GENETIC DIVERSITY AND DROUGHT TOLERANCE IN ETHIOPIAN DURUM WHEAT (*Triticum turgidum subsp. durum*) GENOTYPES

PHD DISSERTATION

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HAWASSA UNIVERSITY, HAWASSA, ETHIOPIA

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A DISSERTATION SUBMITTED TO THE SCHOOL OF PLANT AND HORTICULTURAL SCIENCE, COLLEGE OF AGRICULTURE, SCHOOL OF GRADUATE STUDIES
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ADVISORS' APPROVAL SHEET

This is to certify that the dissertation entitled "***Genetic Diversity and Drought Tolerance in Ethiopian Durum Wheat (T. Turgidum Subsp. durum) Genotypes***" submitted in partial fulfilment of the requirements for the degree of **Doctor of Philosophy (PhD)** with specialization in Plant Biotechnology to the graduate program of the School of Plant and Horticultural Science and has been carried out by **Bantewalu Hailekidan Dukamo ID. No. PhD/PBio/003/10**, under our supervision. Therefore, we recommend that the student has fulfilled the requirements and hence herby can submit the dissertation to the School of Plant and Horticultural Sciences.

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DECLARATION

I hereby declare that this PhD dissertation is my original work and has not been presented for a degree in any other university, and all sources of materials used for this dissertation have been duly acknowledged.

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DEDICATION

This work is dedicated to my mother, Ejigayehu Bekele, and my father, Hailekidan Dukamo, for their unwavering support throughout my studies.

It is also dedicated to the memory of my late uncle, Walelign Bekele, whose encouragement, support, guidance, and belief in me have significantly shaped my journey.

And

To my beloved aunt, Etaferaw Bekele, who has been like a mother and sister to me. Your encouragement, support, and insightful guidance have been my greatest strength.

BIOGRAPHICAL SKETCH

The author was born on August 27, 1989, in Shakiso, Guji Zone, Oromia Regional State, Ethiopia. He attended Hawassa Haik Primary School from 1994 to 2001 for his elementary education. He then attended Hawassa Tabor Comprehensive Secondary and Preparatory School from 2002 to 2005. After taking the Ethiopian Higher Education Entrance Examination in 2005, he joined Hawassa University in 2006 and earned a Bachelor of Science in Applied Biology in 2009. After graduation, he worked as a Graduate Assistant I in the Biology Department at Hawassa University for a year. In 2010, he enrolled at Haramaya University to pursue a Master of Science in Biotechnology, which he completed in 2012. After obtaining his MSc, he returned to Hawassa University, where he served as a lecturer. In 2018, he began his PhD studies in Plant Biotechnology at the School of Plant and Horticultural Science, Hawassa University, culminating in the submission of this dissertation.

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LIST OF ACRONYMS AND ABBREVATIONS

ABA	Abscisic Acid
bHLH	Basic Helix-Loop-Helix Transcription Factors
bZIP	Basic Leucine-Zipper Transcription Factors
CAT	Catalase
CIMMYT	International Maize and Wheat Improvement Center
CTAB	Cetyltrimethylammonium Bromide
DAPC	Discriminant Analysis of Principal Components
DArT	Diversity Arrays Technology
DREB	Dehydration-Responsive Element-Binding
DSI	Drought Susceptibility Index
EBI	Ethiopian Biodiversity Institute
ECV	Environmental Coefficient of Variance
ERF	Ethylene Responsive Factor
FIS	Inbreeding Coefficient
GAM	Genetic Advance as percentage of mean
GBS	Genotyping by Sequencing
GCV	Genotypic Coefficient of Variance
GMP	Geometric Mean Productivity
GST	Glutathione S-Transferase
HD-ZIP	Homeodomain-Leucine Zipper Transcription Factors
HvDRF1	Hordeum vulgare Dehydration Responsive Factor 1
LD	Linkage Disequilibrium
LEA	Late Embryogenesis Abundant
MASL	Meters above sea level
MP	Mean Productivity
NGS	Next-Generation Sequencing
P5CR	Pyrroline-5-Carboxylate Synthetase
PCA	Principal Component Analysis
PCV	Phenotypic Coefficient of Variance
PIC	polymorphic information content
POD	Peroxidase

QTL	Quantitative Trait Loci
RBSB	Root-to-Shoot Biomass Ratio
RLPL	Root-to-Shoot Length
ROS	Reactive Oxygen Species
RSI	Relative Drought Index
RuBisCO	Ribulose Bisphosphate Carboxylase-Oxygenase
SMN	Stomata Number
SMW	Stomata Width
SSI	Stress Susceptibility Index
STI	Stress Tolerance Index
TaDRF1	*Triticum Aestivum* Dehydration Responsive Factor 1
TASSEL	Trait Analysis by aSSociation, Evolution and Linkage
TdDRF1	*Triticum Durum* Dehydration Responsive Factor 1
TOL	Tolerance
UPGMA	Unweighted Pair Group Method of Association
YI	Yield Index
Yp	Grain yield under non-stressed
Ys	Grain yield under stressed
YSI	Yield Stability Index
ZFPs	Zinc Finger Proteins

PAPERS/MANUSCRIPTS TITLES

1. Genetic Diversity of Ethiopian Durum Wheat (T. Turgidum Subsp. Durum) Landraces Under Drought Stressed and Non-Stressed Conditions published as: Dukamo, Bantewalu Hailekidan, Andargachew Gedebo, Bizuayehu Tesfaye, and Degu, Hewan Demissie, 2023. **Genetic diversity of Ethiopian durum wheat (T. turgidum subsp. durum) genotypes under water stressed and non-stressed conditions**. *Heliyon*, 9(7).

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THESIS ABSTRACT

Genetic Diversity and Drought Tolerance in Selected Durum Wheat (T. Turgidum Subsp. Durum) Genotypes of Ethiopia.

Durum wheat (Triticum turgidum subsp. durum), the second most widely cultivated wheat species after common wheat, plays a crucial role in global food security. Ethiopia, recognized as a secondary center of origin and diversity for durum wheat, harbors a broad and unique genetic reservoir well adapted to diverse agroecological conditions. However, productivity remains low due to limited availability of improved, drought-tolerant varieties and insufficient exploitation of genetic diversity. This study aimed to characterize Ethiopian durum wheat landraces, identify drought-tolerant genotypes, and assess their potential for breeding programs by integrating field, greenhouse, and molecular analyses. The research involved three interlinked components. The first was a field experiment conducted at Dera (1500 masl) and Debrezeit (1920 masl), where 104 genotypes (100 landraces and 4 checks) were evaluated under drought-stressed and non-stressed conditions using an augmented design. Thirteen agronomic traits related to yield, phenology, and canopy status were measured across both environments. Additional data were collected from an extended growing season, and drought tolerance was assessed using various indices. ANOVA, correlation, principal component analysis (PCA), and clustering were used to identify promising genotypes and trait associations. The second component involved a greenhouse experiment at Hawassa University using 20 top-performing landraces and four checks selected from the field study. Genotypes were grown under well-watered (70% field capacity) and drought-stressed (35% field capacity) conditions in a completely randomized design with three replications. Data on morphological, physiological, and biochemical traits were collected, including grain yield, relative leaf water content (RLWC), chlorophyll content, canopy temperature, and proline accumulation. Statistical analyses were employed to evaluate drought responses, including correlation, PCA, cluster, and path coefficient analysis. The third component focused on the molecular characterization of 94 genotypes (86 landraces and 8 improved varieties) using SNP markers generated through DArTSeq technology. Genotyping was performed by SEQART AFRICA, producing 17,092 high-quality SNPs. Genetic diversity, population structure, and linkage disequilibrium (LD) were analyzed to assess genome-wide variation and support genotype selection.

Field results revealed significant genetic variation among landraces across all measured traits. Genotypes ETDW/15DZ23, 34493, ETDW/15DZ4, 34522, MCD3-14, 34217, and 31831 demonstrated superior grain yield under stress and non-stressed conditions. High heritability (h^2b = 32.84–97.87) and genetic advance estimates for traits such as spike length, kernel number per spike, and tiller number indicated strong potential for selection. Drought indices, including stress tolerance index (STI), mean productivity (MP), and yield stability index (YSI), identified ETDW/15DZ23, 34493, and ETDW/15DZ4 as top-performing, drought-resilient genotypes. Strong positive correlations (r = 0.88) between grain yields under stressed and non-stressed conditions further confirmed their stability and

adaptability. The greenhouse experiment revealed significant effects of genotype, treatment, and their interaction ($P<0.001$) for most traits. Under stress, grain yield decreased by up to 68%, RLWC dropped from 93.07% to 44.91%, and proline content increased markedly, indicating drought response. Cluster analysis grouped genotypes based on resilience, with one cluster showing the highest yield (5.99 t/ha) under well-watered conditions, while another showed superior RLWC (65.80%) and yield (2.90 t/ha) under stress. Path analysis underscored the importance of RLWC, proline, and chlorophyll content in drought tolerance. Molecular analysis revealed 14,136 informative SNPs distributed across the A and B genomes, with chromosome 2B having the highest marker density. Population structure analysis indicated considerable variation within and among landraces, with AMOVA showing 51.75% of genetic variation within populations and 48.15% within individuals. The genome-wide LD decay threshold was 4.58 Mbp, with the highest LD values on chromosome 4B. Average polymorphic information content (PIC) and gene diversity values were moderate, indicating a diverse and informative marker set for future breeding applications. This study highlights the significant phenotypic and genotypic diversity within Ethiopian durum wheat and identifies promising genotypes for drought tolerance and yield stability. The integration of field performance, physiological traits, and genomic data provides a robust platform for developing improved durum wheat cultivars. These findings support the use of landraces in breeding programs targeting climate resilience and food security. Future work should focus on multi-environment trials, genome-wide association studies (GWAS), and marker-assisted selection to accelerate genetic improvement and enhance drought tolerance in Ethiopian durum wheat.

Key words: Augmented design; Drought tolerance; Ethiopian durum wheat; Genetic diversity; Grain yield; Greenhouse; Landraces; linkage disequilibrium; Non-stressed condition; physiological trait; population structure; proline content; SNP markers; Stress indices; stress-adaptative; Stressed condition.

CHAPTER ONE

1. GENERAL INTRODUCTION

1.1. Background and Justification

The wheat genus, *Triticum*, is a member of the Poaceae family, also known as the grass family. It comprises many species, including two major wheat species essential for human nutrition: bread wheat (*Triticum aestivum*) and durum wheat (*Triticum turgidum subsp. durum* or *Triticum durum Desf.*) (Gustafson et al., 2009). These two species share some similarities but differ in their genetic (ploidy) and agronomical characteristics. Durum wheat has an allotetraploid genome (2n = 4x = 28 chromosomes), with an estimated genome size of approximately 12.9 to 13 (Gb) and with the formula AABB, while bread wheat has an allohexaploid genome (2n=6x=42 chromosomes) with an estimated genome size of approximately 16 (Gb) and with the formula AABBDD (Ganal and Röder, 2007, De Vita and Taranto, 2019).

Durum wheat is an important cereal crop in Ethiopia, predominantly grown in the highland regions. The major durum wheat-growing regions include Oromia, Amhara, Tigray, and parts of the former Southern Nations, Nationalities, and Peoples' Region (Dibaba, 2019). These regions are characterized by altitudes ranging from 1800 to 3000 meters above sea level, with optimal growth occurring between 2000 and 2500 meters (Hurni, 1998). The ecological conditions suitable for durum wheat production in Ethiopia include moderate to high rainfall (500-1500 mm annually), cool temperatures during the growing season, and fertile vertisols and luvisols.

Durum wheat, a tetraploid cereal grain derived from the domestication of emmer wheat (Triticum turgidum spp. dicoccum), is primarily used for producing pasta, semolina, and other traditional foods (Tuberosa et al., 2014, Maccaferri et al., 2019). It accounts for only 5–10% of global wheat production, averaging 34.3 million tons annually from 2018 to 2022, and covers about 18 million hectares or 8–10% of the world’s wheat-growing area (Blanco, 2024, Grosse-Heilmann et al., 2024). Compared to bread wheat, its cultivation is limited due to several factors, including narrower end-use applications, lower yield potential, and greater sensitivity to environmental stresses (FAOSTAT, 2019, Tadesse et al., 2016) Additionally, global breeding and policy efforts have traditionally prioritized bread wheat because of its

critical role in food security, resulting in more research funding, technological advances, and adoption. In countries like Ethiopia, the durum wheat market remains constrained by limited industrial demand and underdeveloped processing infrastructure, further contributing to its lower production and area coverage despite its genetic value and nutritional importance (CSA/ESS, 2021).

