #49 and genotype #13 were the most genetically distinct, while genotype #47 and genotype #123 showed the closest genetic similarity. Interestingly, the PCoA did not clearly separate genotypes based on their region of origin.

The PCoA biplot further revealed that Quadrant I comprised of 24 genotypes primarily from Gambela and Tigray, demonstrating distinct characteristics. Quadrant II included 18 genotypes, displaying a diverse mix of origins, including Oromia, Tigray, Amhara, and Benishangul Gumuz, with sparse distribution. Quadrant III contained 34 genotypes, predominantly from Oromia and Tigray. Quadrant IV harbored the largest number of genotypes (53), mainly from Gambela, Oromia, and Tigray. Genotypes in Quadrants III and IV showed closer clustering, suggesting a higher degree of relatedness. Genotypes # 90 and #13, located on the horizontal lines separating quadrants, likely have a mixed genetic background, suggesting they may have inherited genetic material from multiple sources. The results of the PCoA indicate that genotypes from Amhara and Oromia cluster together in the upper right quadrant. Additionally, some Oromia genotypes overlap with a few genotypes from Tigray in the lower right quadrant. Interestingly, genotypes from Tigray, SEPR, Benishangul Gumuz, and Gambela are spread across all four quadrants. Over all, the Principal Coordinate Analysis (PCoA) also revealed significant admixture among sorghum genotypes, with no clear separation based on region of origin.

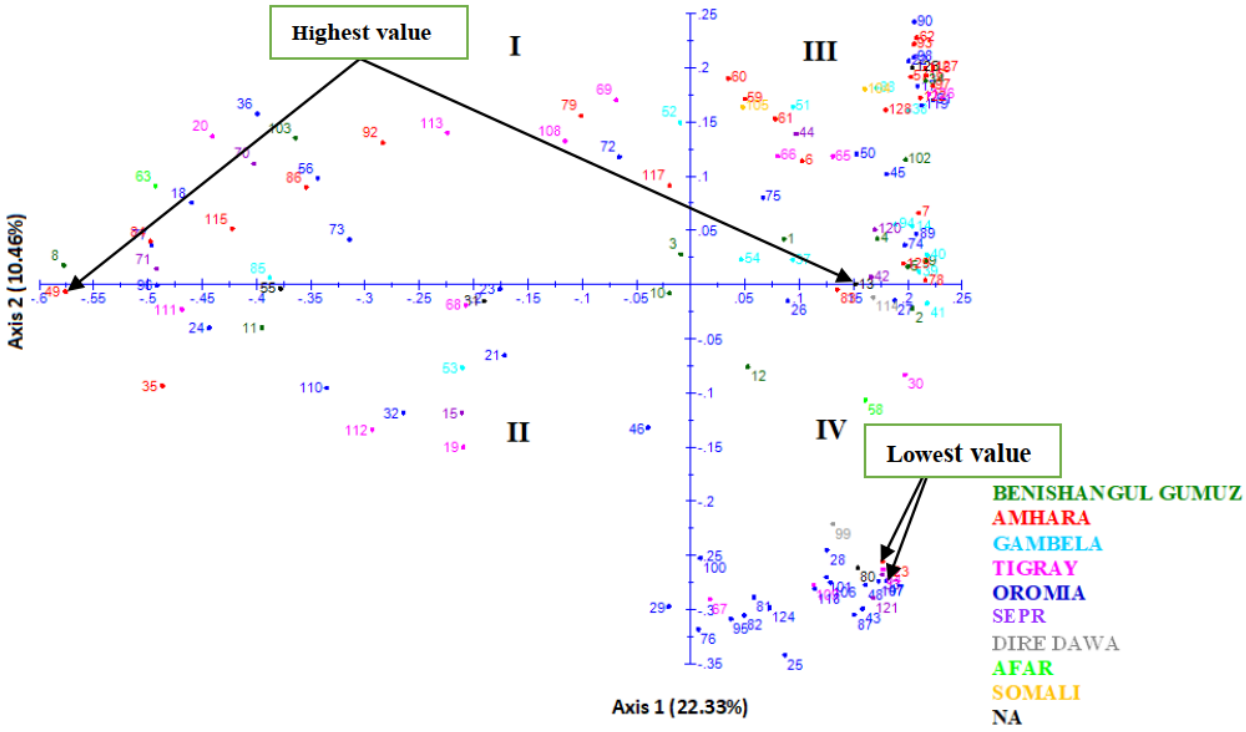


Figure 4.23. Positions of genotypes by entry number on the first and the second principal coordinates indicating relationships among genotypes. The roman numerals represent the four quadrats.

4.4.7. Analysis of Molecular Variance

Molecular variance partitioning through AMOVA demonstrated that the predominant source of genetic diversity (99%) occurred at the intra-regional level, while inter-regional differences contributed minimally (1%) to the total observed variation. A similar pattern was observed when considering population structure, with 99% of the variation occurring within subpopulations and 1% among them (Table 4.18; Table 4.19). The fixation index (Fst) values, a measure of population differentiation, were low (Fst=0.006, p=0.05; Fst=0.008, P<0.05) for both regional and population structure groupings, respectively.Gene flow was high, with Nm values of 41.41 and 27.83 (Table 4.18; Table 4.19), for the regional and population structure groupings, respectively.

Table 4.18. Analysis of Molecular Variance of 129 sorghum genotypes utilizing 8,698 SNPs based on regions of origin

Source of variation	Degrees of freedom	Sum of Square	Mean Square	Estimated Variance	%
Among regions	5	2.731	0.546	0.003(AP)	1%
Within regions	123	60.920	0.495	0.495(WP)	99%
Total	**128**	**63.651**		**0.498(TOT)**	**100%**
P<0.05					Fst=0.006

Table 4.19. AMOVA estimates of 129 sorghum genotypes using 8,698 SNP markers based on population structure

Source of variation	Degrees of freedom	Sum of Square	Mean Square	Estimated Variance	%
Among Pops	4	2.375	0.594	0.004(AP)	1%
Within Pops	124	61.284	0.494	0.494(WP)	99%
Total	**128**	**63.659**		**0.499(TOT)**	**100%**
P<0.05					Fst=0.008

CHAPTER FIVE

5. DISCUSSIONS

5.1. Phenotypic traits variation

5.1.1.Genetic variation

The way plant root systems are architected plays a significant role in their ability to obtain soil moisture, especially in water-limited conditions. This is particularly important for cereals, such as sorghum, which is commonly cultivated in drought-prone environments (Joshi et al., 2017). Research across major cereal crops has demonstrated the value of root architectural features for improving drought tolerance, with documented success in maize (Lobell et al., 2014), rice (Uga et al., 2013), wheat (Richard et al., 2018), barley (Robinson et al., 2018), and sorghum (Mace et al., 2012) Despite these advances, current breeding programs have yet to fully exploit root characteristics for stress resilience (Tracy et al., 2020).This underscores the critical need to characterize both root system architecture and shoot morphological traits to develop crop varieties with enhanced adaptation to abiotic stresses like drought (Wu et al., 2022). Evaluation of 158 Ethiopian sorghum accessions demonstrated highly significant variation ($P < 0.001$) across root and shoot morphological characteristics. This extensive phenotypic diversity presents substantial opportunities for enhancing the crop's resilience to diverse agroecological conditions. The study also revealed significant variations in drought responsive traits among sorghum genotypes, with high heritability (Table 4.2).

The study revealed root angle values spanning from 7.75° to 25.17° across the germplasm. The population average root angle was 14.81°, with approximately 65.8% of accessions displaying values below this mean threshold, indicating a skewed distribution toward narrower root angles. This range is narrower than the 14.5° to 27.37° found in 214 sorghum genotypes by Demelash et al. (2021) and the 16.4° to 26.6° found in 1771 sorghum landraces reported by Menamo et al. (2023). However, it differs significantly, compared to the ranges found in 44 inbred lines of sorghum (14° to 50°) (Singh et al., 2012). Extensive research has established root angle as a critical selection target for enhancing drought tolerance in sorghum, with narrower angles correlating with improved performance in water-limited environments (Singh et al., 2011, 2012; Rostamza et al., 2013). Narrow-angled root systems demonstrate greater root length density, facilitating more efficient soil water extraction (Manschadi et al., 2006; Ali et al., 2015; Liang

et al., 2017), and consistently higher grain yields under drought stress conditions (Mace et al., 2012; Singh et al., 2012; Rostamza et al., 2013; Carvalho et al., 2014; Girma et al., 2020; Demelash & Gedifew, 2023; Solomon et al., 2023).Comparable findings have been reported in other cereal crops, including wheat (Alahmad et al., 2019), and rice (Kato et al., 2006), where steep root angles promote deeper soil exploration and enhanced drought resilience. Additional root system characteristics, including root length and branching patterns, significantly influence water acquisition efficiency in moisture-deficient soils (Ye et al., 2018). In our investigation, root length measurements ranged from 28.67 cm to 71 cm (mean = 48.32 cm), while root counts varied between 3 and 8 per plant (mean = 5.37), as detailed in Appendix 8.2.

In the current investigation, the topmost five genotypes with narrow root angle were #151 (7.75°), #30 (7.87°), #96 (8.25°), #66 (8.87°), and #45 (9.25°) (Appendix 8.2). In contrast, genotypes with wider root angles demonstrate enhanced water extraction from light rainfall (Liao et al., 2006), and phosphorus uptake abilities (Parra-Londono et al., 2018), especially in the topsoil layer, as they have horizontal and shallow root systems. Therefore, the three genotypes with the widest root angles such as #92 (25.17°), #18 (25.06°), and #24 (24.87°) (Appendix 8.2) can be regarded as highly efficient for resource acquisition, especially phosphorus located in topsoil horizons. Varieties derived from wide nodal root angle sorghum accessions are appropriate for growth vonditions with sufficient moisture supply, regardless of water source (precipitation or irrigation) throughout the plant's developmental phases (Enyew et al., 2024).

The present study also revealed a substantial difference ($p < 0.001$) in root length and number of nodal roots, which are a key below-ground trait for arid environment adaptation. Significant variations in these traits have been observed in sorghum at the seedling stage, as reported by Demelash et al. (2021), and also in other crops like barley (de Dorlodot et al., 2007) and wheat (Manschadi et al., 2006). Plants with extended root are better equipped to tolerate arid conditions owing to their capability to penetrate and utilize deep soil moisture, ultimately leading to higher crop yields (Kaydan & Yagmur, 2008; Wasaya et al., 2018; Ye et al., 2018; Baoru Li et al., 2022). The current investigation identified sorghum accessions #86, #142, #125, #21, and #3 as exhibiting superior performance for both root length and number of nodal roots, as detailed in Appendix 8.2. Previous study has shown that higher root-to-shoot ratio is the

common feature of drought tolerant plants (Mathew et al., 2018). The top five genotypes with the highest root-to-shoot ratios in this study were #86, #125, #21, #62, and #129. The current study highlights specific sorghum accessions (#86, #125, #21 - Appendix 8.2) exhibiting optimal combinations of drought-adaptive characteristics, including extensive root elongation, enhanced number of nodal roots, noticeable root-to-shoot biomass ratio, and narrow root architecture angles. These trait combinations position these genotypes as prime candidates for breeding programs targeting improved productivity in water-deficient agroecosystems.

5.1.2. Variance components

In this study, the estimated moderate GCV indicated that the genotypes had a relatively high level of genetic variability, suggesting a diverse genetic origin. The study revealed consistently greater phenotypic coefficient of variation (PCV) relative to genotypic coefficient of variation (GCV) across all examined characteristics. Notably, the minimal disparity between PCV and GCV values suggests limited environmental modulation of trait manifestation, with genetic factors demonstrating predominant regulatory influence. This finding is inconsistent with those reported by Demelash et al.(2021) and Rajarajan et al. (2018). However, Elias et al. (2023) documented substantial difference between PCV and GCV, supporting environmental regulation of these traits.

Broad-sense heritability estimates varied significantly among traits (35.51-76.25%), with root length showing the highest genetic percentage (76.25%) and stomatal area the lowest percentage (35.51%), with population-wide average reached 62.40%.The presence of moderate to high heritability suggests that environmental factors have relatively less influence on the traits studied. This suggests that the degree of heritability for these traits is less influenced by the environment and that significant improvement can be achieved through selection (Tesfaye, 2021). Additionally, these traits are predominantly controlled by genetic factors (Singh et al., 2012; Sohail et al., 2018). Demelash et al. (2021) evaluated 214 sorghum genotypes and found a high average heritability of 81.80%. Mace et al. (2012) reported an average heritability of 73.7% for 141 sorghum RILs.

In the present study, the heritability for nodal root angle is high, with a value of 68.76%. This value is lower than previous studies that reported values of 73.7% (Mace et al., 2012), 78.9% (Menamo et al., 2023), 92.52% (Demelash et al., 2021), and 96% (Joshi et al., 2017); but it is

still higher than a study that reported a heritability of 47% in 44 sorghum inbred lines (Singh et al., 2011), 44.38% in 291sorghum genotypes (Elias et al., 2023), and 30% for stay-green gene introgressed sorghum genotypes (Solomon et al., 2023). The significant heritability observed in the current evaluation indicates that hereditary factors have a major effect on the differences in nodal root angle, while environmental factors play a smaller role. This highlights the significance of root angle as a crucial trait for selective breeding aimed at improving sorghum's ability to withstand drought.

In the current study, the heritability for root length and root dry weight was also found to be high at 76.25% and 74.07%, respectively. Similarly, Demelash et al. (2021) reported a high heritability of 88.86% for root length and a moderate heritability of 40.26% for root dry weight. In contrast, Elias et al. (2023) reported low heritability of 17.33% for root length and moderate heritability of 49.32% for root dry weight. Whereas, Solomon et al. (2023) reported moderate heritability of 44% and 46% for root length and root dry weight, respectively. The combination of high broad-sense heritability and high GAM is a more reliable indicator of the potential impact of selection than considering heritability alone (Johnson et al., 1955). In the present study, the high heritability, GCV, and GAM values of root angle (68%, 20.92% and 35.73%, respectively) indicate that there is a potential for sorghum improvement through selection based on root angle from the genotypes studied. Demelash et al. (2021) also reported high heritability (92.52%) with a high GAM (28.70%) for root angle. While, Elias et al. (2023) reported a moderate heritability (44.38%) and high GAM (24.33%) values in a study of sorghum landraces.

5.1.3. Phylogenetic relationships between genotypes

The tested 158 genotypes were divided into four clusters. Genotypes were clustered irrespective of region. The fifty genotypes from Oromia were clustered into four clusters, with the majority of genotypes (21 genotypes, 42% of 50) in Cl-IV, followed by Cl-III (16, 32%), Cl-I (9, 18%), and Cl-II (4, 8%). However, two genotypes (#90 and #144) originating from Afar and two genotypes from Somali (#89 and #114) were assigned to different clusters. Cluster I had the highest average values for ShL, LAr, ShD, SFW and SDW. Cluster II had the highest average values for RL, RFW, RDW, RHN, RSR, and NR. Cluster II included genotypes #86, #142 and #125, which had the longest roots and larger number of nodal roots. Cluster. III had the lowest

average value for root angle. Cluster III included genotypes #151, #96, and #66, which had narrowest root angles. These accessions were initially collected from main sorghum growing areas in the Tigray, Oromia and Amhara regions of Ethiopia. Genotypes #92, #24, and #97 with wide root angle from the Oromia region were placed under Cluster IV. Therefore, the clustering pattern suggested that genotypes from similar region could be clustered into diverse clusters.This shows a broad regional distribution of genotypes of sorghum with different root and shoot traits of agronomic importance.

5.1.4. Association analysis

There was a strong positive correlation among root-related traits, as indicated by their correlation values. Particularly, the relationships between root length, root dry weight, number of nodal roots, and root fresh weight were highly significant ($r = 0.93$-0.99, $p < 0.001$; Figure 4.2), suggesting similar regulatory mechanisms between RL, RDW, NR, and RFW. The correlation between root angle and root length was negative (r=-0.02), which is consistent with a previous study by Solomon et al. (2023), who reported a negative association between root angle and root length (r=-0.018). Additionally, the correlation analysis revealed that, apart from leaf angle (LAg) and stomatal area (SA), the above-ground traits displayed significant positive correlations with each other ($r = 0.27$- 0.99, $p < 0.001$; Figure 4.2).

A non significant association was evident between leaf angles and shoot length as well as shoot dry weight. Similarly, neither stomata density nor stomata area significantly correlated with shoot length. Moreover, the relationship between the above ground and below ground traits was not significant ($p>0.05$). However, shoot dry weight (SDW) was positively associated with RAg, RHN, and RDW ($p<0.05$) and negatively connected with root-to-shoot ratio ($p<0.001$). Our findings indicate that RSR was negatively interrelated with SDW (r=-0.38), SFW (r=-0.43), LAr (r=-0.43), ShL (r=-0.42), and StD (r=-0.40). These results align with earlier studies conducted by Elias et al. (2023) and Menamo et al. (2023), who reported negative correlations between RSR and SDW of r=-0.43 and r=-0.50, correspondingly. Hence, the significant and negative associations between these traits suggest that it is impossible to simultaneously select both attributes. In this study, the root angle was weakly associated with other root and shoot attributes, as reported in previous studies (Demelash et al., 2021; Elias et al., 2023; Menamo et al., 2023).

5.1.5. PCA biplot and cluster analysis

The biplot clearly showed that root traits were not correlated with shoot traits, as vectors for both variables were positioned in different directions on the biplot. Moreover, the biplot also indicated that many of the genotypes were more closely related with each other and positioned close to each other while some of the genotypes were distant and more divergent from others on biplot. Among these genetically diverse genotypes, genotype #86 stood out for its root to shoot ratio (0.92), longest root length (71cm), highest root dry weight (0.91g), and highest number of roots (8). Genotype #130 is notable for having the highest number of root hairs (45.5), whereas Genotype #91 is distinguished by having the highest leaf angle (45°). Additionally, genotype #42 stands out for its high shoot length (19.5 cm) and leaf area (40.22 cm^2). Genotype #154 exhibited a high shoot fresh weight (4.05 g) and stomatal density (214 stomata/mm^2). Genotypes #25 had the lowest values for ShL, LAr, SFW and SDW, whereas genotype #49 had the lowest values for RSR, RL, RDW, RFW, RH, and NR. Selection among such divergent genotypes may be useful for their use as parents in breeding programs, ultimately leading to the development of cultivars that can address various challenges in sorghum production in moisture-stressed areas (Seetharam & Ganesamurthy, 2013; Sant'Anna et al., 2020). Clustering based on PCA of root and shoot traits differentiated the genotypes into four clusters. The PCA also highlighted phenotypic differences of these clusters. The biplot was efficient in displaying the variables impacting each cluster. For instance, traits related to roots such as root length, root hair number, number of nodal roots, root fresh weight, and root dry weight, root-to-shoot ratio were associated with cluster II. Moreover, the genetic distance between the genotypes were aligning with the outputs of the hierarchical grouping, in which the genotypes placement in each clusters were largely congruent with the clusters shown by the hierarchical analysis. For instance, genotypes #86 #125, #21, and #3 were clustered together in both analyses.

PCA based on geographical region did not show clear grouping of the genotypes by their region of origin. However, there was a tendency of clustering, with some of the genotypes from unknown region (NA) such as genotypes #11, #16, #34, #78, #93 and #134 being grouped together, as they resided close to the biplot origin. Both hierarchical and PCA clustering analyses demonstrated that sorghum genotypes gathered from the identical region were

assembled into dissimilar clusters, indicating high genetic diversity within the same geographical region.This assemblage of genotypes implied that regional origin had no influence on grouping pattern, suggesting that spatial heterogeneity is not a measure of genotypic diversity. The grouping of sorghum accessions gathered from the same geographical area into different groups were also reported in earlier studies (Elias et al., 2023; Enyew et al., 2024). The absence of clear-cut differentiation of the genotypes based on region of origin could be due to the increased outcrossing in cultivated sorghum, gene flow across regions through market systems as well as seed exchange among farmers, and interspecific crossing with its wild relatives (Enyew et al., 2021; Menamo et al., 2021; Mamo et al., 2023).

5.2. Growth and transpiration responses to progressive soil drying

5.2.1. Growth and transpiration attributes of genotypes

The dry down experiment, which examined 40 genotypes, revealed significant genetic variation ($P<0.05$) in both the growth and transpiration parameters, which may have vast potential for improving sorghum's adaptability to a water limited environment. The study also revealed significant variations in the growth and transpiration responses among genotypes, with high heritability (Table 4.7).

Similar to prior researches in sorghum (Li et al., 2011; Tefera, 2021), pearl millet (Kholová et al., 2010), and wheat (Xiong et al., 2006), the present study also found that moisture stress led to a major decline in shoot growth as opposed to root growth (Table 4.10). The decline in shoot biomass in moisture-stressed sorghum plants might be due to decreased water flow from the xylem to nearby cells, water-limiting situations affect cell elongation (Fahad et al., 2017). Because their expansion is dependent on turgor pressure and the amount of water taken in, the number of leaves and the size of each leaf decreased in dry circumstances, this resulted in a drop in plant biomass as well as a decline in leaf quality and plant development (Yang et al., 2012).

The physiological features of drought stress include a reduction in stomata conductance, photosynthetic activity, and transpiration rate (Dwivedi et al., 2008; Fracasso et al., 2016). This adaptation process was also discovered in cowpea (Belko et al., 2013) and pearl millet (Kholova et al., 2009; 2010). Stomata closure and increased mesophyll resistance hinder the diffusion of

CO_2 to the carboxylation sites when there is a water shortage. As a result, there is an imbalance between the rate of electron transport and the rate of CO_2 fixation, which further impedes plant growth under drought conditions (Verma et al., 2018).

Under well-watered conditions, the sorghum genotypes with wide root angles exhibited higher shoot growth compared to the narrow-root genotypes in both experiments, as evidenced by the disparities in above-ground biomass (SDW) between the two groups. Conversely, the narrow root angle genotypes demonstrated greater above-ground biomass accumulation under water stress conditions in both runs. The superior over-ground accumulation in narrow root angle genotypes under moisture-limited environment could be ascribed to their relative ability to explore available soil resources such as water (van der Bom et al., 2024).

There was also significant variation in root dry mass (RDM) between genotypes with narrow and wide root angles both under non-stressed and stressed conditions, though in Experiment 2, this variation was only present under water stress condition (Table 4.11). There was a pronounced disparity in total water extraction between narrow and wide root angle genotypes under water stress conditions in both experiments. The size of water absorbed from the soil is mainly determined by different traits of the roots, such as their depth, density, and angles (Singh et al., 2011; Wasson et al., 2012; Comas et al., 2013). Roots with narrower angles have a tendency to have more root length density (Tsuji et al., 2005; Singh et al., 2011; Lynch, 2013). Previous researchs indicate that sorghum cultivars with greater root length density exhibit enhanced soil water acquisition capacity (Monti and Zatta, 2009; Demissie et al., 2023). Therefore, the faster uptake of soil water observed in narrow root angle genotypes, compared to wide root angle genotypes, can be attributed to the higher root density.