Durum wheat primary center of origin is the Mediterranean region, it has a diverse genetic background extending to other regions (Olmo and Simmonds, 1976). Archaeological evidence suggests that naked emmer wheat reached Ethiopia around 5000 BC, leading Vavilov to propose Ethiopia as a center of origin and diversity for tetraploid wheat (Vavilov, 1951, Vavilov, 1996). However, this hypothesis has faced challenges, with some studies questioning the lack of wild relatives and archaeological evidence in Ethiopia, suggesting it might be a secondary center of origin instead (Engels and Hawkes, 1991). Ethiopian wheat exhibits unique traits compared to global varieties, potentially due to its historical isolation and distinctive agricultural practices (Vavilov, 1951, Kabbaj et al., 2017). Ethiopia remains a valuable reservoir of genetic diversity in durum wheat, significantly contributing to global improvement efforts (Mengistu et al., 2018, Kidane et al., 2019, Alemu et al., 2020c, Mekonnen et al., 2021).

Durum wheat cultivated by smallholder farmers under rain-fed conditions in the highlands, where fertile vertisols and favorable climatic conditions prevail (Dibaba, 2019).. The main production areas include Arsi, Bale, and parts of Shewa, located within Ethiopia's central and southeastern wheat belt, which offers altitudes between 1800 and 3000 meters above sea level, optimal for durum wheat growth (Taffesse et al., 2012, Tadesse et al., 2022). Although a small portion of durum wheat is also grown under irrigation during the off-season in lower elevation areas, the bulk of production occurs during the main rainy season (meher), typically from June to December (Hailu, 2011). Ethiopia's unique agroecology, including bimodal rainfall patterns in areas like Bale Zone, supports diverse sowing and harvesting windows for durum wheat (Gizaw and Assegid, 2021). The crop's cultivation has doubled over the past decade, making Ethiopia the leading wheat producer in Sub-Saharan Africa (Tadesse et al., 2022). Durum wheat covers an estimated 660,000 hectares of Ethiopia's 1.67 million hectares of total wheat area (WorldBank, 2018, Usman et al., 2018).

Even if it is cultivated in various agroclimatic environments, many of which face drought stress as a major constraint to production and productivity (Dukamo et al., 2023). Climate change poses a significant threat, with predictions estimating a 6% reduction in yield per degree Celsius increase in temperature equivalent to a quarter of the global wheat trade (Asseng et al., 2017). Water scarcity is the primary yield-limiting factor for crops. In Ethiopia, where rain-fed agriculture is the dominant practice, drought stress is a leading cause of yield reduction in durum wheat. Developing drought-tolerant wheat varieties with adaptive traits is critical for improving productivity and ensuring food security (Nevo and Chen, 2010). The adoption of drought-resilient genotypes remains one of the most sustainable strategies to mitigate the effects of marginal rainfall and prolonged dry spells (Mir et al., 2012, Mwadzingeni et al., 2016b).

In Ethiopia, durum wheat breeding programs capitalize on the country's extensive genetic diversity to develop high-yielding and stress-resilient varieties (Shumeta, 2024). The development of varieties like Ude and Mangudo, known for their drought tolerance and disease resistance, exemplifies the success of such initiatives (Tola and Alemu, 2024). Selection among local landraces for key traits such as drought tolerance and stem rust resistance are commonly practiced, often using participatory breeding approaches that engage farmers directly in the selection process. This participatory strategy enhances local adaptability while encouraging the adoption of improved cultivars (Weltzien and Christinck, 2017).

Drought stress is one of the most critical abiotic constraints affecting durum wheat production globally, and its impact is particularly severe in Sub-Saharan Africa due to inherently low soil moisture retention and erratic rainfall patterns (Pask and Reynolds, 2013, Xu et al., 2013). In this region, over 60% of soils have limited water-holding capacity, exacerbating the effects of drought on crop growth and yield. Drought affects multiple plant functions, morphological, physiological, and biochemical, leading to reduced photosynthesis, stunted growth, and in severe cases, irreversible tissue damage (Sangtarash, 2010). These responses vary depending on the plant's developmental stage, the intensity and duration of the stress, and the underlying genetic makeup (Nanda et al., 2018). In durum wheat, understanding these stress responses is essential for identifying adaptive traits and selecting genotypes capable of maintaining productivity under water-limited conditions.

Ethiopian durum wheat landraces offer a rich source of drought-resilient genotypes due to their long-term adaptation to diverse and often harsh agroecological zones. Studies by Alemu et al. (2020a) and Kabbaj et al. (2017) demonstrate that these landraces consistently outperform modern cultivars in drought-prone environments. Their superior performance is attributed to adaptive traits such as early flowering, deep rooting, and high chlorophyll retention. Recent efforts to quantify drought tolerance using yield-based indices like the stress tolerance index (STI), mean productivity (MP), and geometric mean productivity (GMP) have proven effective in identifying genotypes with high yield stability (Pour-Aboughadareh et al., 2019, Kumar et al., 2023). Integrating these indices with physiological markers and molecular tools further enhances selection precision, enabling breeders to target resilient lines more effectively.

Beyond yield, Ethiopian landraces also exhibit valuable physiological and biochemical adaptations to drought, including better maintenance of relative leaf water content and elevated proline accumulation, both linked to improved stress tolerance (Negisho et al., 2021, Mulugeta et al., 2023). These traits not only reflect a genotype's ability to withstand water deficit but also contribute to the conservation of genetic diversity under climate-induced stresses. Since drought tolerance is a complex trait governed by multiple genes, evaluating landraces under both stressed and non-stressed conditions provides critical insight into their resilience mechanisms. This approach helps breeders identify and utilize adaptive traits in developing climate-resilient durum wheat varieties, contributing to food security and sustainable agriculture in drought-prone regions.

Several recent studies have demonstrated the relevance of agromorphological, physiological, and biochemical traits in evaluating drought tolerance among Ethiopian durum wheat landraces. Semahegn et al. (2020) and Mengistu et al. (2018) evaluated Ethiopian durum wheat genotypes under moisture-stressed and non-stressed conditions, reporting significant genotypic variability in traits such as plant height, biomass, and grain yield. These studies showed that early flowering and moderate plant height contributed to drought escape and yield stability in water-limited environments. Similarly, physiological traits like chlorophyll content and relative leaf water content (RLWC) have been associated with enhanced drought resilience, as seen in the work of (Cowley and Luckett, 2011, Javed et al., 2022), who found

that genotypes maintaining higher RLWC and chlorophyll under drought exhibited better grain filling and yield. These findings confirm that selecting for such traits can effectively discriminate drought-tolerant landraces adapted to different agroecological conditions in Ethiopia.

Moreover, yield-based drought indices have been widely applied to screen and select promising durum wheat genotypes under stress. Semahegn et al. (2020) utilized indices such as stress tolerance index (STI), mean productivity (MP), and geometric mean productivity (GMP) to identify Ethiopian landraces that combined high yield with drought resilience. Their results indicated that these indices effectively distinguished genotypes with stable yield across environments. Similarly, studies by Kumar et al. (2023) and Tadesse et al. (2022) emphasized that combining yield data with biochemical traits like proline accumulation, found to correlate positively with drought tolerance, provides a comprehensive approach for selecting resilient genotypes. These targeted findings support integrating agromorphological, physiological, and biochemical parameters for identifying and improving drought-tolerant Ethiopian durum wheat lines.

Recent studies have emphasized the critical role of Ethiopian durum wheat landraces as reservoirs of genetic diversity, offering valuable alleles for breeding programs aimed at improving stress tolerance and yield stability. Fufa et al. (2024), employed DArTSeq markers to analyze the genetic diversity and population structure of Ethiopian durum wheat, revealing high within-population diversity and moderate differentiation between populations. These findings were attributed to traditional seed exchange and localized adaptation, supporting the idea that Ethiopian landraces have undergone significant gene flow across regions. Earlier SSR-based studies by Dagnaw et al. (2022) and Asmamaw et al. (2019), also documented considerable diversity across agroecological zones, although their marker systems lacked the resolution offered by DArTSeq. Deres and Feyissa (2023), demonstrated the efficiency of DArTSeq in detecting genome-wide polymorphisms and uncovering hidden genetic structures, reinforcing its applicability for high-resolution diversity studies in durum wheat.

Further genetic diversity metrics from studies using DArTSeq, such as polymorphism information content (PIC), minor allele frequency (MAF), and Nei’s gene diversity, have

confirmed the richness of Ethiopian durum wheat gene pools (Deres and Feyissa, 2023, Fufa et al., 2024). These results are consistent with findings by Mengistu et al. (2016), who observed significant allelic variation among landraces using SNP-based platforms. Moreover, population structure and AMOVA analyses from these studies indicate that most genetic variation exists within rather than between populations, suggesting a broad allelic base preserved through decentralized farming systems. These insights align closely with the goals of current breeding strategies that seek to harness native genetic diversity for developing resilient cultivars.

Ethiopia is among the countries most vulnerable to the impacts of climate change, population growth, and food insecurity. The country's population is projected to continue rising rapidly, intensifying the demand for food and placing pressure on agricultural systems (Adhikari et al., 2015, Lobell and Gourdji, 2012). Compounding this challenge, climate change is increasing the frequency and severity of droughts, especially in rainfed agricultural areas, where the majority of Ethiopian farmers operate (Bodner et al., 2015, Senapati et al., 2019). These changes are expected to significantly reduce crop yields by 2050, worsening food insecurity and raising food prices (De Vita and Taranto, 2019). Addressing these issues requires urgent action to improve crop productivity and resilience through the development of climate-smart, high-yielding cultivars. This, in turn, depends on identifying and utilizing genetically diverse germplasm, especially landraces adapted to local agroecological conditions (Mir et al., 2012, Senapati et al., 2019).

Ethiopia is globally recognized as a center of diversity for tetraploid wheat, including durum wheat, which is valued for its broad range of agro-morphological traits and quality characteristics (Dagnaw et al., 2022, Dinsa, 2023, Dukamo et al., 2023). Despite being the largest wheat producer in sub-Saharan Africa, Ethiopia remains a net wheat importer due to a persistent yield gap—the national average is around 3 tons per hectare, far below the potential 6–8 tons (CSA/ESS, 2021). In response, the Ethiopian government launched a 15-year wheat research strategy (2016–2030) focused on yield improvement, disease resistance, and grain quality. However, durum wheat production continues to face significant biotic and abiotic challenges (Dagnaw et al., 2023, Geleta and Grausgruber, 2013, Haile et al., 2013). The growing replacement of traditional landraces with modern high-yielding varieties raises concerns about genetic erosion, especially the loss of drought-adaptive traits. Without strong

conservation strategies, these valuable genetic resources risk being permanently lost, undercutting future breeding efforts. Immediate documentation, conservation, and utilization of Ethiopia's durum wheat landrace diversity are therefore critical.

Despite advances in wheat improvement, breeding efforts have largely concentrated on bread wheat, with limited attention given to durum wheat, particularly in Ethiopia, where it holds both cultural and agronomic significance. Although previous studies have highlighted the genetic diversity within Ethiopian durum wheat landraces (Alemu et al., 2020c, Kabbaj et al., 2017), there is a notable gap in research specifically addressing their drought tolerance potential. Existing work has primarily relied on yield-based indices (Semahegn et al., 2020), often neglecting a comprehensive evaluation that integrates agro-morphological, physiological, and biochemical traits. Additionally, the phenotypic and genotypic variability of these traits under contrasting moisture conditions remains poorly understood, limiting the identification of heritable drought-resilience characteristics (Kumar et al., 2023, Tadesse et al., 2022). To address these gaps, this study conducted a multi-trait assessment of Ethiopian durum wheat landraces under both drought-stressed and non-stressed conditions, combining analyses of agro-morphological traits, physiological responses, biochemical, and drought tolerance indices. The objective was to identify high-performing genotypes with stable drought tolerance and inform effective selection strategies for breeding. Furthermore, molecular characterization using genome-wide DArTSeq based SNP markers was employed to assess genetic diversity and population structure, providing a foundation for future genomics-assisted improvement of durum wheat.

1.2. Objectives

1.2.1 General objective:

To contribute towards knowledge and understanding of durum wheat genetic variation in response to drought with a view to enhance its productivity and sustainability in Ethiopia.

1.2.2 Specific Objectives:

1. To investigate the genetic diversity of durum wheat landraces in response to drought stress.

2. To evaluate the drought stress tolerance of durum wheat using yield-based drought tolerance indices.

3. To study the stress-adaptive traits expression of durum wheat genotypes under greenhouse conditions.

4. To analyze the genetic diversity and population structure of durum wheat genotypes using DArTSeq-based SNP markers.

1.3. Research Questions

This study aimed to answer the following research questions

1. How do Ethiopian durum wheat landraces perform in terms of grain yield and agronomic traits under non-stressed and drought-stressed conditions? Which physiological traits, such as chlorophyll content and relative leaf water content, contribute most significantly to drought tolerance?

2. Which durum wheat landraces demonstrate superior performance based on stress tolerance indices (e.g., STI, MP, GMP, HM)? Are there significant correlations between stress tolerance indices and grain yield under drought-stressed conditions?

3. What is the extent of genetic diversity and population structure within Ethiopian durum wheat landraces based on SNP markers? How does the genetic diversity vary among different chromosomal groups and genomic regions?

1.4. Research Hypotheses

Ethiopian durum wheat landraces exhibit significant variation in drought tolerance, with specific genotypes demonstrating superior performance in stress tolerance indices, physiological traits, and grain yield stability under drought conditions.

CHAPTER TWO
2. LITERATURE REVIEW

2.1. Taxonomy and Botany of the Genus *Triticum*

The genus *Triticum*, commonly known as wheat, belongs to the family Graminaceae (Poaceae), encompassing a diverse array of species. Among these, bread wheat (*Triticum aestivum*) and durum wheat (*Triticum turgidum*, also known as *Triticum durum Desf*.) stand out as major crops essential to the human diet (Gustafson et al., 2009). Despite their numerous similarities, these two species exhibit distinct genetic (ploidy) and agronomical characteristics. Durum wheat is allotetraploid with a ploidy of 2n = 4x = 28 chromosomes and a genomic formula of AABB, while bread wheat is allohexaploid with a ploidy of 2n = 6x = 42 chromosomes and a genomic formula of AABBDD, both possessing large and intricate genomes (Ganal and Röder, 2007, De Vita and Taranto, 2019).

Extensive research has explored the phylogenetic relationships among wild and cultivated wheat species. *Triticum* species are often highly interfertile, and the evolutionary journey of cultivated wheat has witnessed numerous interspecific hybridization events (Monneveux et al., 2012). The cultivation of wheat species began approximately 10,000 to 8,500 years ago through a series of interspecific hybridizations, followed by spontaneous chromosome doublings, leading to the emergence of fertile polyploid progeny characterized by bivalent chromosome pairing during meiosis (Olmo and Simmonds, 1976, Provan et al., 2004, Salse et al., 2008).

Several studies indicate a close similarity between the B genome in *T. turgidum* and *T. aestivum* and the S genome in section *Sitopsis* (Salse et al., 2008, Huang et al., 2010). Thus, *Sitopsis* species have often been proposed as the B genome donor to polyploid wheat. Cytoplasmic studies have further supported this notion, a similarity between the cytoplasm of *T. aestivum* and the S-type cytoplasm of five *Sitopsis* species of *Aegilops* (Provan et al., 2004). However, detailed genetic comparisons suggest that the evolutionary relationship between the B genome and the S genome of *Ae. speltoides* is not as close as that between the A genome of polyploid wheat species and its identified progenitor, *T. Urartu* (Salse et al., 2008). Despite extensive research, the identity of the B genome donor remains an open question. While evidence points to the *Sitopsis* section of the genus *Aegilops* as the most

probable source, none of the known species within this group possesses all the properties of the B genome (Hao et al., 2020). Therefore, a composite of them is considered the most likely scenario. It has been proposed that the B genome may originate from the SS genome of an *Aegilops* species belonging to the *Sitopsis* section, analogous to present-day *Aegilops speltoides* (Sarkar and Stebbins, 1956). The D genome of bread wheat and *Triticum spelta* is thought to result from a separate hybridization event between an ancestor of the diploid *Aegilops tauschii var. strangulate* DD genome and an *allotetraploid* wheat (McFadden and Sears, 1946).

2.2 Durum Wheat Domestication and Cultivation

The genetic origins of wheat are of particular interest due to its unique polyploid nature, with species of *Triticum* and their close relatives categorized into diploid, tetraploid, and hexaploid groups (Colomba and Gregorini, 2011). Durum wheat is believed to have originated through hybridization between the wild diploid *T. monococcum L*. (A genome donor) and the B genome donor, likely *T. speltoides*, has a long history dating back thousands of years in South Western Asia (Sarkar and Stebbins, 1956, Hao et al., 2020). This region, encompassing present-day Turkey, Syria, Iraq, and Iran, has been cultivating durum wheat for over 10,000 years (Colomba and Gregorini, 2011). The domestication of durum wheat occurred between 8,000 and 12,000 years ago in South West Asia and the Abyssinian region, with the Tigris-Euphrates Valley being the site of the earliest recorded domesticated durum wheat (Sarkar and Stebbins, 1956, Olmo and Simmonds, 1976, Vavilov, 1996). Archaeological evidence suggests that wheat, possibly used in a parched form, has been a staple food since prehistoric times. Today, durum wheat stands as one of the world's most significant crops, extensively cultivated in the arid and semiarid regions of West Asia and North Africa (Al-Karaki, 2012).

Ethiopia is recognized as one of the centers of genetic diversity for tetraploid durum wheat, although recent debates have arisen regarding its status as a primary or secondary center of origin (Vavilov, 1951, Engels and Hawkes, 1991, Tidiane Sall et al., 2019). The diverse agroecology of Ethiopia provides favorable conditions for wheat cultivation, particularly in the highlands at altitudes ranging from 1500 to 3200 meters above sea level, with optimal areas falling between 1900 and 2700 meters above sea level and annual rainfall ranging from 600 to 2000 mm (Hailu, 2011). Statistics from the Central Statistical Agency (CSA/ESS) for

the year 2020/21 indicate that cereals, including tef, maize, sorghum, and wheat, occupy a significant portion of Ethiopia's grain crop area, with durum wheat productivity averaging less than one ton per hectare due to various factors such as limited use of high-yielding improved genotypes, suboptimal production practices, and low fertilizer application rates (Zewdu et al., 2021, Dagnaw et al., 2022).

The domestication process of wheat, potentially initiated between 18,000 and 12,000 BC in the Middle East and Abyssinian region, marked a critical phase in human history (Gustafson et al., 2009, Hailu, 2011, Maccaferri et al., 2019). This domestication process has led to significant morphophysiological changes in wheat, with domesticated varieties differing from their wild progenitors. Notably, one of the earliest and most pivotal modifications in wheat domestication was the acquisition of a non-brittle rachis, which facilitated efficient grain harvesting by preventing premature seed dispersal (Harlan et al., 1973). This process, accompanied by other alterations such as larger seeds, loss of seed dormancy, enhanced grain quality, growth habit, size, coloration, and edibility of economically important organs, led to the development of domesticated forms that relied on human cultivation and enabled large-scale mechanized farming. Modern wheat cultivars primarily encompass hexaploid bread wheat, and tetraploid durum wheat (Gustafson et al., 2009).

2.3 Phenology of Durum Wheat

Understanding the development of durum wheat is crucial for evaluating potential environmental constraints, especially water deficit. Typically, they require between 450 to 650 mm of water throughout their entire growth cycle, which spans approximately 95 to 125 days. In response to drought stress, plants close their stomata to prevent dehydration, leading to reduced transpiration rates (Bodner et al., 2015, Sarto et al., 2017). Temperature plays a pivotal role in driving phenological stages in plants. The accumulation of heat units is commonly used to determine key developmental stages such as flowering and maturity in various field crops (Al-Karaki, 2012). Different phenological stages exhibit varying degrees of sensitivity to drought and high-temperature stress, influenced by both species and genotype variability (Sarto et al., 2017).

Three critical periods significantly affect wheat crop development under drought conditions: floral initiation and inflorescence development, anthesis and fertilization, and grain

formation(Sarto et al., 2017). Each stage of wheat development has specific environmental requirements, particularly regarding water and air temperature. From tillering to physiological maturity, distinct stages such as stem elongation, booting, heading, flowering, and grain filling exhibit unique sensitivities to environmental factors (Khadka et al., 2020). The flag leaf and flowering stages are particularly sensitive to drought stress, while the milk-grain stage shows lower sensitivity. Prolonged water limitation can shorten the growth cycle, impacting emergence, heading, and physiological ripening stages. Both insufficient and excessive rainfall can negatively impact wheat quality and yield (Prasad et al., 2008, Al-Karaki, 2012). Water deficiency during these periods, particularly 15 days before and 5 days after heading, leads to significant reductions in grain yield. The closure of stomata during drought stress enhances water-use efficiency, although the photosynthetic rate may be less responsive (Sarto et al., 2017).

2.4. Agroecological Requirements and Major Wheat-Producing Areas in Ethiopia

Wheat, including both durum and bread wheat, is predominantly cultivated in Ethiopia's highland regions between 1900 and 2700 meters above sea level, where cool temperatures and moderate to high rainfall create favorable conditions for growth (Hurni, 1998, Kabbaj et al., 2017, Mengistu et al., 2018). Durum wheat, locally known as yekoticha sinde or "wheat of the heavy black soils," is typically grown on black swelling vertisols, soils that retain moisture but are prone to waterlogging (Pask and Reynolds, 2013, Habtamu et al., 2016). The crop is mainly cultivated by smallholder farmers across central, northwestern, and northeastern Ethiopia. Major wheat-producing areas include Arsi, Bale, Shewa, Gojam, Gonder, Wello, Tigray, Harerge, Hadiya, and Kembata (Geleta and Grausgruber, 2013, Gizaw and Assegid, 2021, Taffesse et al., 2012). These regions vary in microclimates and elevation, contributing to the country's rich agroecological diversity for wheat cultivation.

Although Ethiopia has significant potential for wheat production, yields remain below the global average due to traditional farming systems, limited input use, and vulnerability to climate-induced stresses (Gizaw and Assegid, 2021, Dibaba, 2019). Simulation studies estimate that up to 6.5 million hectares of land in Ethiopia are suitable for wheat under rain-fed conditions (Hurni, 1998, Minot et al., 2015). However, national breeding programs have historically focused on high-moisture environments, with limited attention to developing varieties adapted to drought-prone and heat-stressed regions. This gap is partly due to a lack

of screening tools and understanding of stress-resilient traits in local conditions. Expanding wheat production into underutilized areas using improved, stress-tolerant varieties could boost national productivity and strengthen resilience to climate change.

2.5 Production and Importance of Wheat

Wheat plays a crucial role in the daily diet of Ethiopians, contributing significantly to calorie and protein intake. It provides an optimal balance of energy, protein, calcium, and iron, with chemical composition values. Wheat's high protein content and iodine levels, surpassing those of other cereals, as its nutritional value and potential contribution to addressing dietary deficiencies (Nadeem et al., 2021, Chaudhary et al., 2023). While durum wheat comprises only a small portion, roughly 5% to 8%, of global wheat production, its significance is profound due to its unique characteristics and its role in the production of essential food items like pasta (Dibaba, 2019). Despite the stabilization of per capita pasta consumption in recent years, the utilization of durum wheat, encompassing both food and feed applications, has been steadily increasing over the last four years (Taffesse et al., 2012, Gizaw and Assegid, 2021, Tadesse et al., 2022)

Beyond pasta production, durum wheat finds application in various food products such as puffed cereals, hot cereals, desserts, and fillers for pastries. Usage patterns vary across regions, with European and American nations predominantly utilizing durum wheat for pasta, while in the Middle East and North Africa, it is divided between local breadmaking and pasta production. Additionally, in regions like the Mediterranean and South Italy, durum wheat contributes to the formulation of various bread types (Nezhadahmadi et al., 2013, Adhikari et al., 2015).