5.2.2. Fraction of transpirable soil water

Across both experiments, there was a substantial disparity ($P < 0.001$) amongst genotypes with respect to FTSW threshold values, with ranges of 0.24 to 0.49 and 0.20 to 0.46, respectively. Previous studies have also reported significant genotypic differences in FTSW thresholds for maize (Gholipoor et al., 2013), sorghum (Gholipoor and Sinclair, 2012), and millet (Kholova et al., 2010). The diverse FTSW thresholds observed among the sorghum genotypes in our study indicate that each genotype responds uniquely to progressive soil drying. Tefera (2021) carried out a dry-down experiment on two sets of sorghum genotypes, categorized as sensitive and

tolerant and reported FTSW ranges of 0.15 to 0.64 in Experiment 1 and 0.16 to 0.53 in Experiment 2. A study conducted by Gholipoor et al. (2012) found variations in FTSW thresholds among sixteen sorghum genotypes, with values spanning from 0.32 to 0.48. Moreover, Kholová et al. (2010) and Gholipoor et al. (2012) observed FTSW thresholds of 0.30 to 0.47 for pearl millet (*Pennisetum glaucum* L. R. Br.) genotypes and 0.33 to 0.60 for maize (*Zea mays*) hybrids, respectively. Likewise, significant differences in FTSW thresholds have been noted in soybean (Purdom et al., 2022) and cotton (Devi and Reddy, 2020) cultivars.

In both experiments, some sorghum genotypes with wider root angles, such as #121 (W) (0.42), #91 (W) (0.45), #137(W) (0.49), and #87(W) (0.46), tended to show relatively higher FTSW thresholds. This suggests that, these particular sorghum genotypes may possess better stomatal control compared to other genotypes. In a similar study, Tefera (2021) reported higher mean FTSW thresholds for drought-tolerant sorghum genotypes and lower mean FTSW threshold values for sensitive ones. In contrast, Kholová (2010) reported a lower FTSW threshold values for tolerant pearl millet genotypes than sensitive genotypes.

Genotypes having superior FTSW threshold are able to thrive in extended periods of drought because they conserve soil moisture by closing their stomata early (Ray and Sinclair, 1998), allowing them to tolerate prolonged drought stress (Ray and Sinclair, 1997; Gholipoor et al., 2012). This restricted transpiration rate is likely a result of strong stomatal control, which is influenced by leaf level hydraulic conductance (Sinclair et al., 2008; Sadok and Sinclair, 2010). Conversely, the results of an outdoor dry-down experiment using two sorghum lines demonstrated that a narrower root angle is associated with a higher FTSW threshold. This was evidenced by the higher FTSW threshold of 0.62 in IS13540 (narrow root angle) compared to 0.53 in IS23143 (wide root angle) (Gowsiga et al., 2021).

5.2.3. Correlation analysis

Shoot dry biomass directly related with root dry biomass under water stress condition (r=0.55* and 0.45*, respectively). Shoot dry mass was also exihibted a positive relationship with total transpiration rate (r=0.48*), total water extraction (r=0.49*) regardless of soil moisture status and negatively correlated with fraction of transpirable soil water (r=-0.57*), under water stress condition. Similarly, total transpiration was positively correlated with total water extracted (r= 0.70*** to 0.99***) regardless of the soil water condition in both experiments. In contrast, total

transpiration showed a negative correlation with fraction transpirable soil water (r=-0.65** and - 0.68**, respectively). Whereas, total water extraction was negatively correlated with FTSW (r=-0.50* and -0.56*, correspondingly), however, these negative correlations were only present under water stress condition (Figure 4.9).

Plants with narrow root angle are better armed to endure arid conditions as they are able to reach soil water resources, ultimately leading to higher crop yields (Kaydan & Yagmur, 2008; Wasaya et al., 2018; Ye et al., 2018; Baoru Li et al., 2022). Thus, prioritizing sorghum genotypes identified in this progressive soil drying experiment that combine key drought adaptive traits including the highest values for biomass accumulation and total water extraction, such as genotypes #151 (N), #25 (N), #96 (N), and #30 (N) can be considered ideal for developing cultivars suitable for moisture limited environments.

Overall, to ensure the consistency of data obtained from this greenhouse experiments, it is recommended to conduct field experiments under both non-stressed and stressed conditions utilizing identical genotypes and parameters. However, it is important to note that some exceptions may apply. Additionally, it is crucial to consider other factors that can affect sorghum production, such as nitrogen (N), phosphorus (P) (Sopandie et al., 2023), and potassium (K) deficiencies, as well as aluminum toxicity (Baligar et al., 1989). These constraints were not taken into account in our analyses, but future studies should consider incorporating these stresses and their potential interactions with root traits (root angle) and drought stress.

5.3. Genome-wide association study using multi-locus approach

5.3.1. Population structure and linkage disequilibrium

Population structure investigation offers vital understanding of the genetic variability among sorghum genotypes and can be helpful in evading mistaken relations between marker loci and traits of interest (Eltaher et al., 2018). Population structure investigation of the 129 sorghum genotypes gathered from diverse geographic regions divided the population into five sub-populations. Genotypes from similar region were grouped into distinct clusters, indicating a significant genetic diversity within the region. These output support that geographic origin has no effect on grouping pattern. The outcomes of this investigation are in agreement with the findings of Morris et al. (2013) ,Wang et al. (2013), Weerasooriya et al. (2016), and Mengistu

et al.(2020), who reported considerable admixture of sorghum genotype from various regions. Sorghum landraces continue to be common among farmers adding to admixtures in germplasm collections. This shows gene flows between regions. Menamo et al.(2021) reported that presumably geographic closeness, mixing of communities across regions, seed exchange and farmers' practice of growing various landraces in the same field would have added to increasing gene flow. The overall admixture across the total population was low (24.8%) lower relative to earlier reports by Girma et al. (2019) and Menamo et al. (2021) who reported 47%, owing to small sample size in the current study. Hence, sorghum landraces remain to be a vital source of planting stock for farmers, stressing the need for detailed evaluation of their genetic diversity for improvement purposes.

The linkage disequilibrium decay distance in the current research was ~ 14. 4 kb, congruent with earlier reports in sorghum of 10 – 30 kb (Wang et al., 2013) and 10 – 15 kb (Hamblin et al., 2005). However, it is less than the ~ 65 kb discovered in 304 sorghum genotypes by Wondimu et al. (2023). This variation may be as a result of the small number of accessions utilized in this study. Previous research indicate that with small sample there is a reduced pool of genetic variability, which can result in rapid linkage disequilibrium decay (Vos et al., 2017), since there are not as many unique combinations of alleles at different loci, making it easier for crossing over to halt linkage between them. Furthermore, minimizing the number of genotypes may also give rise to a decline in valuable genetic information. As a result of small population size, there may be a lesser amount of variability and compromised allelic reachness and limited diversity, which may restrict the information that can be generated from studying linkage disequilibrium patterns (Vos et al., 2017).

5.3.2. Identification and functional characterization of candidate genes

Forty nine reliable QTN were identified on 10 sorghum chromosomes using no less than three multi-locus approaches related to the studied seedling traits. Of these, 28 QTNs (57.1%) were linked to belowground traits such as root angle (RAg), root length (RL), root number (RN), root biomass (RDW), root hair number (RHN) and root-to- shoot ratio (RSR). The aggregate count of QTNs identified in the current study surpasses those discovered in earlier research on Ethiopian sorghum genotypes conducted by Menamo et al. (2023) and Elias et al. (2024). Menamo et al. (2023) and Elias et al. (2024) targeted solely on four main root traits: root angle

(RAg), root length (RL), root number (RN), and root biomass (RDW). Conversely, the current study expands its range to include other traits, including root hair count (RHN), the root-to-shoot ratio (RSR) and several aboveground seedling traits. The detection of robust QTNs for the characters under investigation confirmed that mrMLM was more effective and sturdy relative to the other five models. Similarly, Wondimu et al. (2023) showed that the mrMLM model led to the maximum count of robust QTNs as opposed to the other ML-GWAS models in sorghum agronomic traits. Furthermore, the robust QTNs detected inthis research offer boundless opportunities for prospective genetic enhancement of the traits assessed in the current study.

Genetic relationship among traits is the consequence of gene interactions such as linkage and/or pleiotropy (Saltz et al. (2017). In the present study, 10 pleiotropic QTNs (20.4%) were discovered linked to multiple traits. Nevertheless, no pleiotropic QTNs were discovered between belowground and aboveground traits. Interestingly, the biplot and correlation analysis display minimal association between these traits. Conversely, sturdy relationship was obsereved within root and shoot traits. For instance, S1-6012717 a QTN located on on chromosome 1 was linked to four root attributes such as root length (RL), root number (RN), root dry weight (RDW) and root to shoot ratio (RSR). Four genes namely Sobic.001G078100, Sobic.001G077800, Sobic.001G077900 and Sobic.001G078000 were found close together with S1-6012717. Of these, Sobic.001G078100 and Sobic.001G077800 genes were described as molybdopterin cofactor sulfurase mosc//catalytic/pyridoxal phosphate binding protein and MYB-like DNA-binding domain, respectively.Existing research lacks empirical support for a link between molybdopterin cofactor sulfurase mosc and root growth. However, their involvement in basic metabolic activities supports that they could play a role in modulating root growth implicitly via their effect on vital cellular processes. Molybdopterin cofactor sulfurase (mosc) is participated in the anabolic reaction of molybdenum cofactor, which is important for the functioning of molybdoenzymes engaged in several metabolic pathways. Some molybdoenzymes have been involved in biochemical processes that involve nitrogen and carbon elements (Hänsch et al., 2001; Forde, 2002), which are essential for root developement (Zhang & Forde, 2000). Sobic.001G077800 (myb-like dna-binding domain) which is co-positioned with S1_6012717 QTN. MYB transcription factors have been involved in modulating various metabolic processes, including root development, by monitoring the

manifestation of genes engaged in cell differentiation, cell cycle regulation, and hormone signaling pathways (Gibbs et al., 2014; Li et al., 2020). Some MYB transcription factors have been specifically linked to root growth and development by influencing root meristem activity (Wybouw et al., 2023), lateral root formation (Gibbs et al., 2014; Fernández-Marcos et al., 2017), and response to environmental stresses including drought, high salinity, and low temperatures (Wang et al., 2021).

Three pleiotropic quantitative trait nucleotides (S4-13304368, S4-60963038 and S6-45176048) identified on the 4^{th} and 6^{th} chromosomes were linked to root length, and root biomass, respectively. S4-13304368 QTN closely situated with genes Sobic.004G120200 that code for sentrin/sumo-specific protease and Sobic.004G120233 that code for uncharacterized protein. sentrin/sumo-specific proteases are enzymes that selectively bind and cut small ubiquitin-related modifier (sumo) proteins from target proteins, thus controlling their functionality, persistence, and subcellular localization (Johnson, 2004). Researches recommend that sumo-specific protease have a role in modulating numerous facets of root growth and development (Huang et al., 2009; Yu et al., 2019). sumoylation is a post-translational modification activity that has a decisive part in the control of diverse cellular activities, such as stress adaptations, hormonal communication, and growth (Xu & Yang, 2013). Studies have indicated that sumoylation and desumoylation pathways are engaged in modulating root development in plants. For instance, changed levels of sumoylation have been linked to variations in root development (Zhang & Forde, 2000), root architecture (Srivastava et al., 2021), and adaptations to environmental conditions (Ghimire et al., 2020). Additionally, specific sumo proteases play a key role in fine-tuning the function of TF and other proteins important for root growth (Mercier et al., 2021).

Of the six QTNs discovered for root angle (RAg), two co-positioned with Sobic.004G189600 and Sobic.009G112400 which direct the synthesis of Phosphopantothenate-cysteine ligase/Phosphopantothenoyl cysteine synthetase and F-box domain // Galactose oxidase/kelch, beta-propeller, respectively. While the remaining are unpredicted gene products. Phosphopantothenoyl cysteine synthetase direct the biosynthesis of coenzyme A (CoA), which is critical in diverse methabolic activities linked to root morphogenesis in plants (Kupke et al., 2003). CoA is vital for several metabolic activities, like fatty acid biosynthesis, amino acid

metabolism, and the tricarboxylic acid (TCA) cycle, which delivers energy and raw materials for root development (Yang et al., 2020; Lv et al., 2021). Variations in the level of coenzyme A might possibly influence root growth angle by modulating root enlargement and branching (Hu et al., 2015). Besides, CoA and its variants participate in signaling pathways associated with growth and stress coping mechanisms in plants (Guo et al., 2021). For instance, acetyl-CoA is the source of acetyl group for lysine acetylation on histone proteins, which controls transcriptional activity genes involved in root morphogenesis (Ma et al., 2016). Moreover, fluctuations in coenzyme A production can effect nutrient homeostasis and root architecture, possibly persuading root growth angle as plants become accustomed to resource availability or environmental challenges (Su et al., 2020). Another gene Sobic.009G112400, co-situated with S9-48742385, produces an F-box domain protein related to root angle (RAg). Prior studies confirmed that F-box proteins are vital for monitoring hormonal responses, and seedling development (Yan et al., 2011; Abd-Hamid et al., 2020; Elias et al., 2023). F-box proteins, as constituents of the Skp1-cullin-f-box (scf) E3 ubiquitin ligase complex, are known to participate in several facets of root growth at early stage (Hong et al., 2017). F-box proteins can act on particular proteins engaged in root morphogenesis involving protein modification followed by successive degradation, thus impacting root architecture in seedlings (Sadanandom et al., 2012), hormonal communication (Gray et al., 1999), and other regulatory proteins (Moon et al., 2004) that control root development processes.

An additional QTN (S3-65025755), significantly influencing root length (RL), is positioned near the Sobic.006G075200 gene, which codes for an aluminum-activated malate transporter (ALMT). The ALMT gene family is responsible for producing membrane transporters that facilitate the movement of malate ions across cell membranes (Sasaki et al., 2004; Liu et al., 2009). Under aluminum stress, plants can trigger ALMT transporters to exude malate ions into the rhizosphere, a process that neutralizes toxic aluminum ions and safeguards roots from damage (Sasaki et al., 2004). Studies indicate that ALMT transporter expression and functionality play a role in root growth traits, including elongation (Kobayashi et al., 2007; Liu et al., 2009). By regulating malate production, these transporters enhance aluminum tolerance and promote root elongation in stressed plants (Sasaki et al., 2004; Hong et al., 2017). Beyond aluminum detoxification, ALMT-mediated malate release influences root physiology by modulating ion absorption, nutrient availability, and soil pH, all of which can indirectly alter

root length (Liu et al., 2022). Additionally, the interplay between ALMT transporters and aluminum stress shapes how roots adapt to environmental challenges and other stressors conditions (Das & Ganesan, 2022).

The QTN (S3-62564964), associated with root dry weight (RDW), is situated close to the Sobic.003G226200 gene, which encodes a neutral ceramidase-related protein. Neutral ceramidase is an enzyme critical to sphingolipid metabolism, where it breaks down ceramide into sphingosine and a fatty acid (Chen et al., 2015). Sphingolipids serve as essential signaling molecules, regulating key cellular processes such as growth, differentiation, stress adaptation, and programmed cell death (Zienkiewicz et al., 2020).Studies indicate that sphingolipid metabolites—including ceramides and sphingosine, play a role in plant development, particularly in root biomass accumulation (Ramírez-Chávez et al., 2004). By converting ceramide to sphingosine, neutral ceramidase influences signaling pathways that govern root cell division, elongation, and differentiation, ultimately affecting root biomass (Worrall et al., 2008). Environmental stresses like drought, salinity, and pathogen infection can alter neutral ceramidase activity and sphingolipid levels (Guo & Wang, 2012). Shifts in ceramidase expression may disrupt sphingolipid balance, thereby modifying root growth, biomass production, and plant resilience under stress (Li et al., 2015).

The Sobic.006G090500 gene represents another genetic factor contributing to root dry weight variation, producing a chloride channel (CLC) protein that plays a fundamental role in plant nitrate transport systems (Subba et al., 2021). These specialized membrane proteins orchestrate the controlled movement of chloride ions across cellular boundaries (Wei et al., 2019), while simultaneously contributing to several vital plant functions. Their activities help coordinate ionic equilibrium, manage water balance through osmotic regulation, maintain cellular pH stability, and facilitate the acquisition of essential nutrients (Wei et al., 2019). Experimental evidence reveals that CLC proteins significantly influence root system development, with measurable effects on final biomass yields (Nguyen et al., 2016). Beyond their primary role in chloride transport, these proteins indirectly modulate the cellular concentrations of potassium, sodium and calcium ions - all crucial elements for optimal plant development (Colmenero-Flores et al., 2007; Li et al., 2017). This comprehensive ion management system directly affects root functionality by governing water relations, nutrient uptake efficiency, and cellular turgor

maintenance, which collectively shape root morphology and biomass production (Chen et al., 2016; Li et al., 2017). Under environmental pressures, CLC proteins demonstrate remarkable adaptability (Chen & Jiang, 2010; Wang et al., 2015). When challenged by saline conditions, water scarcity, or heavy metal contamination, plants dynamically adjust CLC operations to preserve ionic homeostasis and cellular water balance, enhancing root survival capacity (Diédhiou & Golldack, 2006; Baek et al., 2018; Um et al., 2018). Emerging research indicates these transport proteins may also participate in auxin-related developmental pathways that coordinate root growth patterns and biomass allocation (Burdach et al., 2014). Genetic or functional modifications affecting CLC performance can substantially alter the ionic composition of root cells, potentially transforming root architecture, elongation dynamics, and ultimately, the total biomass accumulated (Nguyen et al., 2016).

Candidate gene analysis identified two key genes influencing root hair number (RHN): Sobic.010G181000, which encodes a Pentatricopeptide repeat (PPR) protein, and Sobic.010G181200, responsible for far-red-impaired response 1 (FAR1) production. PPR proteins participate in multiple developmental processes including embryonic growth (Cushing et al., 2005), shoot apical meristem formation (Prasad et al., 2005), and cytoplasmic male sterility restoration (Bentolila et al., 2002). These proteins also help plants respond to environmental stresses like drought, salinity, and cold temperatures (Jiang et al., 2015). The FAR1 protein, conversely, primarily affects phosphorus uptake efficiency (Liu et al., 2017) and drought resistance mechanisms (Tang et al., 2013). Additional candidate genes (Sobic.004G008900 and Sobic.004G008800) showed association with root-to-shoot ratio (RSR), both encoding acid phosphatase-like proteins.These enzymes break down phosphoric acid esters under acidic conditions, playing crucial roles in phosphorus metabolism plants (Duff et al., 1994). Some family members contribute to cuticular wax production (Aarts et al., 1995), while others may affect nutrient distribution patterns (Upadhyay et al., 2022), potentially explaining their influence on root-to-shoot biomass allocation.

Our analysis identified several significant QTNs associated with shoot traits. Three loci (S1_68144065, S6_49860654, and S6_6049929) on chromosomes 1 and 6 correlated with shoot length and leaf area, while S8_2624370 showed pleiotropic effects influencing both shoot length and stomatal density. The S1_68144065 QTN coincides with four genes:

Sobic.001G350300 (encoding an achaete-scute transcription factor/ basic helix-loop-helix (bHLH) domain), Sobic.001G350400 (hydroxyisourate hydrolase), Sobic.001G350501 (histone H3), and Sobic.001G350600 (cytochrome c oxidase subunit 6b-1). Of particular interest is the bHLH transcription factor, known to regulate shoot growth during early development. These bHLH transcription factors serve as master regulators in the shoot apical meristem (SAM), the primary growth center that generates all above-ground plant structures (Poirier et al., 2018). They precisely balance stem cell maintenance with differentiation processes (Katayama et al., 2015), coordinating multiple aspects of shoot formation including cell proliferation rates, expansion patterns, organ initiation timing, and overall shoot architecture (Galli et al., 2015). Beyond developmental control, these transcription factors mediate plant responses to environmental stresses, adjusting shoot growth according to external conditions (Zuo et al., 2023).

Among the stable QTNs identified in this study, S2_72741053 demonstrated a significant association with leaf angle (LAg). This locus is positioned near Sobic.002G349500, a gene encoding a threonine-specific protein kinase. These kinases are known to play critical roles in metabolic regulation, gene expression control, and cellular proliferation (Hardie, 1999). Given their broad influence on plant signaling cascades, threonine-specific kinases may contribute to leaf angle determination by modifying downstream pathways that govern cell expansion and tissue patterning (Dissanayake et al., 2004). Although direct evidence linking these kinases to leaf angle regulation remains limited, future studies exploring their interaction with known leaf architecture regulators could clarify their functional significance (Sojka et al., 2024). Leaf angle defined as the positioning of leaves relative to the stem directly impacts light capture efficiency, water retention, and transpiration rates, all of which are vital for drought resilience.

Our analysis identified several candidate genes influencing shoot dry weight (SDW) and stomatal characteristics. Two genes located on chromosome 7- Sobic.007G175600 and Sobic.007G175702 encode auxin responsive family proteins that regulate plant hormone metabolism and growth processes (Zheng et al., 2020). Nearby, Sobic.007G175900 produces RING zinc finger proteins known to mediate both developmental processes and drought responses (Han et al., 2022). On chromosome 8, the Sobic.008G028600 gene containing a WRKY domain showed association with stomatal density (StD). WRKY transcription factors

participate in diverse physiological functions, including stress responses and developmental regulation (Wu et al., 2005). Recent studies demonstrate their direct impact on stomatal patterning - VvWRKY18 overexpression in grape increased stomatal density and water loss rates (Zhang et al., 2022), while StWRKY48 overexpression reduced drought tolerance in potato through similar mechanisms (Yang et al., 2022).These effects likely occur through WRKY-mediated regulation of hormone signaling pathways, particularly those involving ABA and JA, which coordinate stomatal development (Rushton et al., 2012; Ruan et al., 2019).

Sobic.001G395100, a gene encoding a putative DEAD-box RNA helicase, as potentially influencing stomatal area (SA) - a trait previously associated with thermotolerance (Wang et al., 2016). While direct evidence connecting DEAD-box RNA helicases to stomatal morphology remains limited, emerging research suggests these enzymes may modulate stomatal characteristics through several indirect mechanisms. These helicases appear to affect stomatal development and function through their involvement in multiple cellular processes. First, they participate in gene expression regulation, potentially altering transcripts related to stomatal patterning (Baek et al., 2018). Second, they contribute to abiotic stress responses that influence stomatal behavior (Baruah et al., 2017). Third, they interact with hormone signaling pathways, particularly those involving ABA and JA, which are known to regulate stomatal development (Cai et al., 2018). Supporting evidence comes from studies showing that DEAD-box RNA helicase mutants display ABA hyposensitivity and drought susceptibility, characterized by impaired stomatal closure, increased transpiration rates, and altered leaf temperature regulation (Baek et al., 2018). These findings suggest that through their effects on hormone signaling and stress response pathways, DEAD-box RNA helicases may ultimately influence stomatal area by modifying the developmental processes that determine stomatal size and density.

5.4.Genetic diversity

Studies on genetic diversity using single nucleotide polymorphisms (SNPs) offer high-resolution data, enhancing our understanding of the genetic structure and relationships among sorghum landraces. These studies not only aid in developing climate-resilient cultivars but also promote the extensive utilization of genetic diversity (Enyew et al., 2024). Moreover, grasping the genetic diversity is pivotal for the preservation of sorghum germplasm. By identifying

distinct and valuable genetic resources, we can devise conservation strategies to safeguard these resources for future generations. This is especially critical in light of climate change and other environmental challenges that threaten crop diversity (Enyew et al., 2024). Studies on genetic diversity utilizing SNPs offer high-resolution data, enhancing our comprehension of the genetic structure and relationships among sorghum landraces. These investigations also support in the breeding of climate-adapted crop variety (Afolayan et al., 2019; Wondimu et al., 2021; Kasule et al., 2024).