2.6. Durum Wheat Production Constraints

Ethiopia's durum wheat yields face challenging water-related constraints, primarily drought, significantly affecting major wheat-growing regions, due to heavy reliance on rain-fed agriculture (Taffesse et al., 2012, Tadesse et al., 2022). Waterlogging in vertisols exacerbates these challenges, leading to yield reduction and plant damage. With climate change expected to worsen these events, adaptation strategies are imperative for the sustainability of durum wheat production in Ethiopia (Shiferaw et al., 2013) Changing weather patterns disrupt

traditional farming practices, often leaving farmers vulnerable to waterlogging in some areas and water scarcity in others, both detrimental to yield and grain quality (Adhikari et al., 2015). The reliance on low-input farming practices intensifies the susceptibility of Ethiopian durum wheat to such environmental variability.

Ethiopian durum wheat farming also faces a range of biotic stresses, with diseases like rust posing significant threats and causing considerable losses (Degete and Chala, 2019). The emergence of new, virulent pathogen strains shows the urgency for developing resistant varieties. Insect pests, particularly the Wheat Aphid, compound these challenges, prompting farmers to resort to costly chemical solutions or suffer reduced production due to the unavailability of resistant varieties (Letta et al., 2013, Yu et al., 2017). The diverse agroecological landscape of Ethiopia presents region-specific challenges, with high-altitude areas susceptible to frost damage and low-lying areas experiencing drought stress, both adversely impacting yields (Amare et al., 2019, Tadesse et al., 2022). Additionally, soil degradation, a widespread issue, further diminishes yields, reflecting the pressing need for sustainable soil management practices (Pask and Reynolds, 2013).

Nutrient deficiency, particularly nitrogen and phosphorus, poses a significant barrier to durum wheat production in Ethiopia, limiting plant growth and yields (Sarker et al., 2017). The high cost and limited accessibility of fertilizers exacerbate this issue, necessitating the development of fertilizer-efficient varieties and improving fertilizer access for resource-poor farmers (Abdulkadir et al., 2017) . Pests and diseases, such as rusts and *Septoria tritici blotch*, persist as challenging biotic threats, outpacing the development of resistant durum wheat varieties and leading to extensive crop losses (Mekonnen et al., 2021). This emphasizes the urgency for increased genetic diversity in breeding programs to enhance resilience against emerging pathogens (Negassa, 1986, Blum, 2010, Mwadzingeni et al., 2016a, De Vita and Taranto, 2019, Kidane et al., 2019, Tadesse et al., 2022).

Traditional farming practices and limited access to improved technologies present unique challenges to durum wheat production in Ethiopia. Suboptimal land preparation practices, often reliant on low-efficiency tools, hamper optimal planting times and contribute to delayed or loss of yield (Adhikari et al., 2015, Faysal et al., 2022). Weed management remains laborious and ineffective, further compounded by limited access to improved seeds

and modern agricultural machinery, hindering farmers' ability to maximize yield potential (Shiferaw et al., 2013). Moreover, low adoption of new technologies, high input costs, and inadequate infrastructure and marketing systems further impede productivity, to show the multifaceted challenges faced by Ethiopian durum wheat farmers in achieving sustainable production (Shiferaw et al., 2013).

2.7. Genetic Diversity and Variability

Genetic variability, a fundamental concept in evolutionary biology and agricultural science, refers to the range of genetic differences within a population or species (Fellahi et al., 2013, Bayisa et al., 2020). Often confused with genetic diversity, variability specifically relates to variations in alleles of genes or DNA/RNA sequences within a gene pool. Simply put, it represents the assortment of different forms in phenotype. Genetic diversity, on the other hand, encompasses the overall variability among various genotypes within or between species. This distinction is crucial for understanding population genetics and breeding strategies (Bayisa et al., 2020, Dave et al., 2021).

Throughout history, natural variability and divergence among crop species have been essential resources for agricultural progress. They provide the raw materials for systematic plant breeding, allowing for the improvement of desirable traits and adaptation to changing environments (Abinasa et al., 2011, Colomba and Gregorini, 2011, Dragov et al., 2022). However, over time, these natural sources of genetic variability have been depleted due to selective breeding practices, reliance on a limited number of genotypes, and the introduction of genetically similar cultivars across different regions (Haile et al., 2013, Negisho et al., 2021, Rani et al., 2024). This reduction in genetic diversity poses significant challenges to sustainable agriculture, making crops more vulnerable to pests, diseases, and environmental stresses (Adhikari et al., 2015, Khalili et al., 2018).

The importance of genetic variability in plant breeding cannot be overstated. It serves as the basis for breeding programs, enabling the selection and development of superior crop varieties (De Vita and Taranto, 2019). Proper management of genetic diversity allows for the continuous improvement of plant performance and resilience to changing environmental conditions (Mwadzingeni et al., 2016a, Kidane et al., 2019, Pandurangan et al., 2021). The significance of preserving genetic variability is evident in historical agricultural crises, such

as the potato blight epidemic in Ireland and the corn leaf blight in the United States, which devastated crops due to genetic uniformity. . The success of selection efforts depends on the extent of genetic variability present within plant populations (Farshadfar and Sutka, 2006).

Assessing genetic variability requires the use of various measures to quantify the extent of genetic differences within populations. Common metrics include gene diversity, the number of alleles per locus, the percentage of polymorphic loci, and allele frequencies (Alemu et al., 2020a, Faysal et al., 2022). In the context of Ethiopian tetraploid wheat diversity, studies have highlighted desirable traits such as beardless or half-bearded hard durum wheat, resistance to biotic stresses like rusts and Fusarium head blight, tolerance to abiotic stresses such as drought and heat, and nutritional qualities such as high protein content and micronutrient richness (Amare et al., 2019, Degete and Chala, 2019, Mekonnen et al., 2021). Ethiopia is renowned for its diverse durum wheat landraces, which contribute to the country's agricultural heritage. Durum wheat, a tetraploid wheat species known for its high protein content and gluten strength, encompasses a wide range of landrace varieties adapted to diverse agroecological zones (Taffesse et al., 2012, Bodner et al., 2015, Gizaw and Assegid, 2021). These landraces exhibit unique traits and adaptations, reflecting centuries of farmer selection and environmental pressures. From the rugged highlands to the lowland plains, Ethiopian durum wheat landraces showed resilience and adaptability, providing valuable genetic resources for breeding programs aiming to enhance yield potential, disease resistance, and nutritional quality (Amare et al., 2019, Chaudhary et al., 2023, Mulugeta et al., 2023). Through comprehensive characterization and conservation efforts, the rich diversity of Ethiopian durum wheat landraces can be safeguarded for future generations, ensuring food security and agricultural sustainability (Sarto et al., 2017, Temtme et al., 2018, Zewdu et al., 2021).

2.8. Ethiopian Durum Wheat Landrace Diversity and Characteristics

Ethiopian durum wheat landraces have played a vital role in the country's agricultural landscape, cultivated by generations of smallholder farmers across regions such as Oromia, Amhara, Tigray, and the South Nation Nationalities and Peoples (SNNP) (Engels and Hawkes, 1991, Badebo et al., 2009). Despite their historical significance, these traditional varieties face challenges due to modern agricultural practices, leading to genetic erosion and marginalization (Taffesse et al., 2012, Mann and Warner, 2015, Minot et al., 2015).

However, there is a growing recognition of the potential held by Ethiopian durum wheat landraces in modern breeding programs. Researchers and breeders are increasingly exploring these landraces for traits such as enhanced yield, resilience to biotic and abiotic stresses, and improved nutritional quality(Engels and Hawkes, 1991, Daniel, 2018, Negisho et al., 2021, Taffesse et al., 2012).

Recent studies have revealed the genetic diversity present within Ethiopian durum wheat landraces, showcasing a rich reservoir of variation (Kabbaj et al., 2017) (Engels and Hawkes, 1991, Negisho et al., 2021). Using high-density SNP markers, researchers have identified four distinct subgroups within a panel of 215 landraces, demonstrating the extensive genetic variation inherent in these traditional varieties (Negisho et al., 2021). This diversity is attributed to factors such as Ethiopia's diverse agroecological zones and its role as a secondary center of diversity for durum wheat (Engels and Hawkes, 1991). Additionally, traditional farming practices, including seed exchange between farmers, have contributed to the amplification of genetic variation within landrace populations (Gizaw and Assegid, 2021).

The evolutionary history of Ethiopian durum wheat landraces is further illuminated by the concept of admixture, which refers to the mixing of genetic material from different ancestral sources. Over centuries of cultivation and adaptation, these landraces have interacted with neighboring varieties, wild relatives, and introduced germplasm, resulting in varying degrees of admixture (Negisho et al., 2021). This complex relationship of genetic exchange has shaped the genetic landscape of Ethiopian durum wheat landraces, contributing to their resilience and adaptability in diverse environments (Geleta and Grausgruber, 2013, Haile et al., 2013, Mengistu et al., 2018, Dukamo et al., 2023). While modern breeding efforts often seek to isolate specific traits, the admixture found within landrace populations represents a valuable reservoir of genetic diversity that can be harnessed for future breeding programs.

Population structure analyses have further elucidated the genetic relationships among Ethiopian durum wheat landraces, revealing distinct groupings and substructures. These analyses have identified two main groups: one primarily composed of landraces and another comprising released varieties, advanced lines, and germplasm from organizations such as CIMMYT. Within the landrace group, additional substructure indicates the high degree of

genetic differentiation among Ethiopian durum wheat landraces (Alemu et al., 2020a, Negisho et al., 2021, Mulugeta et al., 2023). Conservation efforts aimed at maintaining this diversity are paramount, as they represent a vital repository of genetic material for future wheat improvement programs. Understanding the population structure of Ethiopian durum wheat landraces enables breeders to strategically utilize these genetic resources in the development of improved varieties tailored to the challenges of modern agriculture.

2.9 Drought Stress in Plants

Drought stress remains a significant challenge for plant growth and development, particularly for crops like durum wheat grown in Mediterranean climates and similar environments. In Ethiopia, where durum wheat cultivation mainly occurs in highland rainfed systems, unpredictable rainfall, and high abiotic stress incidence due to climate change pose challenges (Nezhadahmadi et al., 2013, Bodner et al., 2015). Climate projections indicate a decline in agricultural productivity due to rising temperatures, altered rainfall patterns, and increased occurrences of extreme weather events, exacerbating drought stress and impacting crop yields (Bodner et al., 2015). While progress has been made in breeding wheat cultivars for optimal environments, developing true drought-adaptive genotypes remains elusive, particularly in sub-Saharan Africa. Effective breeding strategies for drought tolerance must consider complex genetic traits and contextual environmental factors to address the challenges posed by climate change and ensure food security.

Drought stress profoundly impacts plant growth and development at various levels, from morphological to molecular, and affects all phenological stages of plant life. This stress arises from factors such as global warming, reduced precipitation, declining groundwater levels, and soil water depletion, leading to severe disturbances in ecosystems (Nezhadahmadi et al., 2013). Drought poses a significant challenge to crop production worldwide, particularly in arid and semiarid regions, causing considerable agricultural losses (Bodner et al., 2015, Nadeem et al., 2019).

The effects of drought on plants encompass morphological, physiological, and biochemical processes, resulting in growth inhibition, stomatal closure, reduced transpiration, chlorophyll depletion, and photosynthesis inhibition (Prasad et al., 2008, Wang et al., 2017). The response of plants to drought stress depends on factors like the developmental stage,

severity and duration of stress, and cultivar type (Prasad et al., 2008). Drought stress leads to reduced water content, diminished water potential, turgor loss, stomatal closure, and decreased cell enlargement and growth, ultimately inhibiting photosynthesis and metabolism and potentially leading to plant death.

All stages of plant growth, from germination to maturation, are dependent on water, and the decline in plant growth due to water unavailability is a common outcome of drought stress (Qaseem et al., 2019). Wheat plants are vulnerable to drought at various life stages, with critical stages such as germination and anthesis being particularly sensitive (Dhanda et al., 2004). Drought stress during the pre-anthesis stages can reduce the number of ear heads and kernels per ear, while stress during later stages can decrease kernel number and weight. Drought stress can result in a significant reduction in wheat yield and yield components, with reported reductions of up to 40% under stressed conditions compared to controls (Mwadzingeni et al., 2016a, Sarto et al., 2017).

2.9.1. Mechanisms of drought resistance

Understanding the mechanisms of drought resistance is crucial for developing stress-tolerant crops. Drought resistance is typically quantified by a crop's yield under drought stress, which depends on factors such as genotype, growth stage, severity, and duration of stress, as well as physiological processes, gene expression patterns, and environmental factors (Blum, 2010). Drought-responsive genes play a critical role in conferring resistance to drought stress, and their expression levels among different varieties can indicate the level of resistance. Drought stress triggers various physiological and biochemical responses in plants, including changes in protein composition, antioxidant production, osmotic adjustment, hormone levels, root growth, stomatal behavior, and photosynthesis inhibition (Mir et al., 2012, Mwadzingeni et al., 2016a). Additionally, drought stress can lead to pollen sterility, grain loss, and altered hormone synthesis in susceptible wheat genotypes (Mwadzingeni et al., 2016a). Understanding these mechanisms is essential for developing strategies to enhance drought tolerance in crops and mitigate the adverse effects of drought stress on agricultural productivity.

2.9.2. Mechanisms of drought tolerance

The determination of season length for crops under rain-fed conditions is crucial for agricultural planning and management (Dhanda et al., 2004). Typically, season length is defined as the period during which precipitation equals or exceeds 50% of potential evapotranspiration, as determined by various environmental factors such as radiation, wind, and temperature. One of the primary objectives of crop breeding is to develop cultivars that can adapt to drought conditions by maturing early enough to complete their life cycle within the available season length (Mathew et al., 2019, Senapati et al., 2019). This strategy, known as selection for earliness, aligns the phenology of the crop with the pattern of water availability in the environment.

Flowering or physiological maturity, key stages in the plant's life cycle, are highly heritable traits that significantly influence the plant's response to drought (Akram, 2011, Bodner et al., 2015). Selection for earliness, which accelerates these critical stages, can be relatively straightforward. However, early maturing cultivars may incur a yield "penalty" when rainfall exceeds the average, as they have a limited period to capture solar radiation compared to later maturing cultivars (Sangtarash, 2010).

Precipitation patterns are highly variable and unpredictable, especially in tropical regions. Therefore, no season can be considered "average," and successful crop varieties must exhibit resilience to fluctuations in rainfall from year to year (Mathew et al., 2019). Drought-tolerant varieties are characterized not only by their ability to survive under drought conditions but also by their capacity to maintain or even increase production levels despite drought stress. Traits that enhance survival at the seedling stage may not necessarily contribute to increased production and may have limited value in the selection for drought tolerance (Sangtarash, 2010).

2.10 Durum Wheat Breeding for Drought Tolerance

Conventional breeding methods have historically been used to enhance drought resistance in plants by identifying and selecting desirable traits among sexually compatible cultivars or genotype (Eibach and Töpfer, 2015, Nadeem et al., 2021). However, conventional breeding approaches face limitations such as being time-consuming and labor-intensive, with outcomes that are often unpredictable due to the partially uncontrolled nature of crosses.

Additionally, conventional breeding relies on the availability of suitable genes within the existing gene pool, which may constrain the breeding process.

In contrast, genetic engineering offers a more targeted approach to improving drought resistance by identifying and transferring specific genes associated with stress tolerance into plants (Mir et al., 2012, Kidane et al., 2019). This approach allows for the precise introduction of novel genes that confer drought-resistant traits, potentially overcoming the limitations of conventional breeding methods. Gene expression experiments have identified numerous genes that are induced or repressed under drought stress, providing valuable targets for genetic engineering efforts (Mwadzingeni et al., 2016a, Maccaferri et al., 2019).

Various genes associated with drought resistance, such as dehydration-responsive element-binding (DREB) factors, zinc finger proteins, and NAC transcription factors, have been cloned and utilized in genetic engineering strategies to enhance drought tolerance in crops (Mondini et al., 2012, Mondini et al., 2015). Techniques such as Agrobacterium-mediated transformation and particle gun methods have been employed to introduce transgenes related to drought resistance into a wide range of crops including rice, wheat, maize, sugarcane, tobacco, Arabidopsis, groundnut, tomato, and potato (Latini et al., 2008).

Furthermore, molecular marker technologies have been utilized to identify and transfer drought-related quantitative trait loci (QTL) into crop varieties through marker-assisted selection (MAS). This approach allows breeders to efficiently introduce beneficial QTL associated with drought tolerance into elite crop varieties, thereby accelerating the development of drought-resistant germplasm (Rehman Arif et al., 2020, Colasuonno et al., 2021, Pandurangan et al., 2021, Megerssa et al., 2022).

2.10.1. **Morphological responses for drought tolerance in durum wheat**

Research conducted by Khan et al. (2013) revealed important correlations and direct effects of various morphological traits on grain yield in wheat (Khan, 2013). Plant height and 1000-kernel weight showed significant positive correlations with grain yield, so that taller plants with heavier kernels tend to have higher grain yields (Savé et al., 1995, Khadka et al., 2020). On the other hand, heading and maturity days exhibited considerable negative correlations

with grain yield, suggesting that longer periods to heading and maturity may negatively impact grain yield (Ahmad et al., 2008, Haile et al., 2013, Dukamo et al., 2023).

Furthermore, the study found that maturity days and 1000-kernel weight had significant positive direct effects on grain yield, that early maturity and larger kernel size contribute positively to grain yield (Mengistu et al., 2018, Dukamo et al., 2023). Additionally, while kernels per spike showed a direct positive effect on grain yield, the magnitude of this effect was low. The indirect effects of heading days and plant height on grain yield were mainly mediated through maturity days and 1000-grain weight.

The association between plant height and grain yield was found to be significant under both genotypic and phenotypic levels, with taller plants generally associated with higher grain yields. However, there was a negative correlation between plant height and heading days, that taller plants tended to have longer reproductive phases, which could contribute to higher grain yields (Khoury et al., 2022).

In the context of drought stress, the morphological traits of wheat are significantly influenced. Under drought conditions, traits such as plant height, grains per spike, spikes per plant, and 1000-grain weight exhibit a decreasing pattern (Zewdu et al., 2021, Vályi-Nagy et al., 2023). This reduction in yield-contributing traits ultimately leads to lower grain productivity under drought stress. Plant height, in particular, is affected by both genetic factors and environmental conditions, with drought stress influencing its regulation (Qaseem et al., 2019).

Studies have shown that drought stress can lead to a reduction in plant height and stem length in wheat crops. This reduction is attributed to decreased turgor pressure, resulting in reduced cell expansion and division (Ghaffari et al., 2012, Habtamu et al., 2016, Wang et al., 2017, Nadeem et al., 2019). The decrease in plant height can also be linked to increased leaf senescence under drought stress conditions. The adverse effects of water deficit stress on sensitive stages of wheat growth, such as reproductive, booting, and grain-filling stages, can be minimized by developing genotypes with drought tolerance (Samarah, 2005, Habtamu et al., 2016)

Understanding the relationships between morphological traits and grain yield, as well as their responses to drought stress, is crucial for successful wheat breeding programs aimed at improving drought tolerance and enhancing grain yield under challenging environmental conditions. The impact of drought on plants spans across various life stages, necessitating a comprehensive understanding of plants' responses to drought stress for advancements in genetic engineering and breeding (Mwadzingeni et al., 2016a, Taranto et al., 2023). Research indicates that characteristics such as early maturity, smaller plant size, and reduced leaf area are associated with drought tolerance (Mir et al., 2012, Mwadzingeni et al., 2016a, Chaudhary et al., 2023, Taranto et al., 2023). Under drought stress, the length and area of the flag leaf in wheat tend to increase, while the width remains relatively unchanged. However, leaf extension may be limited to maintaining a balance between water absorption by roots and the water status of plant tissues (Blum, 2010, Bodner et al., 2015). Drought can also lead to a reduction in leaf area, subsequently diminishing photosynthesis.

Additionally, factors such as the number of leaves per plant, leaf size, and leaf longevity may decrease under drought stress conditions. Roots play a crucial role in drought response, as they are the first organs to be induced by drought stress (Qaseem et al., 2019). In arid conditions, roots continue to grow in search of water, while shoot growth is limited. This differential growth response of shoots and roots to drought represents an adaptation to water scarcity, with the root-to-shoot ratio increasing under drought conditions, often associated with the abscisic acid (ABA) content of roots and shoots (Bodner et al., 2015, Habtamu et al., 2016). Despite these adaptations, the growth rate of wheat roots may be diminished under moderate to high drought conditions.

In wheat, several agronomic traits are used to characterize cultivars for drought tolerance. Reduced plant height, which is strongly related to harvest index in rain-fed cereal crops, is particularly important in water-limited environments (Blum, 2010, Nanda et al., 2018). Yield components such as spikelets per spike, kernels per spike, productive tiller number, and thousand seed weight are also relevant for drought screening and breeding for terminal drought stress tolerance (Mwadzingeni et al., 2016b, Magwanga et al., 2018). Morphological characteristics like root length, tillering, spike number per square meter, grain number per spike, peduncle length, spike weight, stem weight, awn length, and grain weight per spike

also influence wheat tolerance to soil moisture shortage (Mwadzingeni et al., 2016a, Amare et al., 2019, Senapati et al., 2019).

Various criteria are employed for selecting high-yielding genotypes, including mean yield, mean productivity, and relative yield performance under drought-stressed and favorable environments (Darzi-Ramandi et al., 2016, Amare et al., 2019). The stability of grain yield for each genotype can be estimated using the drought susceptibility index (DSI), derived from yield differences between stress and non-stress environments. Combining high-yield stability with high relative yield under drought stress is considered a useful selection criterion for characterizing genotypic performance under varying degrees of drought stress (Mevlut and Sait, 2011, Gholinezhad et al., 2014, Nanda et al., 2018, Pour-Aboughadareh et al., 2019).

2.10.2. **Physiological response of plants to drought stress**

Physiological responses to drought stress play a critical role in plant survival and productivity. Researchers have highlighted the importance of understanding these mechanisms to identify and develop genotypes with drought-resistant traits (Ghaffari et al., 2012, Bodner et al., 2015, Kumar et al., 2018b). Plants can mitigate the adverse effects of drought stress by enhancing their tolerance to stress conditions. This includes increasing root density, reducing transpiration rates, maintaining low stomatal conductance, exhibiting leaf rolling, slow wilting, and delaying senescence.

Several physiological processes are significantly affected by drought stress, including transpiration, photosynthesis, and stomatal conductance. For instance, water deficiency can lead to reductions in chlorophyll content, which is crucial for photosynthesis (Savé et al., 1995). The decrease in chlorophyll content under drought stress is often attributed to oxidative stress, pigment photooxidation, and chlorophyll degradation. However, the response of chlorophyll content to drought stress may vary depending on the severity and duration of the stress (Mir et al., 2012, Monneveux et al., 2012, Wang et al., 2017, Nanda et al., 2018)

Higher chlorophyll content in leaves is positively correlated with photosynthesis and crop productivity, making it an important selection trait in breeding for drought tolerance (Kumar

et al., 2018b). Reduced photosynthesis under drought stress conditions is primarily attributed to factors such as decreased stomatal opening frequency and reduced CO2 fixation, which can lead to inhibition of the RuBisCO enzyme activity and ATP development (Bodner et al., 2015, Nanda et al., 2018)

Additionally, leaf Relative leaf water content (RLWC) serves as an important indicator of plant water status and metabolic activity within tissues. Under drought stress, RLWC decreases significantly compared to well-watered plants, impacting metabolic activities and overall plant survival (Blum, 2010). Osmoregulation mechanisms play a crucial role in maintaining turgor pressure, facilitating soil water absorption, and sustaining plant metabolic activities under stress conditions (Kumar et al., 2018b, Nanda et al., 2018, Kumar et al., 2021).

Moreover, during drought stress, reductions in RLWC and water potential contribute to a decline in photosynthetic CO2 fixation. Stomatal closure is the primary mechanism to reduce water loss, leading to decreased CO2 diffusion into leaves and lower intercellular CO2 concentrations. Additionally, structural changes in mesophyll cells and intercellular spaces may further affect CO2 diffusion and assimilation during drought stress (Bodner et al., 2015, Kumar et al., 2018b).

2.10.3. **Biochemical response of plants to drought stress**

Osmotic adjustment is a crucial physiological adaptation for drought tolerance in plants. It involves the accumulation of solutes within cells to decrease water potential, thereby maintaining cell turgor and volume (Gao et al., 2014, Aman et al., 2020). Various solutes such as sugars, organic acids, amino acids (e.g., proline), and inorganic ions contribute to osmotic adjustment. This process plays a significant role in maintaining turgor potential and supporting essential physiological processes like stomatal opening, photosynthesis, shoot growth, and root growth (Hailu, 2011, Khalili et al., 2018). Genotypes with higher osmotic adjustment ability are less affected by drought stress compared to those with lower ability. Osmotic adjustment helps in maintaining stomatal conductance and accumulating solutes, which extends the time available for CO2 assimilation and increases the net assimilation rate under drought conditions. Wheat genotypes with higher osmotic adjustment capabilities exhibit lower yield reductions due to drought-induced stress (Mondini et al., 2012).