5.4.1. Diversity parameters

The high genetic distance (mean= 0.69) suggests a remarkable level of genetic variation between the genotypes examined. The mean genetic distance is greater than the values reported by Silva et al. (2020), who indicated a mean genetic distance of 0.33 across 160 sorghum lines using 29,649 SNP markers, and Emrey et al. (2022), who documented a mean genetic distance of 0.28 in 37 inbred lines based on 7,339 Single nucleotide polymorphisms (SNP) markers. Additionally, the moderately high level of gene diversity or expected heterozygosity (mean = 0.51) detected the study likely reflects the variability present in the sorghum genotypes from various origins. The genetic analysis revealed significant variation within the Ethiopian sorghum genotypes, highlighting its potential as a rich reservoir of unique genetic variants to enhance sorghum breeding efforts.

Polymorphic information content (PIC) serves as a key metric for evaluating marker polymorphism levels. Based on the classification by Botstein et al. (1980), markers can be categorized into three groups: those with PIC values below 0.25 are deemed minimally informative, values between 0.25 and 0.5 are moderately informative, and values above 0.5 are considered highly informative. In the current analysis, the mean PIC value was 0.32, with more than half (53%) of the markers demonstrating PIC values greater than this threshold. This suggests that the SNP markers employed in the study effectively distinguished between the sorghum genotypes examined. This mean PIC value is comparable to the findings of Mudaki et al., (2023), who documented an average PIC of 0.31 using 6,977 SNP markers in 169 genotypes, and Sejake et al. (2021), who found a mean PIC value of 0.30 using 110 SNP markers in 100 genotypes. However, it is higher than the results of Afolayan et al. (2019), who showed a PIC mean of 0.24 using 4,980 SNP markers in 157 genotypes, Enyew et al. (2022),

who found a mean PIC value of 0.24 using 3,001 SNP markers in 359 individuals representing 24 accessions, and Yahaya et al. (2023), who reported a mean PIC value of 0.26 using 7,516 SNP markers in 200 genotypes. Similarly, Adedugba et al. (2023) reported a mean PIC value of 0.26 using 7,078 SNP markers in 50 genotypes. The results demonstrate that the SNP markers employed in this research successfully captured meaningful genetic variation across the 129 sorghum genotypes analyzed. This confirms their utility for evaluating genetic diversity in sorghum populations.

The current study revealed an average observed heterozygosity of 0.15, which corresponds with the results obtained by Yahaya et al. (2023) in their recent investigation. When compared with previous studies, this value appears intermediate - being notably lower than the 0.22 and 0.29 heterozygosity levels documented by Afolayan et al. (2019) and Enyew et al. (2022) respectively, but substantially higher than the 0.05 reported by Mudaki et al. (2023). These findings are consistent with established genetic patterns in self-pollinating species, where heterozygosity levels typically remain quite low, as noted by Poehlman (2013) in his work on crop genetics.

5.4.2. Analysis of molecular variance

The analysis of molecular variance (AMOVA) conducted in this study demonstrated that the majority of genetic diversity (99%) was distributed within populations and regions of origin, rather than between them. This finding highlights the substantial genetic variation present among individual accessions, even when grouped by population structure or geographical origin. The fixation index (Fst) was calculated at 0.005 and 0.009 for regions and population structures, respectively, indicating low genetic differentiation among genotypes from different population structures and regions. Additionally, a gene flow value of 48.79 and 27.83 suggests a pronounced genetic admixture among neighboring regions (Table 4.16; Table 4.17). The higher levels of genetic variation observed within regions (populations) are consistent with the results of Afolayan et al. (2019), Wondimu et al. (2021), and Kasule et al. (2024), who documeneted significant genetic variation within populations of sorghum accessions from Ethiopia, Nigeria, and Uganda, with values of 94%, 98%, and 88%, respectively. Similar results have been documented in earlier studies that employed SSR markers, including those by Tirfessa et al. (2020), Nemera et al. (2022), and Mamo et al. (2023), who identified significant

genetic variation within Ethiopian sorghum populations, with values of 99.62%, 98%, and 98%, respectively. Furthermore, Menkir et al. (1997) and Ayana et al. (2000b) reported comparable results using RAPD markers, indicating that 87% and 77% of the genetic variation was found within regions of ethiopian sorghum genotypes, respectively. Contrary to the overall trend, Mengistu et al.(2020) and Enyew et al. (2022) observed a larger amount of genetic dissimilarity between sorghum populations than within populations. Mengistu et al.(2020), using 5060 SNPs in 342 genotypes, found 58.8% of the variation among populations and 40.4% within. Similarly, Enyew et al. (2022), analyzing 3001 SNPs in 359 individuals from 24 landrace accessions, reported 64.5% among-population variation and 35.5% within. Higher among-population diversity observed by Mengistu et al. (2020) and Enyew et al. (2022) may result from limited gene flow, strong local adaptation, and traditional seed practices.

5.4.3. Clustering analysis

The absence of distinct genetic clustering among sorghum populations based on geographic origin aligns with earlier findings (Amsalu & Endashaw, 2000; Desmae et al., 2016; Wondimu et al., 2021). This pattern likely reflects substantial gene flow, attributable to widespread seed exchange practices among farmers in neighboring regions where sorghum cultivation is predominant. Genotypes #49 and #13 are the most distinct genotypes, as they are part of divergent and broadly separated clusters. This indicates that these genotypes may have a higher potential for producing heterotic hybrids or superior progeny through crossbreeding. Consequently, they should be regarded as valuable parents for a successful future breeding initiative. Principal coordinate analysis (PCoA) of genotypes revealed that a clear distinction according to region of collection was not also noticeable. The result found by AMOVA analysis (higher genetic variation within regions than among regions) is in consistent with the results found by cluster analysis, i.e., sorghum genotypes from different regions of collection clustered in one cluster. This is likely due to the strong interpopulation mixing between regions, which reduces genetic differentiation among populations.The existence of larger proportion of genetic variation within regions shows the necessity of a high attention for germplasm conservation and utilization within regions.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

Sorghum continues to exhibit lower average grain yields relative to other cereal crops, primarily due to multiple biotic and abiotic stresses, where drought represents the most significant constraint. Global food security faces intensifying pressures from climate change and rapid population expansion, creating urgent challenges. Accelerating the rate of genetic improvement in crops has become essential to address rising food demands. Ethiopia serves as both a center of origin and a key reservoir of genetic diversity for sorghum. The country possesses a vast collection of sorghum landraces with diverse traits, providing a rich genetic pool for crop improvement. However, there is a scarcity of detailed information necessary for their effective utilization and conservation. This study was designed to provide critical insights for enhancing the sustainable utilization and conservation of these important genetic resources, while also supporting their effective integration into crop improvement initiatives.

The present study identified significant variation among sorghum genotypes. This variation provides a valuable opportunity to discover contrasting genotypes, which can play a crucial role in advancing sorghum improvement programs.The results from the progressive soil drying experiment provided evidence that genotypes with narrow root angles show higher average values for shoot and root biomass accumualtion, total water extraction, and lower fraction of transpirable soil water under water stress conditions compared to wide root angle genotypes which could enhance sorghum productivity and drought resilience when cultivated in water-limited field environment

The genome-wide association study (GWAS) identified SNPs markers linked to important phenotypic traits and detected potential candidate genes corresponding to these traits. The markers and candidate genes identified in this research require additional exploration and validation to assess their potential application in marker-assisted selection (MAS), aiming to improve the effectiveness of cultivar development. Moreover, the population structure analysis revealed a considerable level of admixture in sorghum genotypes, underscoring their utility as donors of favorable genetic variants for sorghum improvement.

The genetic diversity analysis revealed significant genetic variation among sorghum genotypes, with minimal regional differentiation. The absence of strong genetic disparity of sorghum between the regions of origin could be described by a high gene transfer because of far-reaching exchange of seeds among farmers across neighboring regions where sorghum is a main crop or the similarity in farmers' selection criteria across various regions. In conclusion, this study showed that with targeted breeding efforts, local landraces can significantly improve sorghum productivity in Ethiopia's drought-prone regions. From the outcomes of this inquiry, the following recommendations have been proposed to ensure the efficient utilization and conservation of these valuable resources.

6.2. Recommendations

1. The information generated from this study needs to be used by sorghum breeders to select parental lines for breeding programmes. For example, genotypes #49 and #13, with the highest pair-wise genetic dissimilarity coefficient can be candidates for crossing in breeding programs.
2. Both the phenotypic and genetic analyses indicate that sorghum genotypes from different regions of Ethiopia do not exhibit significant regional differences. As a result, a comprehensive collection from any region of the country would effectively preserve most of the genetic diversity found in Ethiopian sorghum. Still, incorporating underrepresented populations could be crucial for identifying new variants that might enhance breeding program. Furthermore, alongside *ex situ* conservation efforts, *in situ* conservation, specifically the conservation of sorghum landraces in farmers' fields are central in maintaining this valuable diversity and ensuring its availability for future use.
3. It is recommended to conduct field experiments under both well-watered (well irrigated) and water-stressed (water limited) conditions using the same genotypes with wide and narrow root angles, along with consistent parameters, to validate the findings from the drydown experiment under greenhouse condition.
4. The findings from the GWAS have identified SNP markers linked to traits and potential candidate genes that could deepen our comprehension of the genetic basis governing essential early-growth traits in sorghum. Thus, subsequent studies could leverage these markers to increase the accuracy and productivity of sorghum improvement programs.

Collectively, the phenotypic and genomic information gathered for these accessions constitutes a valuable foundation for enhancing sorghum through breeding initiatives.

Future line of work

The scope of this study, constrained by a limited genotype set, necessitates additional research to substantiate the identified markers and candidate genes influencing the genetic blueprint of the studied traits. To enhance the robustness of these findings, future investigations should employ a more extensive and genetically diverse sorghum panel, evaluated under controlled conditions and across multiple environmental conditions.

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8. APPENDICES

Appendix 8.1. Details of all 158 sorghum genotypes examined in this study

Entry #	EBI Accession No	Melkassa ID	Race	Region	Zone	Woreda	Annual mean Temperature (°C)	Annual Precipita-tion (mm)	Altitude (m.a.s.l)
#1	200697	ETSL 101289	Caudatum	Amhara	South Wollo	Dessie Zuriya	15.2	1145	2491
#2	16443	ETSL 100491	Bicolor	Oromia	East Shewa	Arsi Negele	17.7	915	1951
#3	223563	ETSL 101418	Durra	Oromia	Jimma	Sokoru	19.3	1354	1867
#4	216736	ETSL 101396	Durra Caudatum	Gambela	Zone 1	Itang	27.4	933	428
#5	228917	ETSL 101453	Bicolor	Oromia	Illubabor	Bure	20	1505	1688
#6	242038	ETSL 101715	Caudatum	Amhara	North Wollo	Bugna	17.7	815	2208
#7	210961	ETSL 101346	Durra Caudatum	Oromia	West Hararghe	Chiro	NA	NA	1850
#8	71160	ETSL 100813	Caudatum Bicolor	Amhara	North Wollo	Kobo	22	815	1512
#9	228115	ETSL 101431	Caudatum	Amhara	South Wollo	Legambo	11.6	1207	3162
#10	73964	ETSL 101097	Durra Caudatum	Tigray	Western Tigray	Taetay Koraro	20.3	905	1909
#11	2014WestColl#6	ETSL 100198	Durra Caudatum	Benishangul Gumuz	Metekel	Pawe	23.7	1608	1098
#12	Bambasi-3	ETSL 100238	Durra Caudatum	Benishangul Gumuz	Asosa	Bambasi	21.9	1222	1572
#13	70960	ETSL 100785	Caudatum Bicolor	Oromia	West Hararghe	Chiro	17.4	1133	2122
#14	71550	ETSL 100861	Bicolor	Oromia	Illubabor	Ale	18.6	1933	1951
#15	201401	ETSL 101293	Bicolor	Gambela	Zone 1	Gambela	27.6	1148	445
#16	69734	ETSL 100284	Kafir Caudatum	NA	NA	NA	NA	NA	NA
#17	69402	ETSL 100644	Durra Bicolor	Gambela	Zone 1	Itang	27.4	933	428
#18	72554	ETSL 100964	Durra Caudatum	Amhara	South Wollo	Kalu	20.6	898	1671

Appendix 8.1 (continued)

#19	74665	ETSL 101181	Guinea Caudatum	SEPR	Bench Maji	Dirashe Special Woreda	18.7	1303	2075
#20	238403	ETSL 101568	Bicolor	Tigray	Central Tigray	Naeder Adet	22.1	710	1825
#21	73050	ETSL 101021	Durra Bicolor	Amhara	North Wollo	Woldiya	18.5	994	1908
#22	2001PaweColl#104	ETSL 100152	Caudatum Bicolor	Amhara	North Gondar	West Belesa	19.3	1151	2226
#23	241728	ETSL 101712	Caudatum Bicolor	SEPR	Bench Maji	Konso Special Woreda	22.1	507	1379
#24	16476	ETSL 100502	Bicolor	Oromia	West Shewa	Waliso	17.6	1253	2057
#25	245060	ETSL 101766	Bicolor	Oromia	Illubabor	Bedele	18.4	1850	2016
#26	73045	ETSL 101016	Caudatum	Amhara	North Wollo	Woldiya	18.5	994	1908
#27	71109	ETSL 100805	Bicolor	Oromia	West Hararghe	Tulo	19.4	1018	1830
#28	101643	ETSL 101643	NA	NA	NA	NA	NA	NA	NA
#29	71630	ETSL 100889	Guinea Bicolor	Gambela	Zone 1	Gambela	27.6	1148	445
#30	74071	ETSL 101118	Guinea Bicolor	Tigray	Western Tigray	Taetay Koraro	20.3	905	1909
#31	210976	ETSL 101353	Caudatum	Oromia	West Hararghe	Tulo	19.4	1018	1830
#32	69055	ETSL 100565	Bicolor	Oromia	West Shewa	Dendi	16.9	1078	2278
#33	Degalit Yellow	ETSL 101848	Durra	NA	NA	NA	NA	NA	NA
#34	100419	ETSL 100419	NA	NA	NA	NA	NA	NA	NA
#35	69380	ETSL 100634	Bicolor	Gambela	Zone 1	Gambela	27.6	1148	445
#36	IS38279	IS 38279	Bicolor	Amhara	South Wollo	Ambassel	18	1118	2025
#37	215525	ETSL100286	NA	Gambela	Zone 2	Gog	25.5	1346	551
#38	214849	ETSL 101389	Caudatum Bicolor	Amhara	South Wollo	Ambassel	18	1118	2025
#39	237306	ETSL 101536	Durra Caudatum	Tigray	Western Tigray	Taetay Koraro	20.3	905	1909
#40	73784	ETSL 101081	Durra Caudatum	Tigray	Southern Tigray	Enderta	17.3	589	2269
#41	234862	ETSL 101506	Bicolor	Oromia	Illubabor	Gechi	18.4	1881	2033

Appendix 8.1 (continued)

#42	2001PaweColl#013	ETSL 100063	Durra Bicolor	Benishangul Gumuz	Metekel	Dangur	23.7	1608	1098
#43	100392	ETSL 100392	NA	NA	NA	NA	NA	NA	NA
#44	71937	ETSL 100928	Caudatum Bicolor	Oromia	West Hararghe	Habro	20.3	1089	1774
#45	71653	ETSL 100900	Caudatum Bicolor	Gambela	Zone 1	Gambela	27.6	1148	445
#46	71891	ETSL 100916	Caudatum Bicolor	NA	NA	NA	NA	NA	NA
#42	2001PaweColl#013	ETSL 100063	Durra Bicolor	Benishangul Gumuz	Metekel	Dangur	23.7	1608	1098
#47	200122	ETSL 101255	Bicolor	Oromia	West Shewa	Ejerie (Addis Alem)	14.9	1165	2548
#48	241266	ETSL 101686	Caudatum	Oromia	East Hararghe	Haramaya	17.9	799	2016
#49	73102	ETSL 101059	Durra	Amhara	South Wollo	Kalu	20.6	898	1671
#50	16445	ETSL 100492	Bicolor	Oromia	East Shewa	Arsi Negele	17.7	915	1951
#51	69354	ETSL 100620	Durra Bicolor	Gambela	Zone 1	Gambela	27.6	1148	445
#52	16133	ETSL 100445	Durra Caudatum	Oromia	West Wollega	Mana Sibu	20.1	1614	1684
#53	70749	ETSL 100761	Caudatum Bicolor	Oromia	West Hararghe	Tulo	19.4	1018	1830
#54	211239	ETSL 101362	Durra Caudatum	Amhara	North Wollo	Woldiya	18.5	994	1908
#55	2001PaweColl#101	ETSL 100150	NA	Amhara	North Gondar	West Belesa	19.3	1151	2226
#56	69447	ETSL 100662	Caudatum Bicolor	Gambela	Zone 1	Gambela	27.6	1148	445
#57	16437	ETSL 100485	Bicolor	Oromia	East Shewa	Arsi Negele	17.7	915	1951
#58	16060	ETSL 100427	Caudatum	Tigray	Central Tigray	Edaga Arbi	22.1	710	1825
#59	Jamiyu	ETSL 101851	NA	Tigray	Southern Tigray	Raya Azebo	22.3	790	1562
#60	74139	ETSL 101142	Bicolor	Tigray	Southern Tigray	Raya Azebo	22.3	790	1562
#61	Membgazi	ETSL 100267	Durra Bicolor	Benishangul Gumuz	Asosa	Asosa	21.9	1222	1572
#62	202508	ETSL 101299	Bicolor	Amhara	North Wollo	Guba Lafto	18.5	994	1908
#63	223552	ETSL 101410	Bicolor	Oromia	Illubabor	Dedesa	18.4	1850	2016
#64	101190	ETSL 101190	NA	NA	NA	NA	NA	NA	NA
#65	19666	ETSL 100550	Caudatum	Tigray	Western Tigray	Kafta Humera	27.6	611	586
#66	244699	ETSL 101751	Bicolor	Amhara	Wag Himra	Abergele	23.2	673	1581

Appendix 8.1 (continued)

#67	210937	ETSL 101337	Caudatum	Oromia	West Hararghe	Habro	20.3	1089	1774
#68	77133	ETSL 101249	NA	NA	NA	NA	NA	NA	NA
#69	69542	ETSL 100707	Bicolor	Oromia	Illubabor	Dedesa	18.4	1850	2016
#70	69229	ETSL 100593	Durra Caudatum	Oromia	West Hararghe	Chiro	20.2	1035	1710
#71	235791	ETSL 100297	Bicolor	SEPR	South Omo	Dimeka	23.5	719	1106
#72	75119	ETSL 101224	Bicolor	Oromia	Jimma	Mana	18.7	1849	1903
#73	70073	ETSL 100720	NA	Oromia	Jimma	Mana	18.7	1849	1903
#74	71569	ETSL 100877	Durra Caudatum	Gambela	Zone 1	Gambela	27.6	1148	445
#75	75005	ETSL 101215	Bicolor	Oromia	West Shewa	Ejerie (Addis Alem)	14.9	1165	2548
#76	228923	ETSL 101459	Bicolor	Oromia	Illubabor	Bure	20	1505	1688
#77	101061	ETSL 101061	NA	NA	NA	NA	NA	NA	NA
#78	100872	ETSL 100872	NA	NA	NA	NA	NA	NA	NA
#79	74220	ETSL 101163	Caudatum	Tigray	Southern Tigray	Raya Azebo	22.3	790	1562
#80	236731	ETSL 101524	Caudatum	Oromia	West Shewa	Jeldu	17.6	1253	2057
#81	69213	ETSL 100587	Caudatum Bicolor	Amhara	South Wollo	Kalu	20.6	898	1671
#82	237790	ETSL 101556	Bicolor	Oromia	West Shewa	Becho	17.2	1148	2182
#83	229835	ETSL 101464	Caudatum	Benishangul Gumuz	Metekel	Mandura	24	1600	1043
#84	238391	ETSL 101563	Bicolor	Tigray	Central Tigray	Enticho	19	635	1980
#85	206148	ETSL 101313	Bicolor	Oromia	Illubabor	Ale	18.6	1933	1951
#86	73090	ETSL 101051	Bicolor	Amhara	North Wollo	Woldiya	18.5	994	1908
#87	9152	ETSL 100340	Durra	Oromia	East Hararghe	Deder	15.6	1111	2288
#88	100044	ETSL 100044	NA	NA	NA	NA	NA	NA	NA
#89	231201	ETSL 101477	Durra	Somali	Jigjiga	Jigjiga	19.4	712	1656
#90	73010	ETSL 100983	Caudatum	Afar	Zone 1	Asayita	28.7	144	356
#91	101358	ETSL 101358	NA	NA	NA	NA	NA	NA	NA
#92	69533	ETSL 100702	Bicolor	Oromia	Illubabor	Bure	20	1505	1688
#93	100898	ETSL 100898	NA	NA	NA	NA	NA	NA	NA

Appendix 8.1 (continued)

#94	229832	ETSL 101461	Caudatum	Benishangul Gumuz	Metekel	Mandura	24	1600	1043
#95	73095	ETSL 101056	Durra	Amhara	North Wollo	Woldiya	18.5	994	1908
#96	16128	ETSL 100443	Bicolor	Oromia	Kelem Wollega	Yemalogi Welele	NA	NA	2124
#97	70961	ETSL 100786	Bicolor	Oromia	East Hararghe	Kersa	18	857	1999
#98	100040	ETSL 100040	NA	NA	NA	NA	NA	NA	NA
#99	K019	ETSL 100030	Durra Caudatum	Benishangul Gumuz	Asosa	Kurmuk	27.5	933	654
#100	239247	ETSL 101654	Bicolor	Amhara	North Wollo	Meket	12.9	1028	2951
#101	16071	ETSL 100431	Caudatum Bicolor	Tigray	Central Tigray	Chila	22.1	710	1825
#102	100046	ETSL 100046	NA	NA	NA	NA	NA	NA	NA
#103	100005	ETSL 100005	NA	NA	NA	NA	NA	NA	NA
#104	239219	ETSL 101640	Durra Caudatum	Amhara	Oromia	Bati	20.4	926	1655
#105	16116	ETSL 100438	Caudatum Bicolor	Oromia	East Wollega	Sebo	18.8	1923	1933
#106	16208	ETSL 100474	Durra Caudatum	Oromia	East Wollega	Dale Wabera	20.6	1527	1515
#107	210962	ETSL 101347	Durra Caudatum	Oromia	West Hararghe	Chiro	NA	NA	2090
#108	25130	ETSL 100560	Bicolor	NA	NA	NA	NA	NA	NA
#109	DagalitYellow-1	ETSL 100310	Caudatum	Amhara	North Wollo	Kobo	22	815	1512
#110	38306	IS 38306	Durra	Amhara	South Wollo	Ambassel	18	1118	2025
#111	100214	ETSL 100214	NA	NA	NA	NA	NA	NA	NA
#112	38412	IS 38412	Caudatum Bicolor	Amhara	Shewa	Robi	23	1199	1263
#113	75000	ETSL 101210	NA	Oromia	West Shewa	Ejerie (Addis Alem)	14.9	1165	2548
#114	231464	ETSL 101491	Caudatum	Somali	Jigjiga	Jigjiga	19.4	712	1656
#115	69073	ETSL 100568	Bicolor	SEPR	Bench Maji	Dirashe Special Woreda	18.7	1303	2075
#116	228833	ETSL 101444	Caudatum	Oromia	East Hararghe	Fedis	NA	NA	NA
#117	75132	ETSL 101231	Durra Bicolor	Oromia	Jimma	Mana	18.7	1849	1903
#118	75007	ETSL 101217	Bicolor	Oromia	West Shewa	Ejerie (Addis Alem)	14.9	1165	2548

Appendix 8.1 (continued)

#119	2014WestColl#32	ETSL 100178	Durra Caudatum	Benishangul Gumuz	Asosa	Komesha	24.2	1181	1109
#120	70998	ETSL 100792	Durra Caudatum	Oromia	West Hararghe	Tulo	19.4	1018	1830
#121	2001PaweColl#057	ETSL 100107	Durra Caudatum	Benishangul Gumuz	Metekel	Dibate	24	1600	1043
#122	69164	ETSL 100577	NA	Benishangul Gumuz	Metekel	Wenbera	15.9	1955	2488
#123	75458	ETSL 101247	Caudatum	Amhara	North Wollo	Bugna	17.7	815	2208
#124	70568	ETSL 100745	Bicolor	SEPR	Bench Maji	Konso Special Woreda	22.1	507	1379
#125	239116	ETSL 101586	Durra Bicolor	Dire Dawa city Adm	Dire Dawa	Dire Dawa	24.6	637	1193
#126	228852	ETSL 101451	Bicolor	Dire Dawa city Adm	Dire Dawa	Gurgura	24.6	637	1193
#127	200199	ETSL 101262	NA	Oromia	East Hararghe	Haramaya	17.9	799	2016
#128	71629	ETSL 100888	Guinea Caudatum	Gambela	Zone 1	Gambela	27.6	1148	445
#129	100170	ETSL 100170	NA	NA	NA	NA	NA	NA	NA
#130	16447	ETSL 100494	Durra Bicolor	Oromia	East Shewa	Arsi Negele	17.7	915	1951
#131	75457	ETSL 101246	Caudatum Bicolor	Amhara	North Wollo	Bugna	17.7	815	2208
#132	241227	ETSL 101671	Bicolor	Oromia	West Hararghe	Mesela	15	1181	2445
#133	237304	ETSL 101535	Durra Caudatum	Tigray	Western Tigray	Laelay Adiyabo	27.6	611	586
#134	100867	ETSL 100867	NA	NA	NA	NA	NA	NA	NA
#135	16112	ETSL 100436	Caudatum Bicolor	Oromia	West Wollega	Begi	16	1845	2534
#136	2001PaweColl#014	ETSL 100064	Durra Caudatum	Benishangul Gumuz	Metekel	Dangur	23.7	1608	1098
#137	69498	ETSL 100689	Bicolor	SEPR	Bench Maji	Dirashe Special Woreda	18.7	1303	2075
#138	238382	ETSL 101561	Bicolor	Tigray	Central Tigray	Laelay Maychew	16.7	753	2406
#139	228836	ETSL 101446	Durra Caudatum	Oromia	East Hararghe	Babile	NA	NA	NA
#140	210935	ETSL 101335	Durra Caudatum	Oromia	West Hararghe	Habro	20.3	1089	1774

Appendix 8.1 (continued)

#141	2014WestColl#22	ETSL 100167	Durra Caudatum	Benishangul Gumuz	Asosa	Sherkole	27.5	940	662
#142	75009	ETSL 101218	Caudatum Bicolor	Oromia	West Shewa	Ejerie (Addis Alem)	14.9	1165	2548
#143	100837	ETSL 100837	NA	NA	NA	NA	NA	NA	NA
#144	73645	ETSL 101074	Caudatum	Afar	Zone 1	Asayita	28.7	144	356
#145	69396	ETSL 100642	Caudatum Bicolor	Gambela	Zone 1	Itang	27.4	933	428
#146	74663	ETSL 101180	Kafir Caudatum	SEPR	Bench Maji	Dirashe Special Woreda	18.7	1303	2075
#147	201319	ETSL 101292	Bicolor	Amhara	North Wollo	Guba Lafto	18.5	994	1908
#148	206154	ETSL 101316	NA	Gambela	Zone 1	Gambela	27.6	1148	445
#149	Ag061	ETSL 100007	Durra Bicolor	Benishangul Gumuz	Kamashi	Agalometi	24.2	1181	1109
#150	241707	ETSL 101698	Caudatum Bicolor	SEPR	Bench Maji	Konso Special Woreda	22.1	507	1379
#151	74195	ETSL 101160	Bicolor	Tigray	Southern Tigray	Raya Azebo	22.3	790	1562
#152	15931	ETSL 100400	Durra	Oromia	West Hararghe	Chiro	NA	NA	2225
#153	100465	ETSL 100465	NA	NA	NA	NA	NA	NA	NA
#154	101084	ETSL 101084	NA	NA	NA	NA	NA	NA	NA
#155	239193	ETSL 101629	Durra Caudatum	Amhara	South Wollo	Kalu	20.6	898	1671
#156	100639	ETSL 100639	NA	NA	NA	NA	NA	NA	NA
#157	38254	IS 38254	NA	NA	NA	NA	NA	NA	NA
#158	Variety	Dagim	NA	NA	NA	NA	NA	NA	NA

Appendix 8.2. Mean performance of seedling shoot and root traits of sorghum genotypes

Entry	LAg	ShL	LAr	ShD	RL	NR	SFW	SDW	RFW	RDW	RHN	RAg	StD	RSR	SA
#1	29.04	13.25	27.21	2.45	46	5	2.74	0.94	1.6	0.59	31.25	16.34	164.75	0.63	7636.33
#2	33.78	13.5	26.79	2.1	39	4.25	2.69	0.94	1.43	0.49	27.75	23.34	176.75	0.53	5299.38
#3	27	15	30.57	2.52	64.5	7	3.07	1	2.03	0.82	39	19.63	163	0.82	9246.23
#4	38.5	15	30.62	1.86	49.5	5.5	3.08	1	1.67	0.63	32.5	11.38	184	0.63	6839.5
#5	31.34	13	26.24	1.43	44.25	5	2.64	0.93	1.55	0.56	30.25	14.84	151	0.61	8470.06
#6	27.17	15.5	31.37	2.05	53	6	3.15	1.01	1.76	0.67	34	12.88	161	0.67	6022
#7	28.67	15	30.64	2.01	45.34	5	3.08	1	1.58	0.58	30.67	23.13	181.34	0.58	6940.91
#8	33.32	12.75	26.44	2.46	39.75	4.5	2.66	0.94	1.44	0.51	28.25	15.61	163.25	0.54	6349.1
#9	34.23	14.63	29.82	2.04	50.75	5.5	3	0.99	1.7	0.64	35.5	15.57	161	0.65	6933.44
#10	27.42	16	32.54	2.6	51.25	5.5	3.27	1.04	1.72	0.65	34.25	15.5	172	0.63	6646.19
#11	28.25	13.5	26.84	1.67	42.5	5	2.7	0.94	1.51	0.54	29.5	21.13	157.5	0.58	4968.41
#12	33.78	12.5	25.27	1.58	41.25	4.5	2.54	0.92	1.48	0.52	28.75	14.82	144.25	0.57	8717.95
#13	30.67	13	26.31	2.02	49.25	5.5	2.7	0.94	1.66	0.62	32.25	11.82	160.75	0.67	6482.15
#14	26	12	23.08	1.95	44.5	5	2.32	0.88	1.56	0.56	30.5	13.43	145	0.65	5311.25
#15	32.59	14.5	29.47	2.04	61.5	7	2.96	0.99	1.96	0.78	38	16.5	181	0.79	5222.58
#16	32.75	14.75	30.25	1.88	48	5	3.04	0.99	1.61	0.61	32.25	12.76	154	0.62	6662.78
#17	29	13	26.29	1.98	47.25	5.25	2.64	0.93	1.62	0.6	31.5	13.07	164	0.65	5745.73
#18	35.59	18	37.14	2.08	49.25	5.5	3.73	1.11	1.61	0.63	31.25	25.07	198.5	0.57	6245.26
#19	29.25	16	32.06	2.08	49.25	5.25	3.22	1.03	1.67	0.62	32.25	14.89	185.75	0.61	5821.32
#20	25.45	13.25	27.32	2.06	44.25	4.75	2.76	0.95	1.55	0.56	30.25	13.75	150.5	0.59	7320.97
#21	31.34	13.84	27.68	1.86	65.34	7	2.78	0.95	2.04	0.83	39.34	10	142.34	0.88	6027.41
#22	34	9.34	18.96	1.82	45.34	5	1.91	0.88	1.58	0.58	31	13.13	148.34	0.66	6814.39
#23	34.17	14.75	29.22	2.06	41.5	5	2.94	0.98	1.49	0.53	32.5	13.13	169.5	0.54	7127.24
#24	32.67	9.17	18.42	1.92	60	6.34	1.87	0.9	1.92	0.76	37.34	24.88	128	0.84	6171.04
#25	30.56	8.67	17.76	1.9	50	5.67	1.79	0.79	1.69	0.64	32.67	9.63	131	0.81	5586.45
#26	37.17	16	32.56	1.95	40.67	4.34	3.27	1.06	1.45	0.52	28	12.09	189	0.49	6452.39
#27	42	12	24.4	2.52	45.67	5	2.45	0.87	1.57	0.57	30.34	11.17	147.34	0.64	6319.59

Appendix 8.2 (continued)

#28	33.83	11.67	23.6	1.86	55.34	6	2.37	0.89	1.79	0.7	34.67	14.5	152.67	0.79	6059.88
#29	27.17	13.67	27.72	1.8	39	4.34	2.79	0.96	1.44	0.49	27.67	12.84	167	0.52	7270.9
#30	32.84	10.34	20.71	2.11	49.67	5.67	2.08	0.89	1.69	0.63	33	7.88	134	0.71	8310.69
#31	22.67	13	26.74	1.34	41	4.5	2.69	0.93	1.48	0.52	28.5	17.38	141.5	0.56	7319.07
#32	32.17	12	24	1.81	52.5	6	2.41	0.89	1.75	0.67	34	13.25	149	0.75	4881.52
#33	27.67	13	26.13	1.93	48	5.67	2.63	0.92	1.64	0.61	32	14.67	146.34	0.66	8973.72
#34	42.34	14	28.18	1.98	50	5.5	2.83	0.96	1.69	0.64	33	14	165	0.67	7431.22
#35	33.17	16	32.16	1.91	36.34	4	3.23	1.03	1.37	0.46	26.67	12.42	171	0.46	7173.19
#36	29.34	14.67	29.91	1.96	44.67	5.34	3.01	0.99	1.57	0.57	30.67	13.75	167	0.58	6569.46
#37	41.51	16	32.93	1.95	49	5.34	3.31	1.04	1.66	0.62	34.67	13.42	170.34	0.6	7088.24
#38	37	13	26.54	1.92	54	6	2.67	0.94	1.78	0.69	34.5	14.5	151.5	0.74	6491.25
#39	34	18.34	37.84	2.55	39	4	3.8	1.12	1.43	0.49	27.67	14.67	190.67	0.44	7357.31
#40	26.37	13.67	28.01	2.08	39.67	4.34	2.82	0.96	1.44	0.5	28	21.34	156.67	0.53	6567.49
#41	34.84	16	33.09	2.21	35.34	4	3.33	1.04	1.34	0.45	26	14.59	174	0.43	6699.31
#42	34.66	19.5	40.22	2.87	61.5	7	4.04	1.16	1.96	0.78	38	16.5	212	0.67	7528.44
#43	27.67	13.67	27.68	2.1	38.34	4.34	2.78	0.95	1.41	0.49	27.34	13.59	152.34	0.52	8353.48
#44	29	11	21.67	2.16	54.67	6	2.18	0.86	1.79	0.69	34.67	22.63	138	0.81	6888.41
#45	32.33	10.34	20.15	1.36	42.34	4.67	2.06	0.84	1.51	0.54	29.34	9.25	129	0.64	5602.18
#46	30.34	15.67	32.06	2.4	51.67	5.67	3.22	1.02	1.73	0.66	33.67	13.59	181.67	0.64	7858.92
#47	35.67	15.34	31.2	2.03	51.67	6	3.14	1.01	1.73	0.66	33.67	12.42	166.34	0.65	5594.57
#48	33.17	16.34	33.86	2.68	41.34	4.67	3.4	1.06	1.48	0.52	31.34	15.75	199.67	0.5	7636.83
#49	35.83	16	32.86	1.92	28.67	3	3.3	1.04	1.18	0.37	23	14.13	174	0.36	6718
#50	32.09	15	30.22	1.99	48.5	5.5	3.04	1	1.66	0.61	32	14.5	172.5	0.62	5737.23
#51	36.45	15.34	30.5	1.97	35.34	4	3.07	1	1.34	0.45	26	9.84	180	0.45	6304.83
#52	31.95	15.34	31.46	2.02	53.67	6	3.16	1.02	1.77	0.68	34.34	14.42	177	0.67	4817.9
#53	35.34	12.67	26.16	1.98	36	4	2.61	0.93	1.36	0.46	26.34	13.38	149.34	0.49	7675.19
#54	33.45	15	31.23	2.29	54.34	6	3.14	1.01	1.79	0.69	34.34	15	169	0.68	8074.59
#55	28.33	12	24.2	2.28	60	7	2.43	0.89	1.92	0.76	37	16.25	167	0.86	5633.91

Appendix 8.2 (continued)

#56	34.11	16.67	33.82	1.76	61	7	3.4	1.05	1.95	0.77	38	13.67	187	0.74	6332.37
#57	31.45	14	27.98	1.68	52	6	2.81	0.96	1.74	0.66	33.67	12.13	165	0.69	7161.67
#58	28.83	18.5	36.47	2.79	50.5	6	3.86	1.13	1.76	0.64	34	13.75	204	0.57	8466.93
#59	39.34	13.34	26.72	1.81	55	6	2.69	0.94	1.81	0.7	35.34	14.92	159	0.75	7421.38
#60	35.56	13	26.49	2.12	64	7	2.69	0.94	2.02	0.81	39.34	14.75	145.34	0.87	7296.6
#61	28.5	15.34	31.43	1.86	54.34	6	3.16	1.02	1.79	0.69	34.67	14	178	0.69	8282.16
#62	31.67	11	22.08	1.74	60.67	7	2.22	0.9	1.94	0.78	37.34	13.84	150	0.87	6057.91
#63	35.5	14.67	30.47	1.95	51.67	6	3.06	1	1.73	0.65	33.67	15.42	167.67	0.66	5529.15
#64	34.67	15.67	31.88	2.13	37.34	4	3.21	1.03	1.42	0.48	27.67	12.84	174.34	0.47	7154.79
#65	31.22	14	28.61	1.97	51.34	6	2.88	0.97	1.72	0.65	33.67	14	161	0.68	6936.39
#66	37.23	13.67	27.68	1.97	53.34	5.67	2.78	0.95	1.76	0.67	34.34	8.88	156.34	0.71	6159.24
#67	33.67	13.67	27.45	2.09	52	6	2.76	0.95	1.73	0.66	33.67	11.75	155.34	0.7	6270.4
#68	24.5	12	23.8	1.93	50.5	6	2.39	0.89	1.69	0.64	33	9.25	155.5	0.72	7909.15
#69	41.84	13.67	27.85	2.06	49.67	5.34	2.8	0.96	1.68	0.63	32.34	12.5	165	0.67	6524.21
#70	29.34	16.34	33.72	2.04	53.67	6	3.39	1.06	1.78	0.68	34.34	23.42	188	0.65	7350.06
#71	30.92	12.5	24.89	2.03	49	5.5	2.5	0.91	1.66	0.62	33	14.13	170.5	0.68	5607.36
#72	32.67	12.5	24.16	1.86	45	5	2.43	0.9	1.57	0.57	30.34	12.59	147.34	0.64	5943.79
#73	34	14	29.47	1.86	54	6	2.96	0.98	1.78	0.68	35	16.25	172	0.7	5370.91
#74	36.07	13.67	27.95	2.54	44	5	2.81	0.96	1.55	0.56	30	13.42	158.67	0.59	8284.47
#75	31.01	12	23.95	1.96	60	7	2.41	0.94	1.96	0.76	38	11.88	149	0.81	7412.04
#76	39.75	17.5	35.95	2.57	60.5	6.5	3.61	1.09	1.95	0.76	38	13.88	190.5	0.71	6758.34
#77	32.84	14.67	29.84	2.36	61	6.67	3	0.99	1.95	0.77	38	13.88	169.67	0.79	7273.82
#78	38.48	14.34	29.08	2.31	46.34	5	2.92	0.98	1.6	0.59	31	22.67	181.67	0.61	8441.53
#79	35.11	14.67	30.54	2	44.34	5	3.07	1	1.55	0.56	30.34	13.92	167.34	0.57	6299.42
#80	35.95	13.67	27.88	2.02	43.67	5	2.8	0.96	1.54	0.56	30	14.25	164.34	0.59	7058.38
#81	25.17	18	36.24	2.16	44.5	5	3.64	1.1	1.56	0.56	30.5	19.07	188	0.52	6491.25
#82	32.5	12	25.14	2.05	45	5	2.53	0.91	1.57	0.57	30.5	15.08	153	0.63	5867.23
#83	30.67	15	30.25	2.1	62.34	7	3.09	1	1.98	0.79	38.34	13.34	169.67	0.8	5589.9

Appendix 8.2 (continued)

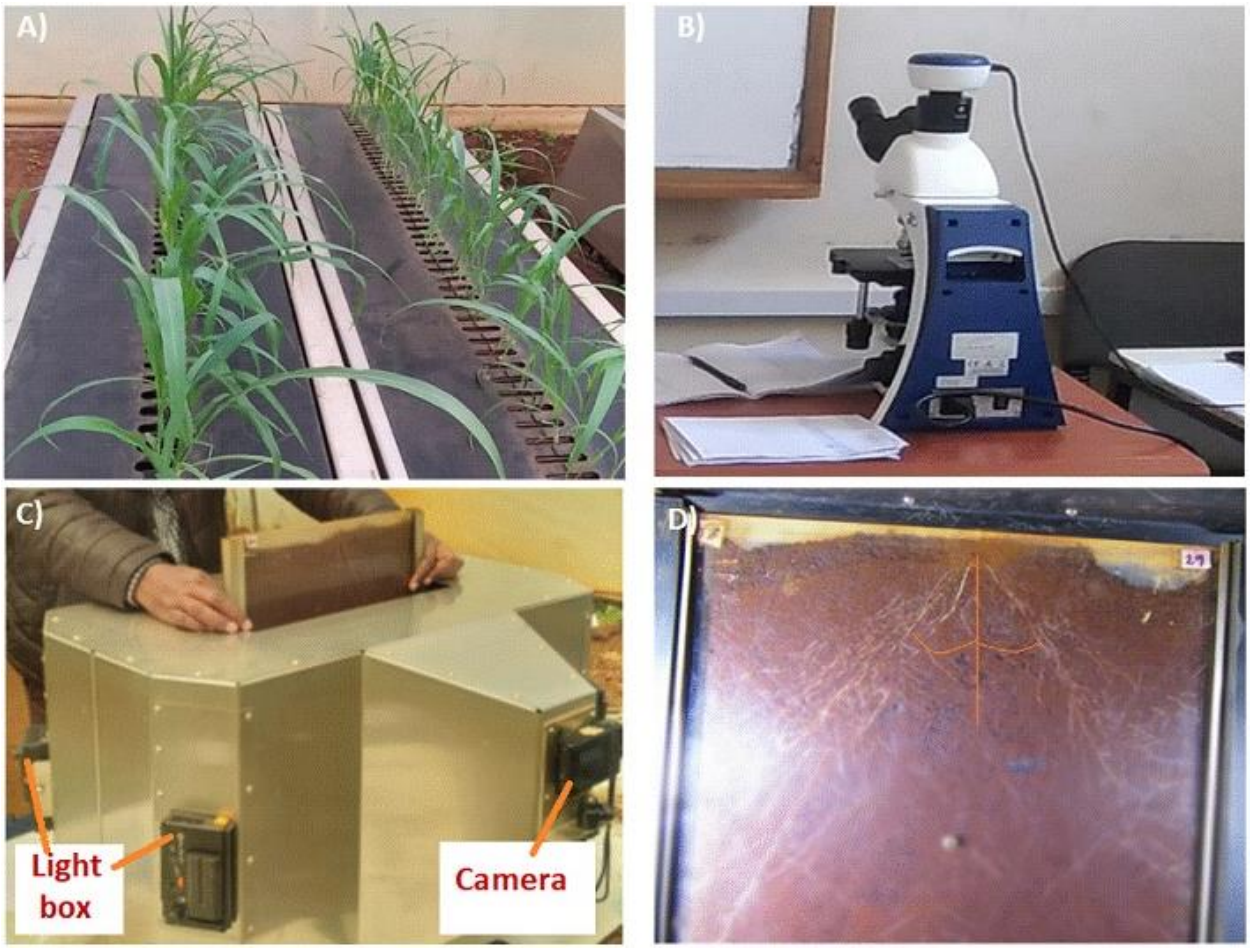
#84	38.95	13	26.54	2	40.5	4.5	2.67	0.94	1.48	0.51	28.5	22.75	166	0.55	5344.86
#85	26.82	16	32.36	1.84	52	6	3.25	1.03	1.73	0.66	37.34	12.42	179.67	0.65	7907.35
#86	37.17	15.5	30.77	2.16	71	8	3.09	1.01	2.19	0.91	42.5	12.5	171	0.92	6808.33
#87	35.73	16	32.33	2.5	39.67	4.34	3.25	1.03	1.44	0.5	28	21.67	170.67	0.49	6459.61
#88	32.28	11.67	23.8	1.97	46	5	2.39	0.92	1.54	0.58	30	21.54	142.34	0.64	7112.48
#89	32.56	11.67	23.7	1.99	41.34	4.67	2.38	0.89	1.49	0.52	28.67	13.17	142.67	0.59	5484.41
#90	40.17	13	26.29	2.28	39.5	4	2.64	0.93	1.43	0.5	28	11.5	174	0.54	6253.5
#91	45	18.67	38.24	2.03	39.34	4.34	3.84	1.12	1.43	0.49	27.67	22.98	192.67	0.44	7020.01
#92	30.34	16	32.6	1.89	38.34	4	3.28	1.04	1.41	0.47	27.34	25.17	171.67	0.46	6958.04
#93	35.56	14	28.74	2.07	49	5.34	2.89	0.97	1.66	0.64	32	15.84	179.34	0.66	6332.38
#94	34	13	26.65	2.09	41	4.34	2.68	0.94	1.48	0.54	28.67	14.98	173.67	0.58	6563.55
#95	26.45	11	22.01	1.7	44.34	5	2.21	0.86	1.56	0.56	30.34	12.5	135.67	0.66	6521.25
#96	40.5	13.34	27.28	1.87	51.34	5.67	2.74	0.95	1.72	0.65	33.34	8.25	162.34	0.7	8454.3
#97	26.56	14.34	24.33	1.89	48.67	5.34	2.95	0.98	1.65	0.62	32	24	143.34	0.63	6803.59
#98	26.11	13.17	26.89	1.76	46	5	2.7	0.94	1.59	0.58	31.34	11.5	162.67	0.62	6706.69
#99	33.78	15	30.47	2.27	46.67	5.34	3.06	0.99	1.61	0.59	31.34	15.17	167	0.61	7607.95
#100	26.01	18.34	38.2	1.93	49	5.34	3.84	1.12	1.66	0.62	32	12.67	211	0.56	5531.63
#101	16.5	14.5	28.88	1.89	51.5	6	2.9	0.97	1.72	0.65	33.5	13.38	157.5	0.68	5867.06
#102	31	16.5	34.06	2.27	50	5.5	3.42	1.06	1.69	0.63	32.5	16.75	187.5	0.6	7028.37
#103	31.34	12	24.1	1.93	53.34	6	2.42	0.9	1.77	0.67	34.34	13.59	134	0.75	4815.79
#104	28.31	17.67	36.44	1.51	48.34	5.34	3.66	1.1	1.65	0.61	32	22.5	184	0.56	5929.15
#105	33.97	11	22.44	2.09	50	5.67	2.26	0.9	1.69	0.64	33	13.59	152	0.71	7167.08
#106	32.78	15	30.47	1.87	55	6	3.06	1	1.8	0.7	35	13.92	168.34	0.7	6470.59
#107	26.59	19	39.33	2.01	45.5	5	3.95	1.14	1.58	0.57	30.5	23.88	195	0.5	9475.68
#108	35.5	12.25	24.49	2.29	31.5	3.5	2.46	0.9	1.25	0.4	24.5	10.25	146.5	0.45	5621.94
#109	33.5	15	30.22	2	48	5	3.04	1	1.63	0.61	31.5	16.63	173.5	0.61	7071.17
#110	28.89	14.34	29.61	1.9	42.34	4.67	2.98	0.99	1.51	0.54	29.34	13.86	177	0.55	6055.94
#111	39.02	14.34	29.24	2.81	49.34	5.34	2.94	0.97	1.67	0.63	32.34	12.42	171.67	0.65	7493.21