Proline, one of the key solutes involved in osmotic adjustment, plays a crucial role in maintaining cell turgor and protecting cells from severe dehydration. Its accumulation in plant tissues increases under drought stress, and higher levels of proline are associated with enhanced drought tolerance (Zadehbagheri et al., 2014, Mwadzingeni et al., 2016b). Proline acts as a signaling molecule, regulating various cellular processes including mitochondria function, cell production, and cell death. Additionally, soluble sugars contribute to drought tolerance by protecting cells from damage in two ways: by forming hydrophilic interactions with membrane proteins, preventing protein denaturation, and by forming a biological glass in the cytoplasm, restricting the movement of reactive compounds and extending the life of dehydrated tissue. Total soluble sugars play a significant role in osmotic adjustment under severe drought stress conditions(Mondini et al., 2012, Sissons, 2022).

Breeding methodologies for wheat should focus on identifying and selecting traits related to drought tolerance at the physiological, cellular, biochemical, and molecular levels. Traits such as canopy temperature depression, root parameters, above-ground biomass, Relative leaf water content, carbon isotope discrimination, chlorophyll content, stomatal conductance, and osmotic adjustment are important for selecting genotypes with enhanced drought tolerance (Kumar et al., 2018b). These traits should exhibit genetic variability, a high genetic correlation with yield, and high heritability to be considered for inclusion in breeding programs. Evaluation of physiological traits should be fast, easy, and cost-effective to facilitate efficient screening of large genotype populations for drought tolerance (Nanda et al., 2018, Kumar et al., 2021).

2.10.4. **Molecular Responses for Drought Tolerance in Durum Wheat**

The determination of genetic diversity within and between wheat populations is essential for understanding the genetic structure and improving quantitative traits such as drought tolerance. With recurrent droughts associated with climate change limiting global wheat production, there is an urgent need for improved drought-tolerant wheat cultivars (Haile et al., 2013, Bodner et al., 2015). Recent advancements in technologies such as high-throughput phenotyping, next-generation sequencing (NGS), and genetic engineering offer promising avenues for enhancing drought tolerance in wheat (Elshire et al., 2011, Avise, 2012, Mengistu et al., 2016, Egea et al., 2017, Rehman Arif et al., 2020).

NGS technologies enable comprehensive de novo genome sequencing and gene expression analysis under stress conditions. These techniques have facilitated the development of cost-effective genotyping by sequencing (GBS) approaches, which eliminate ascertainment biases and the need for prior genome sequence information (Elshire et al., 2011). This allows for the analysis of the structurally complex wheat genome and aids in understanding drought tolerance mechanisms at the molecular level (Elshire et al., 2011, Maccaferri et al., 2015).

Several genes have been identified as being influenced by drought stress and play roles in producing drought stress-related proteins and enzymes, including dehydrins, vacuolar acid invertase, glutathione S-transferase (Megerssa et al.), late embryogenesis abundant (LEA) proteins, and ABA-responsive genes (Latini et al., 2008, Gao et al., 2014, Magwanga et al., 2018). Proline, a crucial protein involved in drought stress tolerance, is synthesized from pyrroline-5-carboxylate synthetase (P5CR), and its accumulation contributes to enhanced drought tolerance in plants (Latini et al., 2008, Mondini et al., 2015, Fayaz et al., 2019).

Molecular markers play a crucial role in detecting drought-induced genes and facilitating marker-assisted breeding (MAB) techniques. Single nucleotide polymorphisms (SNPs) are the most abundant and powerful molecular markers due to their high abundance in the genome, uniform distribution, and cost-effectiveness. SNPs are ideal for constructing genetic maps and conducting genome-wide association mapping, leading to the discovery of thousands to millions of SNPs in recent years (Mondini et al., 2012, Maccaferri et al., 2015, Alemu et al., 2020a).

Marker-assisted selection (MAS) allows for the selection of desirable traits, such as drought tolerance, by tracking the presence of quantitative trait loci (QTLs) linked to these traits. QTL mapping has identified genomic regions associated with drought resistance traits in wheat, providing valuable understanding into the genetic basis of drought tolerance. Mapping of QTLs for drought resistance has been conducted on various chromosomes in tetraploid wheat, enabling the identification of candidate genes and markers for drought tolerance breeding programs (Rehman Arif et al., 2020, Alemu et al., 2021, Colasuonno et al., 2021, Megerssa et al., 2022, Mulugeta et al., 2023, Pundir et al., 2023)

CHAPTER THREE

GENETIC DIVERSITY OF ETHIOPIAN DURUM WHEAT (*T. TURGIDUM SUBSP. DURUM*) LANDRACES UNDER DROUGHT STRESSED AND NON-STRESSED CONDITIONS.

Abstract

Ethiopia, being a major center of origin and diversity for durum wheat, possesses a highly variable genetic pool with diverse agroecological adaptations. Durum wheat landraces are an important source of genetic variation for breeding programs. Ethiopian durum wheat landraces are genetically diverse but underutilized, with limited evaluation under non-stressed and drought stressed conditions. This hinders identification of drought-tolerant, high-yielding genotypes, underlining the need for comprehensive genetic diversity assessment to guide effective breeding strategies. This study was conducted to study the genetic diversity of Ethiopian durum wheat genetic resources under two contrasting environments namely drought-stressed and non-stressed. It was carried out on 100 landraces and 4 local checks using an augmented design. Data were collected on 13 traits comprising yield and yield components, phenology, and canopy condition. The analysis of variance revealed significant differences between landraces for different traits with different sources of variation. Several landraces were found to outyield the checks under both environmental conditions. Intermediate to high estimates of the phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in a broad sense (h^2b), and genetic advance in percent of the mean (Jeyabalasingh et al.) were observed for all the studied traits except for days to flowering under non-stressed, thousands seed weight at stress, and days to maturity, leaf chlorophyll concentration measurement, and canopy temperature measurement under both conditions. The estimation of variability parameters showed that genotypic variation was higher than environmental variation for most traits. The number of tillers, spike length, kernel per spike, and grain yield indicated higher values for h^2b and GAM (74.42% and 20.86; 83.2% and 28.24; 70.79% and 28.0; and 89.54% and 74.71) under non-stressed and (97.87% and 98.22; 71.27% and 28.51; 75.52% and 43.9; and 90.04% and 103.68) under stressed condition, respectively. Spikelets per spike, kernel per spike, and thousands of seed weight were positively correlated with grain yield. Grain yield exhibited a weak negative correlation with days to heading and days to maturity. Principal components analysis revealed that six traits were the major loadings on the first

two principal components that describe 37.9% and 41.0% of the total morphological variance under non-stressed and stressed conditions, respectively. Cluster analysis grouped the landraces into six clusters, with each cluster showing variation in performance for different traits under non-stressed and stressed conditions. The intracluster distance was maximum in cluster I (D2 = 7.68) and (D2 = 8.19) under non-stressed and stressed conditions respectively and the intercluster distance was found to be maximum between clusters I and IV (D2 = 11.02) and clusters I and II (D2 = 10.33) under non-stressed and stressed conditions respectively. The presence of significant genetic variability among the evaluated durum wheat landraces suggests an opportunity for improvement of grain yield through the hybridization of genotypes from different clusters and subsequent selection. Genotypes with superior agronomic traits that outperform the best checks are identified as potential parents for yield improvement programs for drought stress.

Key words: Augmented design; Diversity studies; Drought tolerance; Durum wheat; Grain yield; Landraces

3.1. INTRODUCTION

Wheat is an important cereal and the main food crop for people over the entire world. According to the Food and Agriculture Organization (FAO) report (2020), wheat in Ethiopia covers 1.78 million hectares of a cooler and intermediate agroecology domain. Predominantly grown by subsistence farmers under rain-fed conditions, wheat is the 4th most important cereal crop in the country, with a production of 5.8 million tons (Central Statistical Agency, 2021). Although Ethiopia is the largest wheat producer, it is still reliant on foreign wheat imports to satisfy its annual domestic demand (Duarte et al., 2010, Minot et al., 2015, WorldBank, 2018).

Wheat is grown in a wide range of agroclimatic environments. However, many of these environments have drought stress as one of the major challenges to their production and productivity. The detrimental effect of periodic drought stress during the growth and development seasons adversely affects production and reduces the yield of wheat (Dhanda

et al., 2004). Low water availability and drought stress under the rain-fed-based crop production system of Ethiopia are one of the largest causes of wheat yield reduction. It is estimated that for wheat, a yield loss of 6% per degree of temperature increase due to climate change corresponds to a quarter of all global wheat trade (Asseng et al., 2015).

Drought induces significant alterations in plant physiology and biochemistry. Some plants have a set of physiological adaptations that allow them to tolerate conditions of drought stress (Savé et al., 1995). The degree of adaptation to the decrease in water potential caused by drought may differ among species. Drought stress causes various morphological and biochemical changes in plants. As drought stress worsens, functional damage and plant part loss increase. (Sangtarash, 2010).

Fundamentals of wheat improvement rely on the study of genetic diversity, as it is a basis for elucidating the genetic structure and improving quantitative traits like drought tolerance. Evaluation of genetic diversity levels among adapted and elite germplasm can provide predictive estimates of genetic variation among segregating progeny for pure-line cultivar development (Fellahi et al., 2013, Eibach and Töpfer, 2015, Slafer et al., 2015, Wang et al., 2017).

There exists enormous variation among different indigenous genotypes of durum wheat in terms of quantitative characteristics that are directly related to yield and yield-related traits which are very important for crop improvement programs through breeding and selection. Grain yield is a complex quantitative trait (Sarker, 2020) and is directly and positively influenced by yield-related traits such as the number of tillers, number of grains per spike, thousand grain weight, and spike length (Afrooz et al., 2014, Desheva and Kyosev, 2015). Success in crop improvement generally depends on the magnitude of genetic variability (Azad et al., 2022) and the extent to which the desirable characteristics are heritable (Sarker et al., 2022).

Several approaches and analyses like those of principal components and cluster distances among and between groups of cultivars studied based on morphological and growth attributes have been suggested by many researchers for the estimation of genetic diversity (Sarker et al., 2017, Sarker et al., 2018, Azam et al., 2023). Correlation coefficient analysis

is helpful to select for yield using more than one character (Sodagar et al., 2020). It is the measure of the degree of symmetrical association between two variables or characters which helps us in understanding the nature and magnitude of association among yield and yield components and provides an opportunity for indirect selection (Faysal et al., 2022, Kulsum et al., 2022). Hierarchical cluster analysis has been used to estimate genetic dissimilarity and similarity, and Principal component analysis to determine the factors that contribute to the variation of quantitative characters in durum wheat.

Durum wheat production in Ethiopia experiences a lot of challenges, and various studies have been carried out to tackle different problems. As its production and quality are affected by abiotic and biotic factors (Temtme et al., 2018, Degete and Chala, 2019, Zemede et al., 2019), research has focused mainly on improving grain yield (Alemu et al., 2021, Kidane et al., 2017) and disease resistance (Letta et al., 2013, Liu et al., 2017, Muleta et al., 2017, Miedaner et al., 2019, Megerssa et al., 2020, Megerssa et al., 2022). As is known, Ethiopian durum wheat landraces are unique sources of useful traits. For example, the study by Negassa (1986) showed that landrace from Gamogofa and Harar were highly resistant to a virulent race of powdery mildew and are used as potential parents for resistant breeding, although collections have not been used to their full potential in breeding programs.

Several attempts have been made toward the characterization of Ethiopian durum wheat landraces and released varieties. Molecular characterization and association mapping, which reviles an abundance of high diversity in durum wheat by (Mengistu et al., 2018, Kidane et al., 2019), and (Alemu et al., 2020a) was the prominent work. Much effort is also paved on characterization for biotic stress, such as resistance for stem rust, stripe rust, and other quality-related traits. However, studies on the genetic diversity of durum wheat landraces in response to drought tolerance are scant. The selection of drought-tolerant durum wheat genotypes has paramount importance in expanding its production to the untapped potential production areas. Here we report on the diversity of Ethiopian Durum wheat landraces in their response to drought stress.