Appendix 8.2 (continued)

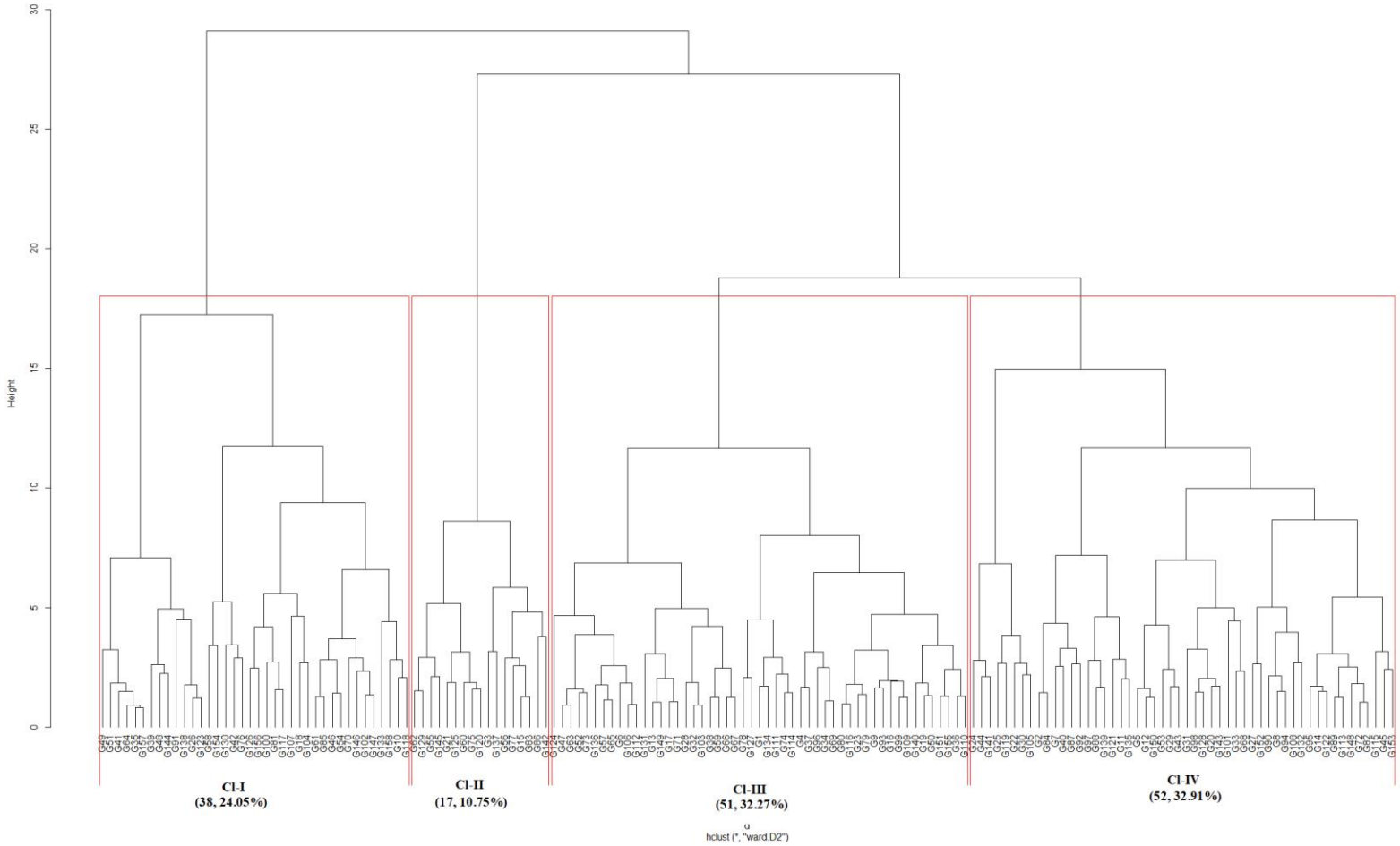
#112	32.96	15	30.77	2.01	53.34	6	3.09	1	1.77	0.68	34.34	11.67	170.34	0.68	6792.77
#113	29.34	11	22.26	1.88	41	4.5	2.24	0.87	1.48	0.52	28.5	12	133.5	0.6	5023.01
#114	36.67	14.67	29.64	2.74	43.34	5	2.98	0.99	1.53	0.55	30	11.59	174	0.56	7976.21
#115	31.11	9.34	17.89	1.66	36.34	4.34	1.8	0.8	1.37	0.46	27	12.92	112.34	0.58	6634.39
#116	38.17	14	28.33	1.9	43.5	5	2.85	0.96	1.53	0.55	30	15.63	173	0.58	7472.36
#117	25.67	17.34	35.58	1.98	47.67	5.34	3.58	1.08	1.54	0.6	29.67	14.75	187	0.56	6451.41
#118	30.34	16.34	33.26	2.67	47.67	5	3.34	1.04	1.63	0.61	31.67	10.09	179.34	0.59	6576.34
#119	32.17	10	20.78	1.78	55.34	6	2.09	0.87	1.81	0.7	35.34	13.34	123.34	0.81	6788.83
#120	32.23	13	26.79	1.86	63.67	6.67	2.69	0.94	2.01	0.81	39	11.42	159.67	0.86	7136.09
#121	26.12	11	22.44	1.9	46.67	5	2.26	0.87	1.61	0.59	31.34	22.84	159	0.69	5144.73
#122	29.56	11.84	23.87	1.99	46.67	5	2.4	0.89	1.62	0.58	31.67	14.5	133.67	0.66	6143.5
#123	40.45	16	32.79	2.12	42	4.34	3.3	1.04	1.5	0.53	29	13.5	192	0.52	6023.48
#124	35.34	14	28.28	2.37	52.34	6	2.84	1.21	1.75	0.76	33.67	12.42	163.67	0.64	5824.76
#125	31.5	14	28.53	1.76	69	7.5	2.87	0.97	2.14	0.87	41.5	12.25	164	0.91	6446.98
#126	28.01	16.67	34.68	1.41	42.34	4.67	3.49	1.07	1.51	0.53	36.34	13.75	178.34	0.5	6325.49
#127	37.12	13.67	28.64	2.45	42.67	5	2.88	0.97	1.55	0.54	33.67	22.09	162	0.56	7180.25
#128	25.45	12.67	25.99	1.82	41.67	4.67	2.61	0.93	1.49	0.53	29	12.84	168.67	0.57	6286.14
#129	26.45	11	22.18	1.94	60	6.67	2.23	0.88	1.92	0.76	37.34	15.75	147.34	0.87	6156.28
#130	39.5	17.5	36.64	2.67	63.5	7	3.68	1.09	2.01	0.79	45.5	12.5	181	0.73	8502.52
#131	35.25	13.5	27.23	1.81	44.5	5	2.74	0.95	1.71	0.56	33	10	177.5	0.6	4769.21
#132	27.23	12.67	26.12	2.37	36.34	4	2.63	0.93	1.37	0.46	26.67	13.67	160.34	0.5	5280.75
#133	21.37	16	32.66	2.97	51.34	6	3.28	1.04	1.72	0.65	33.67	22.42	184.67	0.63	7072.1
#134	31	13.67	27.88	2.91	47	5	2.8	0.96	1.62	0.6	31.34	14.25	166	0.63	8009.66
#135	25.89	13.34	28.01	2.11	47	5	2.82	0.98	1.61	0.6	31	21.5	166.67	0.62	5056.46
#136	27.56	13.67	27.75	2	52.67	6	2.79	0.96	1.71	0.67	33	16.09	177.67	0.7	7011.16
#137	26.56	14.34	29.01	1.98	63.34	6.67	2.92	1.02	2.01	0.8	38.67	22.09	157.34	0.79	6895.08
#138	38.72	16.67	34.19	2.13	42.67	5	3.44	1.06	1.52	0.54	29.34	13.92	182.67	0.52	7184.61
#139	30.83	12	25.04	2.26	48.5	5	2.52	0.91	1.65	0.62	32	22.25	136.5	0.68	7681.91

Appendix 8.2 (continued)

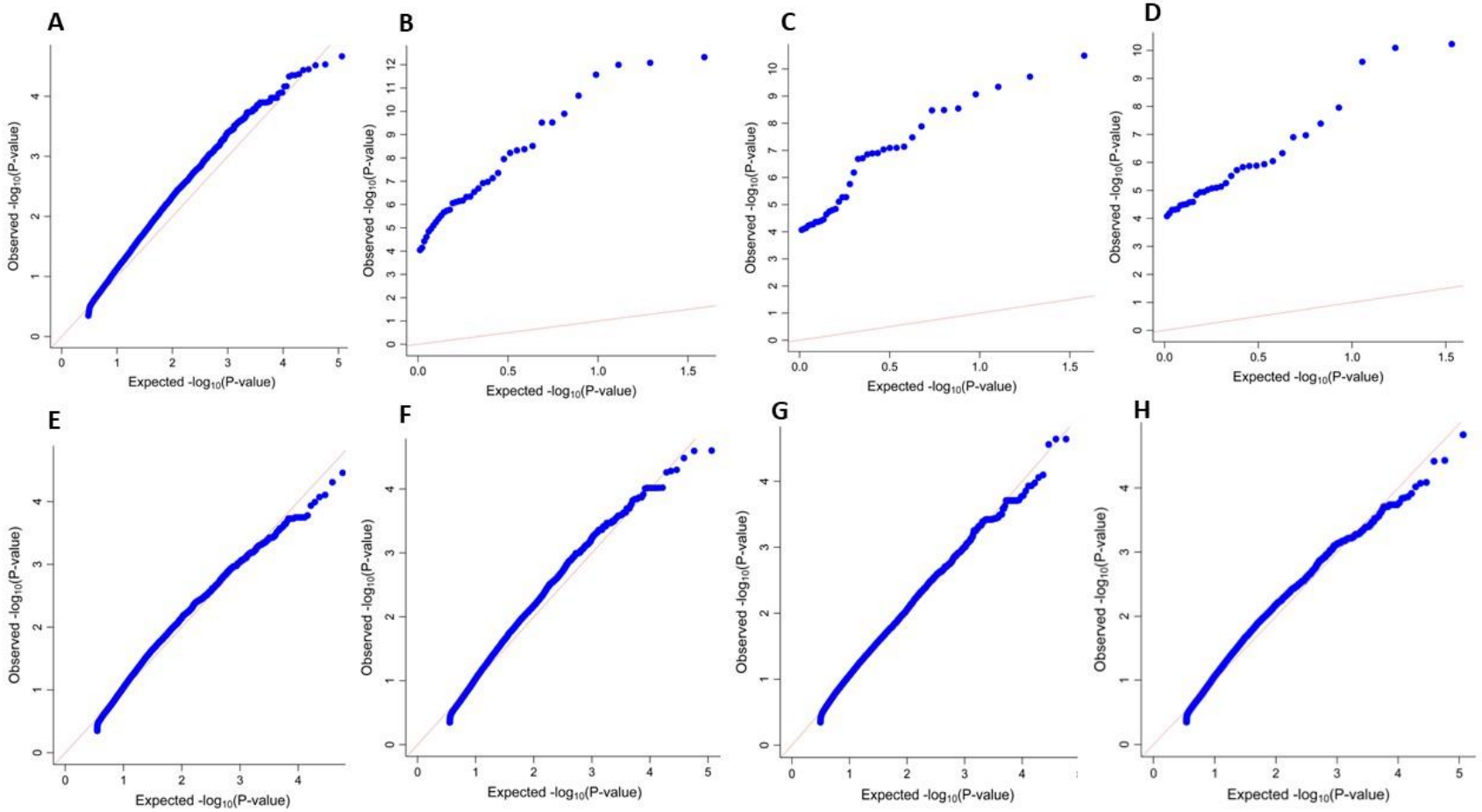
#140	31.95	15.67	32.2	2.05	46.34	5	3.24	1.03	1.6	0.59	31.34	13.92	198.67	0.58	4968.11
#141	33.45	9.34	18.52	2.05	54.34	6	1.86	0.84	1.79	0.69	35	21.67	125.67	0.82	8079.01
#142	29.45	17.67	36.44	2.13	69.34	7.67	3.66	1.1	2.15	0.87	41.67	11.92	204.34	0.8	5495.21
#143	23.45	12.34	24.89	1.91	42	5	2.5	0.92	1.5	0.53	29	11.67	137.34	0.58	6605.85
#144	33.78	18	37.37	2.45	42.34	4.67	3.76	1.11	1.51	0.54	36.34	13.25	196.34	0.49	7634.85
#145	27.45	12.67	26.02	2.11	56.34	6	2.62	0.93	1.84	0.71	35.34	14.84	164	0.77	6302.85
#146	29.45	18	36.94	2.04	53	6	3.71	1.11	1.76	0.67	36.34	13.28	187	0.61	6908.8
#147	31.67	16.5	33.21	2.07	51	6	3.34	1.05	1.71	0.65	33.5	14.75	193	0.62	7662.89
#148	35.78	12.34	24.4	2.08	40.67	4.67	2.45	0.9	1.47	0.52	28.67	13.5	150.67	0.58	6404.19
#149	32.17	12.5	26.14	1.88	48.5	5.5	2.63	0.93	1.64	0.61	31.5	9.63	153.5	0.66	6846.7
#150	36.5	13	26.04	1.61	41.5	5	2.62	0.93	1.49	0.53	29	15.13	140.5	0.57	7913.75
#151	30.67	15	30.52	2.13	43.5	5	3.07	1	1.54	0.55	30	7.75	178	0.56	6767.19
#152	32.34	12.5	25.64	2.78	45.5	5	2.58	0.94	1.58	0.58	31	12	148	0.62	5439.13
#153	27.95	9.34	18.79	1.51	42.67	5	1.89	0.81	1.51	0.54	29.34	15.72	146.67	0.67	5126.13
#154	38.33	19.25	40.06	2.19	54.5	6	4.05	1.16	1.77	0.69	34.5	12.5	214	0.6	7264.48
#155	31.67	15.67	32.23	2.11	45	5	3.24	1.03	1.57	0.57	30.67	11.09	187.34	0.56	6844.91
#156	27.67	17.5	35.55	1.46	41	4.5	3.57	1.08	1.47	0.52	29	12.13	201.5	0.48	6693.41
#157	35.84	15.5	31.02	1.99	36	4	3.12	1.01	1.36	0.46	26.5	12.75	172	0.46	7217.25
#158	26.67	16.5	33.71	2.63	43	5	3.39	1.05	1.52	0.55	29.5	12.38	184	0.53	5270.91

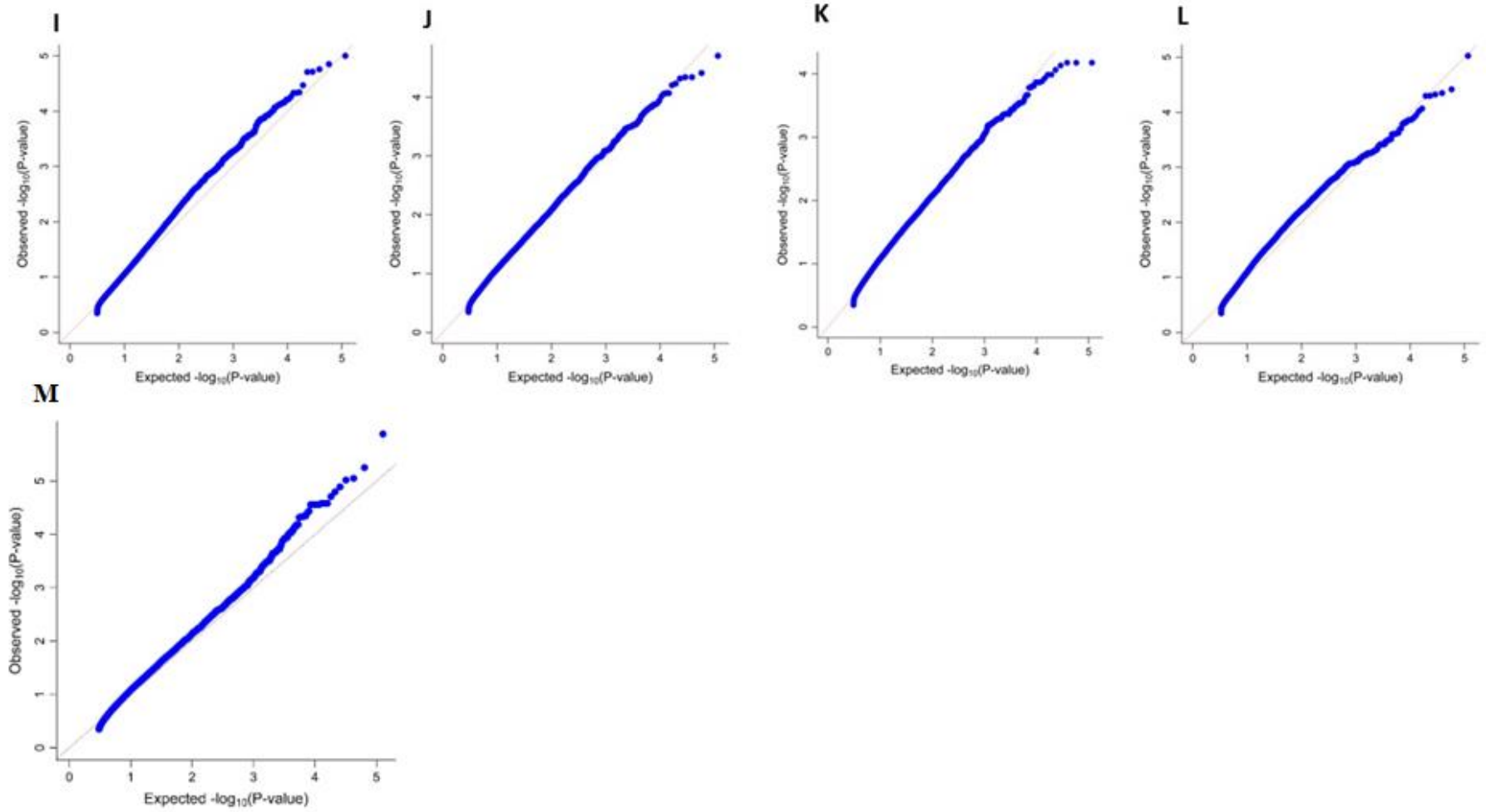


Appendix 8.3. The experimental procedure. A) Root chambers covered with polycarbonate sheet to protect the roots from light; B) A microscope for measuring stomata related traists and counting the number of root hairs; C) Imaging box with mounted camera and light box; D) Measuring nodal root angle



Appendix 8.4. Dendrogram of 158 sorghum genotypes constructed using Ward's method based on 15 traits under greenhouse conditions in 2022/23


A
B
C
D
E
F
G
H
Observed -log10(P-value)
Expected -log10(P-value)



Appendix 8.5. Q-Q plots comparing observed versus expected p-values from the genome-wide association study. Each graph displays the observed -log10(P-values) (shown as blue dots) against the expected -log10(P-values) (represented by a red line) for the following traits: (A) leaf angle, (B) shoot length, (C) leaf area, (D) shoot diameter, (E) root length, (F) number of nodal roots, (G) shoot dry weight, (H) root dry weight, (I) root hair number, (J) root angle, (K) stomatal density, (L) root-to-shoot ratio, and (M) stomatal area.

Appendix 8.6. The genetic membership coefficient showing how each sorghum genotype aligns with the five identified sub-groups.