3.2. MATERIALS AND METHODS

3.2.1. Description of study area

The study was conducted at two test sites, Debrezeit and Dera, during the 2020/21 cropping season. Debrezeit is located at an altitude of 1920 m above sea level and latitude 8.7° 44′ North and 39.0° 58′ East. It has a mean annual rainfall of 931.4 mm and average maximum and minimum monthly temperatures of 27.5 ºC and 11.4 ºc, respectively. Dera is located at an altitude of 1500 m above sea level and latitude 8.3° 20′ North and 39.3° 19′ East and receives a mean annual rainfall of 816.1 mm and average maximum and minimum monthly temperatures of 29.4 ºC and 13.7 ºc, respectively (Semahegn et al., 2020). Monthly average minimum and maximum temperature and rainfall for the 2021 cropping season and the 9-year trend are indicated in Figure 3.1. Dera site was taken as a drought-stressed environment and one of the sites used for stress tolerance trials for field crops, while Debrezeit was considered a non-stressed site.

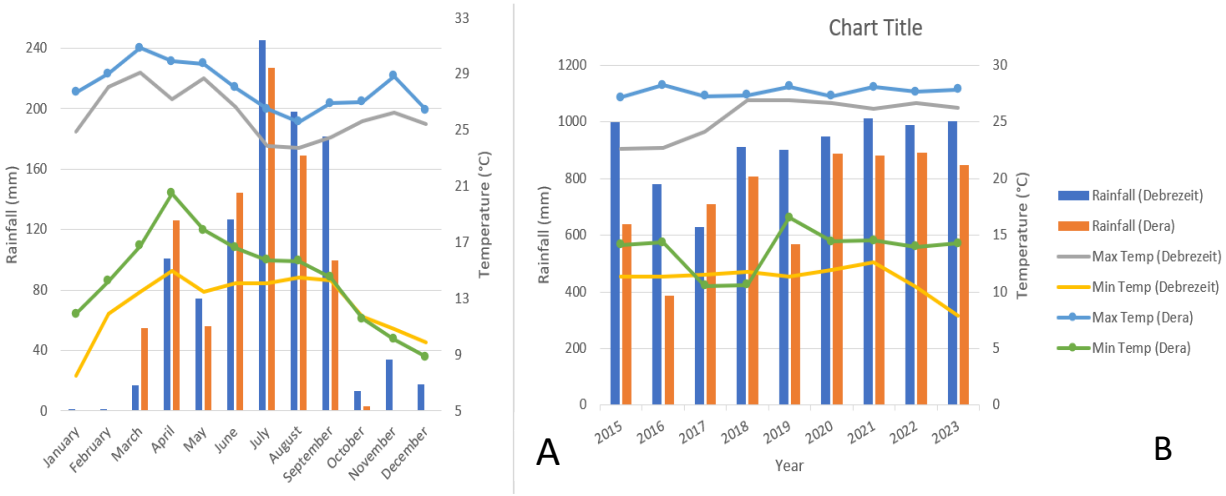

Figure 3.1. Monthly rainfall and temperature distribution during 2021 cropping season (A) and the 9 years trend (B) at Debrezeit and Dera locations.

3.2.2. Experimental Plant Materials

A total of 104 durum wheat genotypes, comprising 100 landraces and four standard checks (Alemtena, Fetan, Kuami, and Ude), were initially included in the study. However, the final analysis was conducted on 99 genotypes due to germination viability issues. The materials were from the Ethiopian Biodiversity Institute (EBI) and the Debrezeit Agricultural Research Center of Ethiopia. Landraces were selected based on the acreage of each seed source region. Thus, more samples were taken from major growing regions (Oromia and Amhara) and fewer samples from minor growing regions, with a criterion of addressing all

agroecological areas. The passport data of the study materials was found in Supplementary Table 1, in the appendix section.

3.2.3. Experimental Design and Field Management

The experiment was laid out in a 24 × 5 augmented block designs with an area of 168 m^2 (24 × 7m) and the space between rows was 20 cm while between blocks were 50 cm. The landraces were randomly planted, and the checks were replicated in each block at random. Planting was done by hand drilling using a seed rate of 120 kg/ha for each genotype. Planting at Dera location was done 15 days after the Debrezeit site. Nitrogen and phosphorous fertilizers were applied at the rate of 100 kg/ha urea (applied in the split; half at the seedling stage and the remaining half at the booting) and 100 kg/ha of Di-ammonium phosphate (DAP) at planting. All other management practices were uniformly applied to all plots.

3.2.4. Data Collection

Data were collected on the following traits of wheat according to the IBPGR descriptors for wheat (IBPGR, 1985). These data were collected in different developmental stages of the wheat plant. Days to heading (DTH) will be calculated as the number of days between the sowing date and the date when 50% of all the shoots in a plot had fully emerged spikes. The number of tillers (TN) will be recorded at physiological maturity and plant height (PH) will be measured in centimeters (cm) from the ground to the tip of the spike days to 50% flowering (DTF). Days to maturity (DTM) will be calculated from sowing date to 50% senescence of the spikes. The spike length (SL) measured in cm, the number of spikelets per spike (SPS) and the numbers of kernels per spike (KPS) will be recorded after harvesting from the main tillers of five randomly selected plants. Thousand seed weight (TSW) is determined using a sensitive balance measured from randomly sampled 1000 seeds after harvest and expressed in g/1000 seed. Biomass yield (BY) is measured as the total dry weight of all above-ground plant parts and is expressed in grams per plot. Finally, grain yield per plot (GY) will be determined as the weight (grams) of the grain from a plot.

3.2.5. Data Analysis

The data were analyzed using SAS 9.3 (SAS, 2011) and R software. Analysis of variance (ANOVA), Pearson’s correlation coefficient (r), and principal component analysis (PCA)

were performed. The PCA biplots were plotted. The statistical model used for the augmented design was

$$Y_{ij} = \mu + \beta_i + c_j + \tau_{k(i)} + \varepsilon_{ij}$$

Where μ is the mean, β_i is blocks effect, c_j are checks $\tau_{k(i)}$ is each new entry and ε_{ij} is error The mean square for the analysis of the variance of the check genotypes is computed by using the formula in Table 3.1.

Table 3.1. The mean square formula for the analysis of variance of the check genotypes in the augmented design.

ANOVA			
Source	df	SS	MS
Total	rc-1	$\sum i \sum j\, x_{ij}^2 - \frac{G^2}{rc}$	
Blocks	r-1	$\frac{1}{c}\sum j\, R_j^2 - \frac{G^2}{rc}$	
Checks	c-1	$\frac{1}{r}\sum i\, C_i^2 - \frac{G^2}{rc}$	
Error	(r-1) (c-1)	$SSTot - SSR - SSC$	$\frac{SSE}{(r-1)(c-1)}$

Standard errors used for computing LSI values are calculated by using

1. Difference between means of two check varieties:

$$\text{Standard error, Sc} = \sqrt{\frac{2MSE}{r}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

2. Difference between adjusted yields of two new selections in the same block

$$\text{Standard error, Sd} = \sqrt{2MSE} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

3. Difference between adjusted yields of two new selections in different blocks:

$$\text{Standard error, Sv} = \sqrt{\frac{2(c+1)MSE}{c}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3$$

4. Difference between the adjusted yield of a new selection and a check mean:

$$\text{Standard error, Svc} = \sqrt{\frac{(r+1)(c+1)MSE}{rc}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 5$$

A least significant difference

LSD = $t_\alpha \times Svc$ ……5

The phenotypic, genotypic, and environmental variance (σ_p^2 , σ_g^2 , σ_e^2) are obtained from the ANOVA tables according to the expected value of the mean square described by Federer and Searle (1976) and Johnson et al. (1955) as follows:

$\sigma_g^2 = \frac{(\text{GMS}-\text{EMS})}{r}$ ……..……6

$\sigma_p^2 = \sigma_g^2 + \text{EMS}$……..…….7

$\sigma_e^2 = \text{EMS}$ ……8

Phenotypic and genotypic coefficients of variation (PCV and GCV) are estimated according to Burton (1951) as follows:

$GCV = \frac{\sigma_g^2}{\sqrt{\bar{x}}} x100$ ……9

$PCV = \frac{\sigma_p^2}{\sqrt{\bar{x}}} x100$ ……10

Where $\bar{x}$ is the mean

The estimates of PCV and GCV are categorized according to Sivasubramanian and Madhavamenon (1973) as follows:

CV (%)	Category
P(G)CV < 10	Low
10 ≤ P(G)CV < 20	Medium
P(G)CV ≥ 20	High

The broad-sense heritability (h^2_b) is calculated according to the method of Lush (1940) as follows:

$h^2\text{b} = \frac{\sigma_g^2}{\sigma_p^2}$ ……..……..11

The estimates of broad-sense heritability (h^2_b) are categorized according to Robinson (1966) as follows:

h^2_b	Category
$h^2_b < 30$	Low
$30 \leq h^2_b < 60$	Medium
$h^2_b \geq 60$	High

Genetic advance (GA) is estimated and categorized according to Johnson et al. (1955) as follows:

$$GA = k\ x\ \sigma_g\ x\ \frac{H^2}{100} \quad \text{.....12}$$

Where the constant k is the standardized selection differential or selection intensity. The value of k at the 5% proportion selected is 2.063 from the table in Falconer (1996).

Genetic advance a percentage of mean (Jeyabalasingh et al.) is calculated according to the method in Comstock and Robinson (1952)

$$\text{GAM} = \frac{\text{Population mean}}{\text{Genetic advance}} \times 100 \quad \text{.....13}$$

Principal component analysis (PCA)

The principal components are calculated by finding the eigenvectors of the covariance matrix of the original variables. The eigenvectors are the directions in which the data varies the most, and the eigenvalues are the corresponding variances. The principal components are then ordered by their eigenvalues, with the first principal component accounting for the most variation in the data, the second principal component accounting for the second most variation, and so on (Jolliffe, 2002, Abdi and Williams, 2010).

Cluster analysis

Cluster analysis was calculated using hierarchical clustering, starting by assigning each object to its cluster, and then iteratively merging the most similar clusters until there is only one cluster left. Euclidean distance was used to measure the similarity between the two clusters. The Euclidean distance between two clusters is the square root of the sum of the squared differences between the cluster means (Ward Jr, 1963, Jain et al., 1999).

D^2 analysis

The genetic distance between two genotypes was measured using D^2 analysis. It is based on the Euclidean distance between the genotypes' vector of principal component scores. The formula for D^2 analysis is as follows:

$D^2 = (Y_1 - Y_2)'(Y_1 - Y_2)$……14

where:

- D^2 is the genetic distance between the two genotypes
- Y_1 is the vector of principal component scores for genotype 1
- Y_2 is the vector of principal component scores for genotype 2

The genetic distance between two genotypes measures how different they are. The higher the genetic distance, the more different the two genotypes are (Mahalanobis, 2018).

3.3. RESULTS AND DISCUSSION

3.3.1. Analysis of variance

The Analysis of variance (Table 3.2) revealed a significant mean sum of squares for different traits with different sources of variation. There indicates a highly significant difference between landraces, eliminating the block effect in all parameters except canopy temperature measurements, and leaf chlorophyll concentration measurements under both non-stressed and stressed conditions. However, the block effects were non-significant for all traits under both conditions except for grain yield under both non-stressed and stressed conditions and thousand seed weights under stressed conditions. The absence of significant differences between blocks is an indication of the homogeneity of the evaluation blocks.

The overall adjusted mean between the two environmental conditions in the separated ANOVA indicates the presence of differences between genotypes in response to the varying environmental conditions. As indicated in Table 3.2, under non-stressed condition, there was a significant difference in the adjusted mean of spike length (8.6), number of spikelets per spike (23.38), and plant height (88.77) than stressed condition (82.83, 6.41, 19.84) respectively. Several researchers also reported the presence of significant differences in major traits in bread and durum wheat genotypes studied under different environmental conditions (Zerga et al., 2016, Berhanu and Haftamu, 2019, Rehman Arif et al., 2020).

Table 3.2. Analysis of variance from augmented block design for the thirteen yield and yield-related traits of 98 durum wheat genotypes under non-stressed and stressed conditions.