SIno	Entry	V1	V2	V3	V4	V5	V	Region
1	#1	0.45707	0.114612	0.177153	1.00E-04	0.251065	V1	Amhara
2	#2	0.068206	1.00E-04	1.00E-04	1.00E-04	0.931495	V5	Oromia
3	#3	1.00E-04	0.083827	0.438532	0.477442	1.00E-04	V4	Oromia
4	#4	0.9996	1.00E-04	1.00E-04	1.00E-04	1.00E-04	V1	Gambela
5	#5	0.015395	0.697188	0.088884	0.003067	0.195466	V2	Oromia
6	#6	1.00E-04	0.026462	0.973238	1.00E-04	1.00E-04	V3	Amhara
7	#7	1.00E-04	1.00E-04	0.9996	1.00E-04	1.00E-04	V3	Oromia
8	#8	0.071635	0.045124	0.025514	0.857628	1.00E-04	V4	Amhara
9	#9	0.035672	0.021288	0.9098	1.00E-04	0.033139	V3	Amhara
10	#10	0.104211	0.076113	0.637794	1.00E-04	0.181783	V3	Tigray
11	#11	0.072116	1.00E-04	1.00E-04	1.00E-04	0.927585	V5	Benishangul Gumuz
12	#12	1.00E-04	0.03925	1.00E-04	0.198216	0.762334	V5	Benishangul Gumuz
13	#14	0.03912	0.011828	0.881866	0.049298	0.017888	V3	Oromia
14	#15	0.026741	0.175984	0.450905	0.317134	0.029236	V3	Gambela
15	#16	0.323112	0.133788	0.259841	1.00E-04	0.283159	V1	NA
16	#17	0.9996	1.00E-04	1.00E-04	1.00E-04	1.00E-04	V1	Gambela
17	#18	1.00E-04	1.00E-04	0.9996	1.00E-04	1.00E-04	V3	Amhara
18	#19	1.00E-04	0.071305	0.369497	0.558997	1.00E-04	V4	SEPR
19	#20	0.025598	1.00E-04	0.761943	0.212259	1.00E-04	V3	Tigray
20	#22	0.47195	0.02826	0.145649	1.00E-04	0.354041	V1	Amhara
21	#23	1.00E-04	0.993502	0.006199	1.00E-04	1.00E-04	V2	SEPR
22	#24	1.00E-04	0.965331	0.001219	0.033251	1.00E-04	V2	Oromia
23	#25	1.00E-04	0.9996	1.00E-04	1.00E-04	1.00E-04	V2	Oromia
24	#26	1.00E-04	1.00E-04	0.9996	1.00E-04	1.00E-04	V3	Amhara
25	#27	1.00E-04	0.992185	0.007515	1.00E-04	1.00E-04	V2	Oromia
26	#29	0.166374	0.384303	0.256667	0.1451	0.047556	V2	Gambela
27	#30	0.082391	0.044967	0.738601	0.029004	0.105037	V3	Tigray
28	#32	1.00E-04	0.540094	0.27702	0.182686	1.00E-04	V2	Oromia
29	#33	1.00E-04	1.00E-04	0.9996	1.00E-04	1.00E-04	V3	NA
30	#35	0.039375	0.019202	0.858102	0.057014	0.026307	V3	Gambela
31	#36	1.00E-04	1.00E-04	0.9996	1.00E-04	1.00E-04	V3	Amhara
32	#37	0.9996	1.00E-04	1.00E-04	1.00E-04	1.00E-04	V1	Gambela
33	#38	1.00E-04	1.00E-04	0.9996	1.00E-04	1.00E-04	V3	Amhara
34	#39	0.000517	0.987349	0.011934	1.00E-04	1.00E-04	V2	Tigray
35	#40	1.00E-04	0.972835	0.026865	1.00E-04	1.00E-04	V2	Tigray
36	#41	1.00E-04	0.9996	1.00E-04	1.00E-04	1.00E-04	V2	Oromia
37	#42	0.095954	1.00E-04	0.022837	1.00E-04	0.88101	V5	Benishangul Gumuz
38	#44	0.092166	1.00E-04	0.625347	0.257462	0.024924	V3	Oromia

“V1”, “V2”, “V3”, “V4”, and“V5”indicate the different inferred populations

Appendix 8.6 (continued)

39	#45	0.065501	0.269741	0.598131	0.038589	0.028038	V3	Gambela
40	#46	0.149764	0.169656	0.336919	0.309012	0.034649	V3	NA
41	#47	1.00E-04	0.814097	0.178884	0.00682	1.00E-04	V2	Oromia
42	#48	1.00E-04	1.00E-04	0.9996	1.00E-04	1.00E-04	V3	Oromia
43	#49	1.00E-04	1.00E-04	0.9996	1.00E-04	1.00E-04	V3	Amhara
44	#50	0.159852	0.119664	0.594863	1.00E-04	0.12552	V3	Oromia
45	#51	0.966132	1.00E-04	1.00E-04	0.003634	0.030034	V1	Gambela
46	#52	1.00E-04	0.002174	1.00E-04	0.058712	0.938914	V5	Oromia
47	#53	0.08689	0.079702	0.754071	1.00E-04	0.079237	V3	Oromia
48	#54	0.015975	1.00E-04	0.879418	0.100822	0.003685	V3	Amhara
49	#55	0.03497	0.015408	0.873064	0.039439	0.03712	V3	Amhara
50	#56	0.985669	1.00E-04	1.00E-04	1.00E-04	0.014031	V1	Gambela
51	#57	1.00E-04	0.9996	1.00E-04	1.00E-04	1.00E-04	V2	Oromia
52	#58	0.2788	0.468671	0.042856	0.100714	0.108958	V2	Tigray
53	#59	0.021249	1.00E-04	0.955809	0.008991	0.013851	V3	Tigray
54	#60	0.003472	0.86413	0.071731	0.019045	0.041623	V2	Tigray
55	#61	1.00E-04	0.216732	0.045312	1.00E-04	0.737756	V5	Benishangul Gumuz
56	#62	0.367313	1.00E-04	0.325037	0.307451	1.00E-04	V1	Amhara
57	#63	1.00E-04	0.958135	0.000518	0.003446	0.037802	V2	Oromia
58	#65	0.073751	0.377301	0.43001	1.00E-04	0.118838	V3	Tigray
59	#66	1.00E-04	0.969151	0.02872	0.001929	1.00E-04	V2	Amhara
60	#68	0.018985	0.94055	1.00E-04	1.00E-04	0.040265	V2	NA
61	#69	1.00E-04	0.98427	1.00E-04	1.00E-04	0.01543	V2	Oromia
62	#70	1.00E-04	1.00E-04	0.767923	0.231777	1.00E-04	V3	Oromia
63	#71	0.218363	0.486258	0.025466	0.144749	0.125165	V2	SEPR
64	#72	1.00E-04	0.992612	1.00E-04	0.007088	1.00E-04	V2	Oromia
65	#74	0.12196	0.039405	0.710783	0.046093	0.081759	V3	Gambela
66	#75	0.067016	0.132983	0.549768	0.193655	0.056578	V3	Oromia
67	#76	0.020942	0.72248	0.068984	1.00E-04	0.187494	V2	Oromia
68	#79	0.043899	0.022043	0.889952	0.007908	0.036198	V3	Tigray
69	#80	1.00E-04	0.99279	0.00691	1.00E-04	1.00E-04	V2	Oromia
70	#81	1.00E-04	0.523895	1.00E-04	0.475805	1.00E-04	V2	Amhara
71	#82	1.00E-04	0.367385	1.00E-04	0.158204	0.474211	V5	Oromia
72	#83	1.00E-04	1.00E-04	0.760049	0.239651	1.00E-04	V3	Benishangul Gumuz
73	#84	1.00E-04	0.521267	0.22291	0.255622	1.00E-04	V2	Tigray
74	#85	1.00E-04	0.9996	1.00E-04	1.00E-04	1.00E-04	V2	Oromia
75	#86	1.00E-04	1.00E-04	0.996098	0.003602	1.00E-04	V3	Amhara
76	#87	1.00E-04	1.00E-04	0.9996	1.00E-04	1.00E-04	V3	Oromia
77	#89	0.006648	0.002689	0.990462	1.00E-04	1.00E-04	V3	Somali
78	#90	1.00E-04	0.52711	0.47259	1.00E-04	1.00E-04	V2	Afar

“V1”, “V2”, “V3”, “V4”, and“V5”indicate the different inferred populations

Appendix 8.6 (continued)

79	#94	0.022746	0.009225	0.89412	0.025888	0.048021	V3	Benishangul Gumuz
80	#95	1.00E-04	1.00E-04	0.979432	0.020268	1.00E-04	V3	Amhara
81	#96	1.00E-04	1.00E-04	0.9996	1.00E-04	1.00E-04	V3	Oromia
82	#97	1.00E-04	0.582115	0.31986	0.097825	1.00E-04	V2	Oromia
83	#99	0.009182	0.031987	0.085312	1.00E-04	0.873419	V5	Benishangul Gumuz
84	#100	0.191856	1.00E-04	0.658767	0.051486	0.097791	V3	Amhara
85	#101	1.00E-04	1.00E-04	0.642998	0.356702	1.00E-04	V3	Tigray
86	#104	1.00E-04	0.01447	0.98523	1.00E-04	1.00E-04	V3	Amhara
87	#106	1.00E-04	0.001603	1.00E-04	0.264126	0.734071	V5	Oromia
88	#107	0.014846	0.025055	0.94334	1.00E-04	0.016658	V3	Oromia
89	#108	0.305094	0.192133	0.360203	0.14247	1.00E-04	V3	NA
90	#109	1.00E-04	1.00E-04	0.9996	1.00E-04	1.00E-04	V3	Amhara
91	#110	0.014049	0.006693	0.961906	0.000541	0.016811	V3	Amhara
92	#112	0.38893	1.00E-04	0.124441	1.00E-04	0.486429	V5	Amhara
93	#113	0.235331	1.00E-04	0.510298	0.072306	0.181965	V3	Oromia
94	#114	0.023733	0.015805	0.849518	0.083415	0.027529	V3	Somali
95	#115	1.00E-04	0.966259	0.033441	1.00E-04	1.00E-04	V2	SEPR
96	#117	1.00E-04	0.148233	0.401506	0.450061	1.00E-04	V4	Oromia
97	#118	0.15009	0.128618	0.601747	1.00E-04	0.119444	V3	Oromia
98	#119	1.00E-04	0.050322	0.054447	1.00E-04	0.895031	V5	Benishangul Gumuz
99	#120	1.00E-04	0.980233	0.019467	1.00E-04	1.00E-04	V2	Oromia
100	#121	0.082962	1.00E-04	0.001382	1.00E-04	0.915456	V5	Benishangul Gumuz
101	#122	0.036998	0.020354	0.867319	0.034766	0.040562	V3	Benishangul Gumuz
102	#123	0.055464	0.019478	0.829863	0.08416	0.011035	V3	Amhara
103	#124	0.124518	0.070771	0.69057	0.005464	0.108677	V3	SEPR
104	#125	0.086425	1.00E-04	1.00E-04	1.00E-04	0.913275	V5	Dire Dawa city Adm
105	#126	0.004794	0.979605	1.00E-04	1.00E-04	0.015401	V2	Dire Dawa city Adm
106	#127	1.00E-04	0.9996	1.00E-04	1.00E-04	1.00E-04	V2	Oromia
107	#128	0.0551	0.013597	0.822509	0.071873	0.036922	V3	Gambela
108	#130	0.00576	0.94781	1.00E-04	1.00E-04	0.046229	V2	Oromia
109	#131	0.9996	1.00E-04	1.00E-04	1.00E-04	1.00E-04	V1	Amhara
110	#132	1.00E-04	0.9996	1.00E-04	1.00E-04	1.00E-04	V2	Oromia
111	#133	0.035972	1.00E-04	0.838275	0.121589	0.004064	V3	Tigray
112	#135	1.00E-04	1.00E-04	1.00E-04	0.052021	0.947679	V5	Oromia
113	#136	0.092812	1.00E-04	0.057829	1.00E-04	0.849159	V5	Benishangul Gumuz
114	#137	0.4724	0.106421	0.1463	1.00E-04	0.274779	V1	SEPR
115	#138	1.00E-04	0.253745	0.238435	0.50762	1.00E-04	V4	Tigray
116	#139	1.00E-04	1.00E-04	0.9996	1.00E-04	1.00E-04	V3	Oromia
117	#140	0.074385	1.00E-04	1.00E-04	1.00E-04	0.925315	V5	Oromia
118	#141	1.00E-04	1.00E-04	1.00E-04	0.83388	0.16582	V4	Benishangul Gumuz

“V1”, “V2”, “V3”, “V4”, and“V5”indicate the different inferred populations

Appendix 8.6 (continued)

119	#142	0.094061	0.117838	0.659641	0.036839	0.091621	V3	Oromia
120	#144	1.00E-04	1.00E-04	0.582838	0.416863	1.00E-04	V3	Afar
121	#145	0.9996	1.00E-04	1.00E-04	1.00E-04	1.00E-04	V1	Gambela
122	#146	1.00E-04	1.00E-04	0.694497	0.305203	1.00E-04	V3	SEPR
123	#147	1.00E-04	0.071006	0.392126	0.536668	1.00E-04	V4	Amhara
124	#148	0.0295	0.008322	0.944061	0.003302	0.014815	V3	Gambela
125	#149	0.066442	1.00E-04	0.018254	1.00E-04	0.915104	V5	Benishangul Gumuz
126	#150	0.467481	0.123103	0.138066	1.00E-04	0.27125	V1	SEPR
127	#151	1.00E-04	0.339678	0.473461	0.186661	1.00E-04	V3	Tigray
128	#152	0.071277	1.00E-04	0.508089	0.417659	0.002875	V3	Oromia
129	#155	0.050394	1.00E-04	0.521834	0.427572	1.00E-04	V3	Amhara

“V1”, “V2”, “V3”, “V4”, and“V5”indicate the different inferred populations

Appendix 8.7. A comprehensive list of 388 quantitative trait nucleotides (QTNs) detected

Trait name	Method	RS#	Chr	Marker position (bp)	QTN effect	LOD score	'-log10(P)'	r2 (%)	MAF	Allele
LAg	mrMLM	S1_72527985	1	72527985	2.3491	5.5222	6.3384	16.8554	0.189	AA
LAg	mrMLM	S1_18776644	1	18776644	3.4919	4.318	5.0848	17.6514	0.0669	GG
LAg	FASTmrEMMA	S2_68197632	2	68197632	1.8889	3.1727	3.8788	4.0332	0.4341	TT
LAg	FASTmrMLM	S2_72741053	2	72741053	-2.8819	5.1948	5.9986	13.086	0.062	TT
LAg	pLARmEB	S3_10258003	3	10258003	1.5949	3.0942	3.7954	7.397	0.1163	AA
LAg	FASTmrMLM	S3_31816443	3	31816443	2.5159	5.5927	6.4115	11.3092	0.0814	AA
LAg	FASTmrMLM	S4_12354434	4	12354434	1.5017	4.3506	5.1189	5.7442	0.1163	GG
LAg	pLARmEB	S6_4082257	6	4082257	-1.2231	4.2575	5.0215	6.2107	0.3062	CC
LAg	FASTmrEMMA	S6_39672553	6	39672553	2.3872	3.6431	4.3762	6.9639	0.4574	AA
LAg	pLARmEB	S6_42277140	6	42277140	1.8769	5.8887	6.7179	7.0576	0.1202	GG
LAg	mrMLM	S7_11107766	7	11107766	-2.0512	4.0221	4.7748	14.8103	0.3071	CC
LAg	mrMLM	S8_245323	8	245323	2.5315	3.947	4.6959	5.8593	0.0551	TT
LAg	FASTmrMLM	S9_55485561	9	55485561	-1.9178	4.4802	5.2543	6.5711	0.0891	AA
LAg	pLARmEB	S10_53874910	10	53874910	2.0011	3.9713	4.7214	11.3552	0.3992	AG
ShL	FASTmrMLM	S1_68144065	1	68144065	0.5209	4.1854	4.946	3.7141	0.4845	GG
ShL	pLARmEB	S1_1279203	1	1279203	1.1576	5.0042	5.8005	6.3399	0.0465	CC
ShL	ISIS EM-BLASSO	S4_59787593	4	59787593	-0.3006	3.577	4.3065	1.3172	0.5	TT
ShL	ISIS EM-BLASSO	S4_817571	4	817571	-0.9426	5.7888	6.6145	3.036	0.0543	CC
ShL	pLARmEB	S4_7479523	4	7479523	-1.0966	7.0482	7.9141	6.7131	0.062	CC
ShL	mrMLM	S4_59251139	4	59251139	-1.1818	3.0464	3.7446	7.1024	0.0591	AA
ShL	mrMLM	S5_12790273	5	12790273	-0.7901	6.7929	7.6513	8.0251	0.4252	TT
ShL	mrMLM	S6_49860654	6	49860654	1.1727	4.6492	5.4307	5.639	0.0748	GG
ShL	FASTmrMLM	S6_6049929	6	6049929	-1.0279	3.911	4.6581	9.1212	0.1008	GG
ShL	mrMLM	S7_59376113	7	59376113	-0.805	4.5871	5.3659	7.8144	0.2441	CC
ShL	pLARmEB	S7_28578574	7	28578574	0.8129	4.4521	5.225	8.3242	0.3798	AG
ShL	pLARmEB	S8_62201039	8	62201039	-0.4213	5.4237	6.2362	2.5129	0.345	GG

Appendix 8.7 (continued)

ShL	pLARmEB	S8_37861295	8	37861295	0.5281	3.4018	4.1215	3.8928	0.2093	TA
ShL	FASTmrMLM	S8_2624370	8	2624370	-0.6327	5.0459	5.8439	5.9817	0.2713	GG
ShL	mrMLM	S8_59077434	8	59077434	-1.0214	5.2356	6.041	6.5846	0.1575	AA
ShL	ISIS EM-BLASSO	S10_53619913	10	53619913	0.4051	3.1401	3.8442	2.1421	0.186	GG
ShL	mrMLM	S10_58031253	10	58031253	0.8888	5.998	6.831	5.8736	0.1772	CC
ShL	pLARmEB	S10_44302656	10	44302656	-0.7905	6.2118	7.0519	7.0266	0.4729	GC
ShL	mrMLM	S10_56681073	10	56681073	-0.8548	4.3227	5.0897	9.1169	0.374	AA
ShL	FASTmrMLM	S10_44302678	10	44302678	-0.9119	5.0702	5.8691	10.1612	0.4729	AC
ShL	mrMLM	S10_59892991	10	59892991	-0.9788	3.8032	4.5448	10.6223	0.378	GG
ShL	mrMLM	S10_11079448	10	11079448	1.8475	7.1521	8.021	16.2581	0.0551	AA
LAr	mrMLM	S1_49600029	1	49600029	1.4915	3.3455	4.0619	4.2784	0.248	GG
LAr	mrMLM	S1_28345135	1	28345135	2.6279	3.7053	4.4418	9.5128	0.0945	GG
LAr	FASTmrMLM	S2_77195660	2	77195660	1.3911	5.0809	5.8803	5.1932	0.1667	AA
LAr	mrMLM	S2_67000059	2	67000059	2.8718	3.1346	3.8384	7.0267	0.0551	AA
LAr	mrMLM	S2_64672108	2	64672108	-4.1297	4.2118	4.9736	15.513	0.0551	AA
LAr	FASTmrMLM	S3_9935681	3	9935681	0.8826	3.3421	4.0583	2.7774	0.2558	GG
LAr	mrMLM	S3_60019380	3	60019380	-2.3075	5.5876	6.4062	4.5366	0.0945	GG
LAr	FASTmrMLM	S4_68110928	4	68110928	1.3357	5.7744	6.5996	5.3726	0.2054	TT
LAr	FASTmrEMMA	S5_5262919	5	5262919	-2.6122	3.2149	3.9235	6.208	0.3411	AA
LAr	mrMLM	S7_5950884	7	5950884	-2.1371	3.6076	4.3388	4.9129	0.126	GG
LAr	mrMLM	S8_33969166	8	33969166	2.5869	4.6741	5.4567	9.5278	0.126	CC
ShD	FASTmrMLM	S1_11122583	1	11122583	0.1038	5.2229	6.0278	4.4272	0.093	TT
ShD	FASTmrMLM	S1_4627194	1	4627194	-0.0933	3.1769	3.8833	5.1749	0.1279	CT
ShD	FASTmrEMMA	S1_4638838	1	4638838	0.2912	4.0393	4.7928	9.8454	0.1279	GG
ShD	ISIS EM-BLASSO	S3_9935697	3	9935697	0.0669	4.1995	4.9607	4.4833	0.2946	TT
ShD	mrMLM	S3_51970970	3	51970970	0.1155	4.6417	5.4229	5.8678	0.189	AA
ShD	FASTmrMLM	S4_66518330	4	66518330	-0.0694	3.2896	4.0027	3.199	0.1667	CC
ShD	mrMLM	S4_56209797	4	56209797	-0.1146	4.7829	5.5701	6.0833	0.1969	GG

Appendix 8.7 (continued)

ShD	mrMLM	S4_51654020	4	51654020	-0.1258	5.7724	6.5976	9.5585	0.3819	CC
ShD	FASTmrMLM	S4_40232458	4	40232458	-0.1104	3.9701	4.7202	10.3186	0.1473	AA
ShD	pLARmEB	S5_34428102	5	34428102	0.0696	3.4405	4.1624	0.5563	0.0969	AA
ShD	FASTmrMLM	S5_71649865	5	71649865	0.0524	3.467	4.1904	3.0926	0.5	TT
ShD	mrMLM	S5_6024238	5	6024238	-0.1083	3.8878	4.6337	6.67	0.2717	AA
ShD	FASTmrMLM	S6_60594858	6	60594858	-0.0841	6.7982	7.6567	7.6748	0.3566	CC
ShD	mrMLM	S6_46576987	6	46576987	0.1871	8.3897	9.2915	7.8446	0.0827	CC
ShD	FASTmrMLM	S7_5819399	7	5819399	-0.0648	3.297	4.0106	2.3926	0.3721	CC
ShD	mrMLM	S7_44897619	7	44897619	-0.3629	8.878	9.7915	27.3012	0.0472	GG
ShD	mrMLM	S9_57701415	9	57701415	-0.2663	6.2446	7.0858	13.4715	0.0433	AA
ShD	ISIS EM-BLASSO	S10_53094195	10	53094195	0.0171	3.7962	4.5374	0.1215	0.093	GG
RL	mrMLM	S1_61299116	1	61299116	-3.2012	13.2522	14.2494	1.6565	0.0433	GG
RL	mrMLM	S1_61140028	1	61140028	3.218	7.0553	7.9214	2.0118	0.0472	TT
RL	mrMLM	S1_56601593	1	56601593	-3.1598	5.5844	6.4029	2.5554	0.0591	AA
RL	ISIS EM-BLASSO	S1_6012709	1	6012709	2.7452	7.0924	7.9596	5.3426	0.2674	TT
RL	ISIS EM-BLASSO	S1_9667940	1	9667940	2.1285	6.9429	7.8057	5.3856	0.4031	CC
RL	FASTmrMLM	S1_6012717	1	6012717	4.2802	6.7111	7.567	8.8329	0.1357	TT
RL	mrMLM	S2_22399949	2	22399949	-2.3479	5.2357	6.0411	0.7987	0.0591	GG
RL	mrMLM	S2_72765031	2	72765031	3.6854	11.4392	12.4055	2.1955	0.0512	AA
RL	mrMLM	S2_8478316	2	8478316	-3.7987	11.8013	12.7742	2.5701	0.0669	AA
RL	mrMLM	S2_76366289	2	76366289	-4.4237	8.0344	8.9273	2.8352	0.0433	CC
RL	mrMLM	S2_12197197	2	12197197	-3.8203	4.9448	5.7387	3.294	0.0512	TT
RL	mrMLM	S3_56637079	3	56637079	1.5756	5.1881	5.9917	0.3173	0.0433	CC
RL	mrMLM	S3_22854034	3	22854034	1.2029	8.7249	9.6348	0.3266	0.0433	CC
RL	mrMLM	S3_8609025	3	8609025	-2.2591	4.2778	5.0427	0.909	0.0591	CC
RL	pLARmEB	S3_6983050	3	6983050	-1.3116	3.4533	4.1759	1.1842	0.4302	GG
RL	mrMLM	S3_62564964	3	62564964	3.1808	12.5955	13.582	1.9656	0.0669	CC
RL	mrMLM	S3_62428745	3	62428745	-2.8192	3.7676	4.5073	3.9825	0.1417	TT

Appendix 8.7 (continued)

RL	mrMLM	S4_56135274	4	56135274	-2.1684	3.1796	3.8861	0.76	0.0591	TT
RL	mrMLM	S4_14854287	4	14854287	2.184	4.5277	5.3039	1.9119	0.1024	GG
RL	mrMLM	S4_57625836	4	57625836	2.9442	12.2952	13.2766	2.0888	0.0945	CC
RL	mrMLM	S4_62032410	4	62032410	4.3565	7.5502	8.4303	2.7496	0.0512	AA
RL	mrMLM	S4_42656476	4	42656476	4.875	12.1351	13.1138	4.2327	0.0433	GG
RL	ISIS EM-BLASSO	S4_12075115	4	12075115	-2.0481	6.8768	7.7377	4.3425	0.3682	TT
RL	mrMLM	S4_13304368	4	13304368	-4.6597	13.6261	14.6291	5.5571	0.0709	GG
RL	FASTmrMLM	S4_60963038	4	60963038	-3.6846	3.9898	4.7409	16.8218	0.1667	GG
RL	mrMLM	S5_1575148	5	1575148	-2.5028	7.0685	7.935	1.6032	0.0748	TT
RL	mrMLM	S5_36541047	5	36541047	2.7233	5.0282	5.8255	1.7871	0.0551	GG
RL	mrMLM	S5_41906698	5	41906698	-3.1982	5.1548	5.9571	2.3086	0.0512	TT
RL	mrMLM	S5_19538615	5	19538615	3.9081	5.4557	6.2694	4.1329	0.063	TT
RL	pLARmEB	S6_47428336	6	47428336	1.9128	3.0459	3.7441	1.0067	0.1008	CC
RL	mrMLM	S6_40852427	6	40852427	3.2803	3.5802	4.3099	1.9165	0.0472	TT
RL	mrMLM	S6_45176048	6	45176048	-4.504	16.2814	17.3219	2.5929	0.0512	GG
RL	mrMLM	S6_55253000	6	55253000	-5.0596	6.2131	7.0532	7.6553	0.0669	CC
RL	mrMLM	S7_61116299	7	61116299	3.2469	9.845	10.78	1.5274	0.0512	CC
RL	mrMLM	S7_2413959	7	2413959	3.9955	11.7291	12.7007	4.0858	0.0591	TT
RL	mrMLM	S8_58152111	8	58152111	2.0422	3.2155	3.9242	0.7428	0.0669	TT
RL	mrMLM	S8_61541182	8	61541182	2.8576	9.1194	10.0385	1.1831	0.0551	GG
RL	mrMLM	S9_677823	9	677823	-1.1847	3.2207	3.9297	0.1302	0.0394	CC
RL	mrMLM	S9_1910433	9	1910433	2.3478	5.3202	6.1288	0.7986	0.0591	GG
RL	mrMLM	S10_1529333	10	1529333	2.5195	3.9109	4.658	0.7012	0.0512	GG
RL	mrMLM	S10_1587181	10	1587181	-1.147	4.6021	5.3816	0.7975	0.4016	TT
RL	mrMLM	S10_57975402	10	57975402	-2.4185	5.2698	6.0765	0.9455	0.063	CC
RL	mrMLM	S10_56429114	10	56429114	-3.7699	4.2559	5.0198	3.2076	0.0512	GG
NR	mrMLM	S1_19486949	1	19486949	-0.2459	4.1495	4.9083	1.2504	0.0591	CC
NR	mrMLM	S1_59520997	1	59520997	-0.3392	3.0351	3.7326	3.5461	0.0945	TT

Appendix 8.7 (continued)

NR	mrMLM	S1_59142051	1	59142051	-0.4398	6.4504	7.2982	3.9995	0.0433	CC
NR	mrMLM	S1_2062348	1	2062348	-0.5219	5.7817	6.6072	9.4275	0.063	TT
NR	mrMLM	S2_38550249	2	38550249	-0.3201	6.3534	7.1981	1.899	0.0512	GG
NR	mrMLM	S2_74985825	2	74985825	0.5438	3.0349	3.7324	6.1136	0.0512	GG
NR	FASTmrMLM	S2_10753898	2	10753898	0.4144	4.0358	4.7891	13.0145	0.4961	TC
NR	pLARmEB	S3_69088913	3	69088913	0.1397	4.7574	5.5435	0.8446	0.3566	CC
NR	mrMLM	S3_60070892	3	60070892	0.4374	3.6183	4.3501	3.5452	0.0551	GG
NR	mrMLM	S3_67146923	3	67146923	-0.4668	5.9727	6.8048	3.5625	0.0512	AA
NR	mrMLM	S3_6736239	3	6736239	0.2292	10.4925	11.4408	3.8401	0.3386	CC
NR	pLARmEB	S3_73699963	3	73699963	-0.4735	3.4018	4.1215	4.8719	0.0736	AA
NR	FASTmrMLM	S3_7008580	3	7008580	-0.3827	5.0042	5.8005	8.1296	0.1395	GG
NR	pLARmEB	S4_57614386	4	57614386	0.3924	4.8989	5.6909	2.8649	0.1047	TT
NR	mrMLM	S4_2212522	4	2212522	-0.3697	6.7424	7.5992	3.3955	0.0787	TT
NR	FASTmrMLM	S4_56727113	4	56727113	-0.3125	3.8183	4.5607	9.3675	0.2364	CC
NR	mrMLM	S5_43597800	5	43597800	-0.2494	3.0575	3.7564	1.2859	0.0433	CC
NR	mrMLM	S6_47443068	6	47443068	0.3174	3.5005	4.2258	2.5034	0.0748	CC
NR	ISIS EM-BLASSO	S6_47465957	6	47465957	0.5284	8.1699	9.0662	10.9913	0.0814	AA
NR	mrMLM	S7_32727569	7	32727569	-0.3589	3.4106	4.1308	5.1526	0.0787	CC
NR	mrMLM	S8_59946324	8	59946324	-0.1942	4.5794	5.3579	1.8778	0.1693	TT
NR	pLARmEB	S8_46226438	8	46226438	-0.2356	4.0025	4.7542	2.1793	0.3876	AA
NR	mrMLM	S8_7393666	8	7393666	0.3547	8.8612	9.7744	3.3813	0.0551	AA
NR	ISIS EM-BLASSO	S9_2329974	9	2329974	0.2085	3.7823	4.5228	4.0928	0.2984	CC
NR	mrMLM	S10_1170277	10	1170277	0.2041	6.682	7.537	3.3187	0.4646	GG
NR	ISIS EM-BLASSO	S10_3188609	10	3188609	-0.2966	4.0987	4.8551	8.8486	0.3217	GC
SDW	FASTmrMLM	S1_9570201	1	9570201	-0.0003	10.5223	11.4712	0.0014	0.3372	CC
SDW	FASTmrMLM	S1_12922040	1	12922040	0.0007	3.8401	4.5836	0.0054	0.2054	CC
SDW	FASTmrMLM	S1_74545695	1	74545695	-0.0038	11.0442	12.0032	0.2299	0.2326	AC
SDW	mrMLM	S1_11756199	1	11756199	-0.03	5.0773	5.8765	4.0202	0.0866	GG

Appendix 8.7 (continued)

SDW	ISIS EM-BLASSO	S1_19245447	1	19245447	0.0164	6.282	7.1244	4.1016	0.3605	CC
SDW	mrMLM	S1_72474262	1	72474262	-0.0727	6.6421	7.4959	15.3549	0.0591	TT
SDW	FASTmrMLM	S2_70143143	2	70143143	0.0027	5.9142	6.7443	0.0244	0.0659	GG
SDW	FASTmrMLM	S2_3944813	2	3944813	-0.0036	8.8733	9.7867	0.2166	0.4225	AA
SDW	FASTmrMLM	S2_9258392	2	9258392	0.0073	3.0584	3.7574	0.3255	0.0581	CC
SDW	FASTmrMLM	S3_3897494	3	3897494	-0.0039	6.9824	7.8464	0.0975	0.0543	TT
SDW	FASTmrMLM	S3_50409159	3	50409159	-0.0029	7.1143	7.9821	0.1021	0.1202	CC
SDW	FASTmrMLM	S3_38412773	3	38412773	-0.0082	15.8734	16.9086	0.3227	0.0465	TT
SDW	mrMLM	S3_10257624	3	10257624	0.0593	4.2709	5.0355	11.0572	0.0551	AA
SDW	FASTmrMLM	S4_784914	4	784914	-0.0006	3.6487	4.3821	0.0014	0.062	CC
SDW	FASTmrMLM	S4_820871	4	820871	-0.0029	8.2786	9.1777	0.0401	0.062	GG
SDW	FASTmrMLM	S4_53315434	4	53315434	0.0025	10.209	11.1516	0.0763	0.1357	CC
SDW	FASTmrMLM	S4_61056825	4	61056825	-0.0057	13.2846	14.2823	0.183	0.062	CC
SDW	mrMLM	S4_15676372	4	15676372	-0.0409	3.5453	4.2731	11.3972	0.1378	GG
SDW	FASTmrMLM	S5_61127990	5	61127990	0.0013	6.5434	7.3941	0.0235	0.1667	CT
SDW	FASTmrMLM	S5_17653588	5	17653588	-0.0017	4.7146	5.4989	0.0262	0.1395	AA
SDW	pLARmEB	S5_14397079	5	14397079	-0.0036	4.2576	5.0216	0.1055	0.469	AA
SDW	FASTmrMLM	S5_2696740	5	2696740	-0.0028	6.929	7.7914	0.1132	0.314	TT
SDW	FASTmrMLM	S5_66956000	5	66956000	-0.0057	8.4483	9.3516	0.3948	0.2326	CC
SDW	FASTmrMLM	S5_67499875	5	67499875	-0.0089	8.1297	9.025	0.8427	0.1124	CC
SDW	pLARmEB	S6_27859562	6	27859562	0.0117	4.6275	5.4081	1.4192	0.4457	TC
SDW	FASTmrMLM	S7_6869900	7	6869900	-0.0006	3.895	4.6413	0.0059	0.4109	CC
SDW	FASTmrMLM	S7_43891501	7	43891501	-0.0022	17.096	18.1468	0.0198	0.0426	AA
SDW	FASTmrMLM	S7_9145212	7	9145212	-0.0039	3.1018	3.8035	0.0604	0.0543	CC
SDW	ISIS EM-BLASSO	S7_2468468	7	2468468	-0.0028	4.386	5.1559	0.1043	0.1783	CC
SDW	FASTmrMLM	S7_58886667	7	58886667	0.0035	10.8016	11.756	0.1776	0.4612	AG
SDW	FASTmrMLM	S7_1504125	7	1504125	-0.0044	6.075	6.9106	0.2692	0.2519	GG
SDW	mrMLM	S7_64332829	7	64332829	-0.0296	3.0872	3.788	5.0875	0.1732	CC

Appendix 8.7 (continued)

SDW	FASTmrMLM	S8_8629741	8	8629741	0.0017	4.1379	4.8962	0.0227	0.3721	AG
SDW	FASTmrMLM	S8_3832378	8	3832378	0.0018	4.4081	5.179	0.0377	0.4806	AA
SDW	pLARmEB	S8_3678042	8	3678042	-0.0216	3.8786	4.6241	4.5873	0.2791	GG
SDW	mrMLM	S8_1658038	8	1658038	-0.0239	3.3893	4.1083	4.6088	0.2874	GG
SDW	FASTmrMLM	S10_54286999	10	54286999	-0.0014	5.8499	6.6778	0.0132	0.0891	TT
SDW	FASTmrMLM	S10_55999407	10	55999407	-0.0105	10.8202	11.7749	0.675	0.093	GG
SDW	pLARmEB	S10_56813120	10	56813120	-0.0526	6.6523	7.5064	6.4997	0.0504	GG
RDW	pLARmEB	S1_74545670	1	74545670	8.29E-05	9.0193	9.9361	3.29E-05	0.1279	GC
RDW	FASTmrMLM	S1_59434357	1	59434357	0.0025	7.0386	7.9043	0.0363	0.1628	AA
RDW	pLARmEB	S1_62391431	1	62391431	0.007	20.1419	21.2274	0.1034	0.0775	AA
RDW	mrMLM	S1_13357003	1	13357003	0.0145	5.0304	5.8277	0.1208	0.0591	AA
RDW	pLARmEB	S1_5896344	1	5896344	-0.0055	13.1262	14.1214	0.2024	0.4922	GG
RDW	mrMLM	S1_23215428	1	23215428	0.0199	3.364	4.0815	0.2152	0.0551	TT
RDW	mrMLM	S1_74410393	1	74410393	-0.0175	12.0341	13.011	0.2866	0.1378	TT
RDW	mrMLM	S1_10463230	1	10463230	0.0385	27.1496	28.2986	0.5969	0.0709	AA
RDW	mrMLM	S1_28914756	1	28914756	-0.0407	20.9625	22.0565	0.7273	0.0591	GG
RDW	mrMLM	S1_12577265	1	12577265	-0.0402	16.6285	17.6735	0.8236	0.0551	CC
RDW	mrMLM	S1_62624633	1	62624633	-0.0598	32.1605	33.3455	1.0326	0.0512	CC
RDW	mrMLM	S1_11550744	1	11550744	-0.0518	20.8157	21.9082	1.1773	0.0709	GG
RDW	FASTmrMLM	S2_76710684	2	76710684	0.0004	3.8428	4.5864	0.0005	0.0543	AA
RDW	mrMLM	S2_76750734	2	76750734	-0.005	3.873	4.6182	0.0083	0.0591	AA
RDW	FASTmrMLM	S2_2586409	2	2586409	-0.0015	5.1221	5.9231	0.0096	0.0736	GG
RDW	mrMLM	S2_2392355	2	2392355	0.0094	7.6204	8.5024	0.0624	0.1181	TT
RDW	mrMLM	S2_2622373	2	2622373	-0.0192	12.6182	13.6051	0.1485	0.0433	GG
RDW	mrMLM	S2_8597955	2	8597955	-0.0233	13.5763	14.5786	0.1988	0.063	AA
RDW	FASTmrMLM	S2_5745105	2	5745105	-0.0107	3.021	3.7176	1.1132	0.2326	GG
RDW	mrMLM	S2_9796969	2	9796969	-0.052	32.1804	33.3656	1.2861	0.0709	CC

Appendix 8.7 (continued)

RDW	mrMLM	S2_63601121	2	63601121	0.0797	45.2689	46.5269	1.8349	0.0827	GG
RDW	mrMLM	S2_16593171	2	16593171	-0.0657	40.8592	42.0953	1.8966	0.0827	TT
RDW	mrMLM	S2_7089638	2	7089638	0.0505	34.1053	35.3028	2.0279	0.0866	CC
RDW	mrMLM	S2_45425225	2	45425225	-0.0575	34.3947	35.594	2.1287	0.0669	AA
RDW	FASTmrMLM	S2_73895386	2	73895386	-0.0201	12.2012	13.181	3.8395	0.3333	TT
RDW	FASTmrMLM	S3_65000490	3	65000490	0.0008	9.083	10.0013	0.0022	0.0659	AA
RDW	pLARmEB	S3_68211791	3	68211791	-0.0008	21.6301	22.7308	0.0025	0.0969	CT
RDW	FASTmrMLM	S3_53323810	3	53323810	-0.0014	4.9528	5.747	0.008	0.093	GG
RDW	FASTmrMLM	S3_59813010	3	59813010	0.0022	5.944	6.7751	0.0143	0.0775	TT
RDW	pLARmEB	S3_7226856	3	7226856	-0.0051	21.039	22.1338	0.0595	0.0543	AA
RDW	pLARmEB	S3_71297866	3	71297866	-0.0046	5.0595	5.858	0.0952	0.1202	GG
RDW	pLARmEB	S3_7121753	3	7121753	0.0041	9.8003	10.7344	0.0968	0.3527	GG
RDW	FASTmrMLM	S3_51816771	3	51816771	0.0056	4.8546	5.6448	0.2113	0.1705	TT
RDW	mrMLM	S3_67503259	3	67503259	-0.0284	23.0738	24.1882	0.2325	0.0472	GG
RDW	mrMLM	S3_73484326	3	73484326	-0.039	34.8312	36.0332	0.4986	0.0551	CC
RDW	mrMLM	S3_63144077	3	63144077	-0.0249	20.9587	22.0527	0.5624	0.1024	GG
RDW	mrMLM	S3_10771486	3	10771486	0.0359	14.6938	15.7127	0.8294	0.0669	CC
RDW	mrMLM	S3_5809364	3	5809364	-0.0489	42.9692	44.216	0.8733	0.0591	CC
RDW	FASTmrMLM	S3_2431542	3	2431542	0.0103	12.4676	13.452	1.0121	0.3023	TT
RDW	FASTmrMLM	S3_53110100	3	53110100	0.0135	6.1485	6.9865	1.5027	0.2713	AA
RDW	mrMLM	S3_33493205	3	33493205	-0.0773	36.9766	38.1914	1.7238	0.0512	AA
RDW	mrMLM	S3_59956759	3	59956759	0.1092	43.8852	45.1365	4.3526	0.063	CC
RDW	mrMLM	S3_35927444	3	35927444	0.1253	51.4186	52.7037	4.534	0.0512	CC
RDW	FASTmrMLM	S4_50469879	4	50469879	-0.0037	3.8236	4.5662	0.0598	0.1202	AA
RDW	mrMLM	S4_1563174	4	1563174	0.0098	7.1692	8.0386	0.0684	0.1693	CC
RDW	mrMLM	S4_3451818	4	3451818	0.0167	7.2772	8.1497	0.1124	0.0551	AA
RDW	mrMLM	S4_7790019	4	7790019	-0.0218	6.9849	7.849	0.1552	0.0512	GG
RDW	mrMLM	S4_57943670	4	57943670	-0.0813	46.0752	47.3369	2.4125	0.0669	CC

Appendix 8.7 (continued)

RDW	pLARmEB	S5_6991039	5	6991039	-0.0008	4.0663	4.8211	0.002	0.0698	CC
RDW	FASTmrMLM	S5_68781533	5	68781533	0.0031	6.5076	7.3572	0.0415	0.1163	GG
RDW	FASTmrMLM	S5_6496091	5	6496091	0.0064	12.0256	13.0024	0.083	0.0543	AA
RDW	FASTmrMLM	S5_1184341	5	1184341	-0.0041	6.4623	7.3105	0.1105	0.1124	AA
RDW	FASTmrMLM	S5_6953582	5	6953582	-0.0061	10.3954	11.3418	0.3583	0.3295	GG
RDW	mrMLM	S5_13380603	5	13380603	-0.0341	19.4001	20.4777	0.5523	0.0512	AA
RDW	mrMLM	S5_2563056	5	2563056	0.0676	41.9037	43.1452	1.3203	0.0472	CC
RDW	mrMLM	S5_73885	5	73885	0.0849	26.3816	27.5245	2.3598	0.0512	CC
RDW	FASTmrMLM	S6_13523739	6	13523739	-0.0026	10.4414	11.3887	0.0489	0.1822	CC
RDW	mrMLM	S6_56254072	6	56254072	0.0356	18.6302	19.6992	0.734	0.0591	TT
RDW	mrMLM	S6_45228619	6	45228619	-0.0456	35.0107	36.2138	0.7591	0.063	CC
RDW	mrMLM	S6_43323853	6	43323853	0.053	36.9465	38.1611	1.0248	0.0591	GG
RDW	mrMLM	S6_51495425	6	51495425	-0.0429	6.9676	7.8312	1.0663	0.0591	TT
RDW	mrMLM	S6_46938261	6	46938261	-0.0421	32.3511	33.5374	1.0859	0.0748	GG
RDW	mrMLM	S6_58123784	6	58123784	-0.0572	47.4394	48.7074	2.7879	0.1024	TT
RDW	pLARmEB	S7_62824948	7	62824948	-0.0016	6.901	7.7626	0.011	0.1783	TT
RDW	FASTmrMLM	S7_59408245	7	59408245	-0.0037	10.5822	11.5323	0.0628	0.1163	CC
RDW	FASTmrMLM	S7_5819395	7	5819395	-0.0059	8.1566	9.0526	0.2889	0.1667	GG
RDW	mrMLM	S7_63084000	7	63084000	-0.0735	42.5907	43.8356	1.7666	0.0551	CC
RDW	FASTmrMLM	S7_32206381	7	32206381	-0.0174	12.9904	13.9834	2.8239	0.2132	GG
RDW	pLARmEB	S8_5393369	8	5393369	0.001	4.3013	5.0673	0.0031	0.1279	CC
RDW	pLARmEB	S8_61269072	8	61269072	0.0026	15.0101	16.0335	0.0122	0.062	AA
RDW	FASTmrMLM	S8_12170538	8	12170538	-0.0031	6.522	7.372	0.0465	0.0775	CC
RDW	mrMLM	S8_61911269	8	61911269	-0.0163	10.0652	11.0048	0.1169	0.0827	AA
RDW	mrMLM	S8_41508858	8	41508858	0.0186	3.0268	3.7238	0.2008	0.0551	CC
RDW	mrMLM	S8_60450793	8	60450793	-0.044	31.647	32.8286	0.7796	0.0433	CC
RDW	mrMLM	S8_14247947	8	14247947	0.0954	57.2997	58.608	4.3229	0.0748	GG
RDW	FASTmrMLM	S9_56346446	9	56346446	0.0003	5.7535	6.578	0.0003	0.124	AA

Appendix 8.7 (continued)

RDW	pLARmEB	S9_56395818	9	56395818	-0.0008	11.7088	12.68	0.0027	0.1279	AA
RDW	pLARmEB	S9_45257130	9	45257130	0.0015	11.9484	12.9239	0.0073	0.1202	TT
RDW	pLARmEB	S9_56705459	9	56705459	-0.003	6.1145	6.9514	0.0451	0.2171	GG
RDW	FASTmrMLM	S9_2243505	9	2243505	-0.0052	17.5064	18.5623	0.1132	0.0698	GG
RDW	pLARmEB	S9_56419671	9	56419671	0.007	15.4608	16.4904	0.3206	0.3062	CC
RDW	pLARmEB	S9_53014493	9	53014493	-0.0077	10.6307	11.5817	0.3953	0.2597	AC
RDW	mrMLM	S9_20479151	9	20479151	0.0408	19.4785	20.5569	0.5455	0.0394	GG
RDW	mrMLM	S9_54447834	9	54447834	-0.0379	23.6738	24.7936	0.7819	0.0669	CC
RDW	mrMLM	S9_17214745	9	17214745	0.0327	33.5973	34.7916	0.8181	0.0866	TT
RDW	mrMLM	S9_56419649	9	56419649	0.0388	30.6544	31.8292	0.8725	0.0591	CC
RDW	pLARmEB	S10_50376411	10	50376411	0.0028	11.8392	12.8127	0.0298	0.155	GG
RDW	mrMLM	S10_46881538	10	46881538	0.0139	4.5546	5.332	0.0774	0.0394	GG
RDW	mrMLM	S10_46856525	10	46856525	0.0095	5.0301	5.8274	0.1136	0.4764	CC
RDW	pLARmEB	S10_52287420	10	52287420	0.0059	3.8533	4.5975	0.1213	0.0969	TT
RDW	FASTmrMLM	S10_16912285	10	16912285	0.0046	4.3915	5.1616	0.1896	0.1783	TC
RDW	pLARmEB	S10_3336757	10	3336757	-0.0075	10.3248	11.2697	0.2741	0.1434	TT
RDW	mrMLM	S10_54187232	10	54187232	-0.0234	29.7926	30.9613	0.45	0.1496	AA
RDW	FASTmrMLM	S10_40512601	10	40512601	-0.0069	10.3732	11.3191	0.4573	0.2713	GC
RHN	FASTmrMLM	S2_75829957	2	75829957	-1.3167	3.066	3.7655	3.7495	0.0698	TT
RHN	pLARmEB	S2_58868368	2	58868368	1.0812	4.2478	5.0113	4.2465	0.1318	TT
RHN	mrMLM	S2_69362046	2	69362046	-1.0282	5.9438	6.7749	5.5385	0.3307	AA
RHN	pLARmEB	S3_26437403	3	26437403	-0.8922	3.2188	3.9277	3.4488	0.438	CC
RHN	FASTmrMLM	S3_900128	3	900128	-0.7754	3.3769	4.0951	3.464	0.2868	AA
RHN	FASTmrEMMA	S3_62350796	3	62350796	-1.733	3.8524	4.5966	4.1379	0.3721	TT
RHN	ISIS EM-BLASSO	S3_16332149	3	16332149	1.2644	4.1576	4.9168	4.5226	0.1085	GG
RHN	FASTmrMLM	S3_46904508	3	46904508	0.8622	3.876	4.6213	5.0408	0.2984	AA
RHN	mrMLM	S3_52286531	3	52286531	-1.2904	5.4305	6.2433	5.1206	0.126	TT
RHN	pLARmEB	S3_47278241	3	47278241	1.1278	5.257	6.0632	7.7445	0.3643	CC

Appendix 8.7 (continued)

RHN	FASTmrMLM	S4_50537111	4	50537111	-0.9331	3.5591	4.2876	4.2069	0.1473	GG
RHN	mrMLM	S4_56055096	4	56055096	0.9673	5.9781	6.8104	5.7195	0.4921	CC
RHN	mrMLM	S4_26397945	4	26397945	-1.3771	5.8004	6.6266	5.8322	0.4528	TT
RHN	mrMLM	S4_54844000	4	54844000	1.2377	5.0188	5.8157	6.2075	0.1811	CC
RHN	mrMLM	S5_69607775	5	69607775	-0.7014	3.2667	3.9785	2.6452	0.3071	CC
RHN	mrMLM	S5_59372539	5	59372539	1.436	4.576	5.3543	3.6107	0.0709	GG
RHN	mrMLM	S5_1988594	5	1988594	-1.0102	6.2705	7.1125	5.7879	0.3386	GG
RHN	mrMLM	S6_57125681	6	57125681	0.9188	3.2826	3.9953	3.2297	0.4567	AA
RHN	pLARmEB	S6_51320382	6	51320382	1.2706	5.714	6.5371	6.1012	0.1512	CC
RHN	FASTmrMLM	S6_46092732	6	46092732	-1.1418	6.9139	7.7759	8.9147	0.4225	GG
RHN	mrMLM	S6_53726998	6	53726998	-1.4273	8.9971	9.9134	11.3316	0.3228	TT
RAg	pLARmEB	S1_63389970	1	63389970	0.6226	3.7354	4.4734	0.7254	0.345	GG
RAg	pLARmEB	S1_10638882	1	10638882	1.0032	4.7494	5.5352	0.9894	0.1434	TT
RAg	mrMLM	S1_53254066	1	53254066	1.8322	5.3709	6.1815	15.3731	0.3425	CC
RAg	pLARmEB	S2_4366810	2	4366810	-0.712	4.6933	5.4767	0.849	0.345	CC
RAg	pLARmEB	S2_59152339	2	59152339	-1.0319	5.4331	6.246	1.4877	0.2519	AA
RAg	pLARmEB	S2_11561699	2	11561699	-1.7416	8.0794	8.9734	5.0076	0.3682	CT
RAg	FASTmrMLM	S2_11561700	2	11561700	-2.1162	6.6392	7.4929	16.8835	0.376	AG
RAg	mrMLM	S3_69688782	3	69688782	0.8576	4.5299	5.3062	3.2861	0.4213	GG
RAg	ISIS EM-BLASSO	S3_7226868	3	7226868	-1.7141	4.513	5.2886	5.2185	0.0659	GG
RAg	pLARmEB	S3_25956821	3	25956821	-2.6266	8.897	9.811	8.1779	0.093	CC
RAg	mrMLM	S3_18779995	3	18779995	-1.7791	3.3467	4.0632	12.5732	0.1732	AA
RAg	FASTmrEMMA	S4_6185292	4	6185292	1.7892	3.2469	3.9575	4.8263	0.4457	CC
RAg	mrMLM	S4_56610955	4	56610955	-2.8944	5.5832	6.4016	7.9835	0.0472	CC
RAg	pLARmEB	S5_64191285	5	64191285	-1.4295	5.1401	5.9418	1.2476	0.0736	CC
RAg	pLARmEB	S6_53563443	6	53563443	-0.8287	3.9795	4.73	1.2334	0.2248	TT
RAg	pLARmEB	S6_8148250	6	8148250	-1.1417	4.1803	4.9406	2.1203	0.1744	CC
RAg	FASTmrMLM	S6_2724950	6	2724950	-0.8762	3.354	4.0709	3.0268	0.469	TT

Appendix 8.7 (continued)

RAg	ISIS EM-BLASSO	S6_43747319	6	43747319	-2.1803	4.9751	5.7702	12.0154	0.0736	AA
RAg	FASTmrMLM	S8_61744018	8	61744018	-1.2505	3.1418	3.846	7.1533	0.2364	CC
RAg	FASTmrEMMA	S9_53230189	9	53230189	-2.5569	3.366	4.0836	5.3352	0.1512	GG
RAg	mrMLM	S9_48742385	9	48742385	-1.4794	5.9456	6.7768	9.0309	0.2559	GG
RAg	mrMLM	S9_33318596	9	33318596	-2.2239	5.3319	6.141	13.4549	0.0945	TT
StD	mrMLM	S1_67789082	1	67789082	6.254	6.3359	7.18	6.3517	0.4843	GG
StD	FASTmrMLM	S3_73487935	3	73487935	-9.3026	8.0536	8.947	9.829	0.1628	CC
StD	FASTmrMLM	S5_67297121	5	67297121	7.7947	10.3275	11.2725	13.1346	0.4341	AA
StD	mrMLM	S6_46534458	6	46534458	19.2383	5.263	6.0695	20.5619	0.0472	AA
StD	mrMLM	S7_538982	7	538982	-7.3362	5.5468	6.3639	6.0226	0.2638	AA
StD	ISIS EM-BLASSO	S8_59140828	8	59140828	-5.6854	4.3406	5.1084	2.4196	0.2287	CC
StD	FASTmrMLM	S8_3510642	8	3510642	7.3299	6.2759	7.1181	7.3579	0.1705	AA
StD	pLARmEB	S9_5036482	9	5036482	7.8706	4.2503	5.0139	9.4638	0.1434	GG
StD	FASTmrMLM	S10_59303713	10	59303713	4.6017	3.2497	3.9605	2.61	0.1512	TT
StD	mrMLM	S10_24713198	10	24713198	11.9427	3.9838	4.7346	4.1651	0.0394	GG
StD	mrMLM	S10_54033361	10	54033361	-6.8273	3.6258	4.358	7.47	0.378	TT
StD	FASTmrMLM	S10_56910652	10	56910652	11.7232	6.6651	7.5196	8.0662	0.062	CC
RSR	pLARmEB	S1_12562809	1	12562809	3.67E-05	3.4114	4.1316	6.91E-06	0.2868	TC
RSR	ISIS EM-BLASSO	S1_1542460	1	1542460	0.002	6.7005	7.5561	0.0128	0.062	GG
RSR	mrMLM	S1_11452607	1	11452607	0.0225	5.8079	6.6343	0.5001	0.0512	AA
RSR	mrMLM	S1_8816738	1	8816738	-0.024	5.6174	6.437	0.887	0.1063	CC
RSR	mrMLM	S1_1611278	1	1611278	0.0285	4.1924	4.9533	0.8967	0.0394	GG
RSR	ISIS EM-BLASSO	S1_4986552	1	4986552	0.0311	3.8671	4.612	2.757	0.1008	TT
RSR	FASTmrMLM	S1_1542459	1	1542459	0.0332	5.2636	6.0701	3.8306	0.0736	AA
RSR	mrMLM	S1_19714940	1	19714940	0.0526	10.6159	11.5666	4.5704	0.0551	GG
RSR	mrMLM	S1_18242491	1	18242491	0.0704	9.5939	10.5235	9.6619	0.0591	AA
RSR	mrMLM	S2_72394972	2	72394972	-0.0463	6.0029	6.836	3.315	0.0669	CC

Appendix 8.7 (continued)

RSR	mrMLM	S3_11571659	3	11571659	0.0018	3.4444	4.1665	0.007	0.0748	AA
RSR	FASTmrMLM	S3_37081587	3	37081587	0.0036	3.3874	4.1063	0.1117	0.3527	CC
RSR	mrMLM	S3_34232053	3	34232053	-0.0362	5.2711	6.0779	2.5619	0.0748	GG
RSR	mrMLM	S3_51439331	3	51439331	-0.036	10.2044	11.1469	3.5636	0.1181	TT
RSR	ISIS EM-BLASSO	S4_64876108	4	64876108	-7.6E-05	6.4285	7.2756	1.9E-05	0.0581	GG
RSR	mrMLM	S4_40571398	4	40571398	-0.0248	3.8943	4.6406	1.0165	0.0551	TT
RSR	mrMLM	S4_2212556	4	2212556	-0.0296	10.8019	11.7563	1.1659	0.0827	CC
RSR	mrMLM	S4_66242639	4	66242639	0.0476	12.5494	13.5351	3.0125	0.0512	TT
RSR	mrMLM	S4_42052991	4	42052991	0.0692	12.8151	13.8052	7.3876	0.0551	GG
RSR	ISIS EM-BLASSO	S4_60963046	4	60963046	-0.0364	6.8521	7.7122	7.3904	0.1124	GG
RSR	ISIS EM-BLASSO	S5_66970311	5	66970311	-4.1E-05	4.8632	5.6538	8.85E-06	0.1977	TT
RSR	ISIS EM-BLASSO	S5_1691960	5	1691960	-0.0046	3.7625	4.502	0.1692	0.4612	TT
RSR	ISIS EM-BLASSO	S5_16428201	5	16428201	-0.0071	11.4183	12.3843	0.2435	0.0891	CC
RSR	ISIS EM-BLASSO	S5_63258485	5	63258485	-0.0155	5.255	6.0612	0.8782	0.1008	TT
RSR	mrMLM	S5_24044614	5	24044614	0.0453	9.4195	10.3453	5.6274	0.1024	AA
RSR	ISIS EM-BLASSO	S6_59553145	6	59553145	0.0006	4.6149	5.3949	0.0023	0.186	GG
RSR	mrMLM	S7_449331	7	449331	-0.0274	8.9765	9.8923	0.5693	0.0394	AA
RSR	mrMLM	S7_20897911	7	20897911	-0.0122	4.3582	5.1268	0.6111	0.4488	CC
RSR	mrMLM	S7_64024650	7	64024650	0.0504	10.1243	11.0651	10.5309	0.2205	GG
RSR	pLARmEB	S8_41506744	8	41506744	0.0107	4.8235	5.6124	0.2118	0.4767	GA
RSR	mrMLM	S8_49903132	8	49903132	0.0148	5.9964	6.8293	0.4669	0.0827	CC
RSR	mrMLM	S8_9503615	8	9503615	-0.031	4.2923	5.0579	1.8712	0.0709	AA
RSR	mrMLM	S9_56233065	9	56233065	0.0231	4.3118	5.0783	1.4161	0.1732	GG
RSR	FASTmrMLM	S9_55975700	9	55975700	0.0146	4.684	5.467	1.7546	0.3333	CC
RSR	mrMLM	S9_4343604	9	4343604	-0.0755	13.3119	14.31	7.5804	0.0472	GG
RSR	mrMLM	S10_54368363	10	54368363	-0.0161	4.2403	5.0034	0.2874	0.0591	CC
RSR	mrMLM	S10_11370589	10	11370589	0.0359	6.9935	7.8578	2.2561	0.0591	GG

Appendix 8.7 (continued)

SA	mrMLM	S1_74573922	1	74573922	-265.169	4.4536	5.2265	5.2794	0.4252	CC
SA	FASTmrMLM	S1_72242744	1	72242744	392.2143	5.0999	5.9	5.9284	0.1008	CC
SA	FASTmrMLM	S2_69466237	2	69466237	-275.438	5.4258	6.2384	5.0781	0.2752	GG
SA	mrMLM	S2_1298521	2	1298521	343.8756	6.1409	6.9787	7.8925	0.2913	GG
SA	pLARmEB	S3_72882254	3	72882254	-227.81	4.2129	4.9748	2.8253	0.186	AA
SA	mrMLM	S3_60263480	3	60263480	223.3821	3.4106	4.1308	3.7087	0.4331	GG
SA	mrMLM	S3_70595312	3	70595312	-335.354	3.3233	4.0384	8.0745	0.2205	GG
SA	FASTmrMLM	S3_18753032	3	18753032	-411.574	5.2719	6.0787	15.6064	0.2984	TC
SA	mrMLM	S4_2704188	4	2704188	227.0783	3.2471	3.9577	3.8437	0.4488	CC
SA	mrMLM	S4_53597075	4	53597075	-267.051	3.8398	4.5833	4.1575	0.2756	AA
SA	mrMLM	S4_61960378	4	61960378	-373.532	4.1961	4.9572	5.4898	0.1378	GG
SA	FASTmrEMMA	S4_8709338	4	8709338	-564.083	4.1232	4.8808	6.9381	0.3372	CC
SA	ISIS EM-BLASSO	S4_65106437	4	65106437	-436.036	5.5959	6.4148	7.3702	0.1279	GG
SA	pLARmEB	S5_7006538	5	7006538	211.2298	3.5223	4.2488	1.8957	0.1279	GA
SA	ISIS EM-BLASSO	S5_53756439	5	53756439	367.7144	4.678	5.4608	5.5671	0.155	GG
SA	pLARmEB	S5_5827150	5	5827150	-647.664	8.5176	9.4226	10.1474	0.062	GG
SA	FASTmrMLM	S5_5827139	5	5827139	-752.327	8.0916	8.986	13.7763	0.0581	GG
SA	pLARmEB	S6_2711430	6	2711430	-380.479	5.6006	6.4196	9.682	0.1512	TC
SA	mrMLM	S6_903787	6	903787	-515.115	6.7973	7.6558	10.8617	0.1378	CC
SA	mrMLM	S7_2009483	7	2009483	359.5146	8.7879	9.6993	9.3965	0.4331	AA
SA	pLARmEB	S8_1238895	8	1238895	-359.837	4.2103	4.972	3.4166	0.0775	GG
SA	FASTmrMLM	S8_914735	8	914735	-370.96	4.0704	4.8254	7.8153	0.124	CC
SA	pLARmEB	S8_43093465	8	43093465	-364.579	7.6039	8.4854	11.1594	0.3411	CC
SA	mrMLM	S9_1173343	9	1173343	-462.095	6.9858	7.8499	10.0203	0.1535	GG
SA	pLARmEB	S10_57811098	10	57811098	143.1382	3.1757	3.882	1.5402	0.438	GA
SA	pLARmEB	S10_58695241	10	58695241	229.0555	4.152	4.911	2.6003	0.1473	AA
SA	FASTmrMLM	S10_2194407	10	2194407	-204.802	4.0536	4.8078	3.9211	0.4845	GG
SA	FASTmrEMMA	S10_54118313	10	54118313	-499.105	3.8284	4.5713	5.8261	0.4457	GG
SA	mrMLM	S10_24172493	10	24172493	455.7066	5.3209	6.1296	10.0375	0.1339	AA

Appendix 8.8. Details of QTNs significantly associated with 10 phenotypic traits and their functional annotation that mapped into sorghum phytozome.

Trait	SNP.ID	Chr	Position(bp)	r2%	Gene name	Gene pos.(Chr:bp)/location	Annotation
LAg	S2_72741053	2	72,741,053	10.34-13.09	Sobic.002G349100	02:72732829..72738225 reverse	Pyridoxamine 5'-phosphate oxidase
					Sobic.002G349200	02:72739017..72739821 reverse	Ubiquitin-related domain
					Sobic.002G349300	02:72743388..72743740 forward	arabinogalactan peptide 12-related
					Sobic.002G349500	02:72744787..72747479 reverse	Threonine-specific protein kinase.
					Sobic.002G349600	02:72751234..72752561 forward	small ubiquitin-related modifier // subfamily not named
	S4_12354434	4	12,354,434	5.74-11.29	Sobic.004G116200	04:12344377..12348950 reverse	similar to Putative uncharacterized protein
	S7_11107766	7	11,107,766	5.72-14.81	No candidate gene found	-	-
	S9_55485561	9	55,485,561	6.57-9.34	Sobic.009G159500	09:55476511..55477870 forward	GINS complex, subunit Psf1
					Sobic.009G159600	09:55482037..55486664 forward	PRONE (Plant-specific Rop nucleotide exchanger) (PRONE)
					Sobic.009G159700	09:55490595..55493430 reverse	similar to Putative uncharacterized protein
RL	S1_6012717	1	6,012,717	3.87-8.83	Sobic.001G078100	01:6023273..6027052 reverse	molybdopterin cofactor sulfurase mosc // catalytic/ pyridoxal phosphate binding protein
					Sobic.001G077800	01:5998746..6005066 reverse	MYB-like DNA-binding domain, SHAQKYF class family protein, expressed
					Sobic.001G077900	01:6013766..6018597 forward	Os03g0766000 protein, Predicted membrane protein

Appendix 8.8 (continued)

					Sobic.001G078000	01:6018490..6019678 reverse	similar to Putative uncharacterized protein
RL	S4_13304368	4	13,304,368	4.83-15.95	Sobic.004G120200	04:13291740..13296676 forward	sf31 - sentrin/sumo-specific protease
					Sobic.004G120233	04:13304597..13307279 reverse	similar to Putative uncharacterized protein
RL	S4_57625836	4	57,625,836	2.08-6.95	No candidate gene found	-	-
RL	S4_60963038	4	60,963,038	7.73-17.42	Sobic.004G236500	04:60961821..60963634 forward	similar to Os02g0748800 protein,SF82 - glutaredoxin
RL	S6_45176048	6	45,176,048	2.59-6.02	Sobic.006G075100	06:45163610..45170683 forward	similar to OSIGBa0092M08.9 protein,glycosyltransferase
					Sobic.006G075200	06:45169574..45172104 reverse	similar to ALMT1-like protein, aluminum-activated malate transporter 8
NR	S1_6012717	1	6,012,717	2.07-9.54	Pleiotropic marker	-	-
SDW	S7_64332829	7	64,332,829	1.55-5.09	Sobic.007G175600	07:64322926..64324665 forward	auxin-responsive family protein
					Sobic.007G175702	07:64327062..64327998 forward	auxin-responsive family protein
					Sobic.007G175800	07:64329968..64332823 reverse	Predicted K+/H+-antiporter
					Sobic.007G175900	07:64334400..64336961 forward	RING Zinc finger proteins
					Sobic.007G176000	07:64338819..64341324 reverse	mate efflux family protein
RDW	S1_28914756	1	28,914,756	0.002-0.72	Sobic.001G254700	01:28908725..28909920 forward	no functionally defined domain
RDW	S1_6012717	1	6,012,717	0.27-7.21	Pleiotropic marker	-	-
RDW	S2_64672108	2	64,672,108	0.006-1.47	Sobic.002G245100	02:64664610..64666990 forward	similar to Os09g0484800 protein,Pirin gene family
					Sobic.002G245200	02:64667513..64670057 reverse	chitinase-related protein

Appendix 8.8 (continued)

					Sobic.002G245300	02:64673497..64676089 forward	similar to Os09g0484900 protein,sodium-dependent dicarboxylate transporter
					Sobic.002G245400	02:64679372..64684579 forward	similar to Os09g0484900 protein,sodium-dependent dicarboxylate transporter
RDW	S3_62564964	3	62,564,964	0.17-1.52	Sobic.003G226100	03:62558333..62560080 forward	similar to Putative uncharacterized protein
					Sobic.003G226200	03:62566976..62573263 reverse	Sobic.003G226200 - similar to Os01g0624000 protein,neutral ceramidase-related
RDW	S4_13304368	4	13,304,368	0.62-4.69	Sobic.004G120200	04:13291740..13296676 forward	sentrin/sumo-specific protease
RDW	S4_60963038	4	60,963,038	0.00-24.97	Sobic.004G236500	04:60961821..60963634 forward	similar to Os02g0748800 protein,SF82 - glutaredoxin
RDW	S5_19538615	5	19,538,615	0.0002-1.17	No candidate gene found	-	-
RDW	S6_45176048	6	45,176,048	1.61-4.92	Sobic.006G075100	06:45163610..45170683 forward	similar to OSIGBa0092M08.9 protein, glycosyltransferase // subfamily not named
					Sobic.006G075200	06:45169574..45172104 reverse	similar to ALMT1-like protein
RDW	S6_47428336	6	47,428,336	0.08-4.21	Sobic.006G090500	06:47427515..47436892 reverse	similar to Chloride channel,chloride channel // subfamily not named
					Sobic.006G090601	06:47436254..47437155 forward	similar to Putative uncharacterized protein
RHN	S10_53619913	10	53,619,913	6.06-10.14	Sobic.010G181000	10:53616350..53619070 reverse	similar to PPR-repeat protein-like,PPR repeat (PPR)
					Sobic.010G181100	10:53619070..53621791 forward	monooxygenase
					Sobic.010G181150	10:53626150..53626796 forward	Threonine-specific protein kinase

Appendix 8.8 (continued)

					Sobic.010G181200	10:53630723..53633877 forward	similar to Os06g0597800 protein,FAR1 DNA binding domain // Zinc finger, SWIM-type // MULE transposase domain // FHY3/FAR1 family
RHN	S9_56346446	9	56,346,446	4.62-6.56	Sobic.009G170100	09:56349612..56351445 revers	similar to Putative uncharacterized protein
RAg	S1_53254066	1	53,254,066	3.71-15.37	No candidate gene found	-	-
RAg	S3_18779995	3	18,779,995	2.73-12.57	No candidate gene found	-	-
RAg	S4_56610955	4	56,610,955	0.76-7.98	Sobic.004G189500	04:56597055..56597804 reverse	similar to Putative uncharacterized protein
					Sobic.004G189600	04:56600892..56603676 reverse	Phosphopantothenate--cysteine ligase / Phosphopantothenoylcysteine synthetase
RAg	S6_2724950	6	2,724,950	0.60-3.77	No candidate gene found	-	-
RAg	S9_48742385	9	48,742,385	2.28-9.03	Sobic.009G112400	09:48741414..48745643 reverse	F-box domain // Galactose oxidase/kelch, beta-propeller
StD	S10_56910652	10	56,910,652	4.97-8.57	Sobic.010G209450	10:56903049..56904446 forward	with no functionally defined domain,
					Sobic.010G209501	10:56904616..56913224 forward	predicted glycosyltransferase like domains
StD	S8_2624370	8	2,624,370	3.08-7.02	Sobic.008G028600	08:2611079..2613556 forward	WRKY DNA -binding domain (WRKY)
					Sobic.008G028700	08:2616054..2634173 reverse	set domain proteins
RSR	S1_28914756	1	28,914,756	1.22E-05-1.64	Sobic.001G254700	01:28908725..28909920 forward	with no functionally defined domain,
RSR	S1_6012717	1	6,012,717	1.41-6.98	Pleiotropic marker	-	-
RSR	S2_10753898	2	10,753,898	5.04-9.51	No candidate gene found	-	-

Appendix 8.8 (continued)

RSR	S3_62564964	3	62,564,964	0.02-1.67	Sobic.003G226100	03:62558333..62560080 forward	similar to Putative uncharacterized protein
					Sobic.003G226200	03:62566976..62573263 reverse	similar to Os01g0624000 protein,neutral ceramidase (ASAH2)
RSR	S4_817571	4	817,571	2.51-3.76	Sobic.004G009100	04:824808..828607 forward	similar to CGI-144-like protein,protein FAM32A (FAM32A)
					Sobic.004G008700	04:803183..803940 forward	Knottin, scorpion toxin-like
					Sobic.004G008900	04:817516..818772 forward	similar to Acid phosphatase-like
					Sobic.004G009000	04:818795..822243 forward	similar to Putative uncharacterized protein, DEAD/DEAH box helicase domain-containing protein (K06877)
					Sobic.004G008800	04:811075..812448 forward	similar to Acid phosphatase-like
RSR	S9_55975700	9	55,975,700	0.03-1.75	Sobic.009G165800	09:55965454..55969018 reverse	similar to Protein BUD31 homolog 2
					Sobic.009G165900	09:55970528..55973152 reverse	similar to Putative uncharacterized protein, Protein kinase domain // Serine-threonine/tyrosine-protein kinase catalytic domain // Serine/threonine/dual specificity protein kinase, catalytic domain // Protein kinase-like domain // Tyrosine-protein kinase, catalytic domain
SA	S1_72242744	1	72,242,744	3.75-7.67	Sobic.001G395100	01:72233322..72238987 reverse	similar to DEAD-box ATP-dependent RNA helicase 24