Source of variation	Env.	DF	BY	CT	DTF	GY	DTH	PH
Landraces (EB)	Non-stressed	97	17010.39 *	10.32 ns	61.11 **	1.34 **	54.78 **	147.97 *
Checks		3	5342.96 ns	14.37 ns	4.6 ns	2.08 **	11.25 ns	65.3 ns
Landr vs. Check		1	287.5 ns	73.69**	990.78**	17.8 **	417.74**	578.85**
Test treatments		93	16924.1 *	9.71 ns	55.12 *	1.13 **	57.31 **	148.84 *
Block (ET)		4	21654.7 ns	6.19 ns	2.32 ns	1.73 **	3.58 ns	8.61 ns
Residuals		12	6861.25	5.45	16.72	0.12	8.87	59.56
Overall adj. mean			806.99	31.88	74.52	2.62	59.18	84.77
CV			10.25	7.39	5.56	12.41	5.09	9.02
Landraces (EB)	Stressed	98	45299.36 *	9.51 ns	64.1 **	1.18 **	36.71 **	94.09 **
Checks		3	11776.21 ns	3.33 ns	1.47 ns	0.79 **	9.13 ns	3.26 ns
Landrce vs. Check		1	260547.16 **	95.53 **	1151.21 **	25.31 **	449.46 **	385.19 **
Test treatments		94	43909.99 *	8.79 ns	53.64 **	0.95 **	33.91 **	93.17 **
Block (ET)		4	23560.94 ns	4.3 ns	38.05 ns	0.89 **	9 ns	57.93 ns
Residuals		12	14340.33	5.3	13.22	0.09	7.3	22.91
Overall adj. mean			605.45	33.23	62.73	1.75	51.46	81.92
CV			19.29	7	5.89	16.1	5.32	5.8

Source of variation	Env	KPS	DTM	LCC	SL	SPS	NT	TSW
Landraces (EB)	Non-stressed	26.46**	59.89**	15.76ns	2.52**	8.35*	7.04**	63.29*
Checks		17.19ns	13.93ns	6.95ns	20.27**	3.47ns	0.56ns	36.75ns
Landrac vs. Check		175.48**	141.79**	40.72*	2.65*	133.6**	55.93**	273.9**
Test treatments		25.09*	60.87**	16.52*	2**	7.33*	6.85**	60.59*
Block (ET)		14.38ns	4.37ns	19.76ns	0.41ns	4.23ns	1.6ns	32.8ns
Residuals		7.33	6.14	6.9	0.34	2.51	1.75	24.94
Overall adj. mean		26.12	106.31	46.58	8.6	21.38	19.26	41.08
CV		10.19	2.34	5.66	6.7	7.28	6.96	11.98
Landraces (EB)	Stressed	49.79**	37.67**	24.06ns	1.98**	11.96**	57.87**	11.91*
Checks		9.21ns	51.33**	6.21ns	16.99**	2.98ns	3.86ns	3.05ns
Landrac vs. Check		1073.18**	245.23**	224.56**	2.44*	183.58**	2.77ns	36.42**
Test treatments		40**	34.61**	23.06ns	1.55**	10.52**	63.09**	11.22*
Block (ET)		28.19ns	15.92ns	24.96ns	0.3ns	0.81ns	1.74ns	16.55*
Residuals		9.79	8.13	15.49	0.44	2.73	1.34	3.84
Overall adj. mean		22.44	95.48	42.45	6.41	17.38	16.33	38.42
CV		13.34	3	9.38	10.29	9.28	7.12	5.13

*, ** Significant at 5% and 1% probability level, respectively. Env.: Environment, DF: Degree of freedom, BY: Biomass yield, CT: canopy temperature measurement, DTF: days to 50% flowering, GY: grain yield per plot, DTH: days to heading, PH: plant height, KPS: kennels per spike, DTM: Days to maturity, LCC: leaf chlorophyll concentration measurement, SL: spike length, SPS: the number of spikelets per spike, NT: number of tillers, and TSW: thousand seed weight

Highly significant differences in plant height among genotypes under stressed conditions were shown in previous studies (Azam et al., 2014, Biswas et al., 2015). The adjusted mean of the number of kernels per spike and thousand seed weight also have a significant difference between non-stressed and stressed conditions. The least significant difference (Supplementary Table 2) was computed to identify the test genotypes that significantly surpassed the best check. Under both environmental conditions, a total of seven landraces out yield the checks namely, ETDW/15DZ4, 34493, ETDW/15DZ23, 34522, MCD3-14, 34217, and 31831. From those out yielded landraces ETDW/15DZ23, 34522, ETDW/15DZ4, 34493 under non-stressed conditions, and ETDW/15DZ4 34493 ETDW/15DZ23 under stressed conditions surpass all four checks.

3.3.2. Agronomic performance of durum wheat genotypes

The mean comparison in the separate ANOVA (Table 3.2) showed the presence of a significant difference between the means of each trait in both environmental conditions. The landraces have significant differences with respect to the checks and each other. For the phenological traits, there is a significant difference between the two environments. The heading, flowering, and maturity days have shown a significant difference between the two environments. The overall mean days for heading, flowering, and maturity under non-stressed condition were 58.08, 73.89, and 109.27, and for the stressed conditions 51.34, 62.82, and 98.14, respectively. Studies by Vályi-Nagy et al. (2023) and Samarah (2005) indicated that drought can accelerate and shortens the period of flowering and grain maturity. it speeds up wheat plant flowering and maturity and caused forced maturity. There is an absence of correspondence between days to heading and maturity. Some of the genotypes having early heading did not show early maturity and late maturing was not matched with late days to heading. It was in agreement with a previous study by Khan (2013) and Zewdu et al. (2021) that for most of the genotypes, they reported an absence of coincidence with each other.

The full table of the adjusted mean values of the studied genotypes for different agronomic traits in both conditions is given in Supplementary Tables 3 and 4. From Table 3.3 which showed the top 10 and bottom 5 performing landraces, grain yield varies with a range of 0.68 t/ha to 6.13 t/ha and from 0.11 t/ha to 5.47 t/ha with an overall adjusted mean grain yield of 2.62 and 1.75 t/ha under non-stressed and stressed conditions respectively. The result showed a yield reduction due to drought stress by around 33.2%. The result is in

agreement with the reports of other authors where drought stress resulted in a significant reduction of grain yield. The studies by Amare et.al. (Amare et al., 2019) and Habtamu et.al. (Habtamu et al., 2016) indicated the performance of grain yield under non-stressed conditions to be higher than the stressed condition by around 30.6%, which is an indication of a significant reduction of yield due to stress. Also, Qaseem et.al. (Mohammadi, 2012) reported a greater loss (around 63.0%) of yield in durum wheat due to drought stress.

The genotypes also showed variation in the adjusted mean of canopy temperature and leaf chlorophyll concentration measurement across the two environmental conditions and within the genotypes too. The mean canopy temperature under non-stressed and stressed conditions was 31.88^{o}C and 33.23 respectively. Canopy temperature readings depend on the environment in which the measurements were taken and there are as many responses in CT as there are environments. Studies indicated the mean CT under non-stressed condition was cooler than that of the stressed condition, which indicates that plants adjust their physiology and minimize the loss of water via the stomata (Jackson et al., 1981, Nanda et al., 2018). The leaf chlorophyll concentration measurement indicates a greater mean value (46.58) in the non-stressed condition than in stressed (42.45) condition. As indicated in (Lobell and Gourdji, 2012, Shiferaw et al., 2013), Changes in the structure of chloroplast such as the change in the shape of chloroplasts, swelling of stromal lamellae, clumpy vacuoles, antenna-depleted PS II, and degradation of chlorophyll molecules during stress results in the reduction in chlorophyll content (Qaseem et al., 2019). The mean performance of genotypes under non-stressed conditions for the number of spikelets per spike, spike length, and thousand seed weight showed a significant increment from that of stressed condition as studies reported that drought stress reduced the number of spikes and grains per plant (Samarah, 2005, Rahman et al., 2009).

Table 3.3. Adjusted mean values of the 10 best-performing genotypes and five bottom-performing genotypes (based on grain yield under drought-stressed conditions) for the thirteen quantitative traits in non-stressed and stressed conditions.

Treatment	BY		CT		DTF		GY		DTH		PH		KPS	
	NS	Stress	NS	Stress	NS	Stress	NS	Stress	NS	Stress	NS	Stress	NS	Stress
Top ten genotype														
ETDW/15DZ4	1048.02	698.64	26.92	29.72	59.6	56.8	5.73	5.47	45.35	45.8	97.75	78.73	28.84	20.56
ETDW/15DZ23	915.21	257.01	29.62	27.92	76.85	65.55	6.13	4.96	64.6	57.8	88.95	79.48	26.49	29.63
34522	830.16	746.31	26.43	29.98	74.6	52.05	6.08	4.66	61.1	49.8	103.49	86.89	31.09	35.05
34493	726.29	588.34	34.72	33.02	72.85	63.55	5.22	4.54	64.6	53.8	85.67	88.63	26.84	31.39
31831	801.48	283	32.01	32.31	72.35	57.55	4.54	3.62	51.6	50.3	67.25	73.79	48.19	36.86
34217	636.46	360.71	26.97	29.27	82.85	75.55	4.34	3.6	66.6	59.8	104.32	93.01	24.57	17.63
Ude	768.72	740.26	31.95	30.55	66	54.6	4.28	3.22	56.4	45	90.46	85.43	27.6	28.22
Fetan	842.11	720.27	28.88	30.88	67.8	54.6	4	3.25	52.8	46	86.03	86.7	30.64	31.42
ETDW/15DZ010	592.41	795.1	29.12	29.42	73.35	69.55	3.9	3.19	58.6	53.3	87.45	91.79	32.37	22.53
31778	590.16	809.2	28.48	28.78	75.35	59.55	3.7	3.04	56.6	47.3	100.03	88.79	33.79	25.2
Bottom five genotypes														
34295	803.03	681.61	28.72	31.02	74.85	69.55	2.92	0.35	61.6	59.8	93.13	82.63	19.99	28.63
31761	927.36	279.2	28.06	28.36	70.35	62.55	0.93	0.36	55.6	53.3	80.92	88.79	28.12	16.15
MCD1429	994.19	975.76	35.33	36.38	57.6	52.05	0.88	0.38	49.35	45.5	94.21	91.11	21.23	18.19
34451	790.79	661.61	36.42	34.72	66.85	62.55	0.7	0.21	54.6	52.8	68.86	74.54	19.61	14.45
DW-PVT LM8	620.69	357.61	32.43	34.73	62.85	46.55	0.68	0.11	44.6	44.8	76.12	83.55	18.99	14.2
Overall adj. mean	806.99	605.45	31.88	33.23	74.52	62.73	2.62	1.75	59.18	51.5	84.77	81.92	26.12	22.44
CV	10.25	19.29	7.39	7	5.56	5.89	12.41	16.1	5.09	5.32	9.02	5.8	10.19	13.34
least sign. Inc. (LSI)	180.78	261.35	5.1	5.03	8.93	7.93	0.75	0.68	6.5	5.9	16.84	10.44	5.92	6.83

Table 3 continued

Treatment	DTM		LCC		SL		SPS		NT		TSW	
	NS	Stress	NS	Stress	NS	Stress	NS	Stress	NS	Stress	NS	Stress
Top ten genotype												
ETDW/15DZ4	103.5	99.3	41.25	43.03	13.02	9.57	24.59	16.7	17.1	10.8	44.42	39.18
ETDW/15DZ23	110.25	94.05	45	35.49	13.04	9.59	27.32	21.5	17.51	14.5	46.5	35.78
34522	114	92.55	38.43	43.1	10.47	8.27	28.68	22	15.17	9.62	41.15	38.16
34493	99.25	91.05	55.01	48.76	12.79	10.34	23.32	15	23.06	20	47.48	37.53
31831	109.25	86.8	46.39	47.53	11.88	8.68	23.43	19	19.81	18.3	59.62	41.17
34217	103.25	99.05	45.7	39.29	11.84	9.39	24.7	18.6	23.23	20.2	48	36.95
Ude	103.8	92.4	46.64	40.47	8.43	6.13	24.74	20	17.31	15.3	43.92	37.46
Fetan	101.2	88.6	44.48	38.92	10.65	8.35	24.41	21.2	17.36	15.4	46.3	37.07
ETDW/15DZ010	109.25	98.8	46.38	50.09	11.68	9.48	23.69	18.9	17.57	16	54.42	32.15
31778	109.25	95.8	49.14	43.39	11.13	8.93	24.43	19.6	16.42	14.9	49.27	36.23
Bottom five genotypes												
34295	106.25	94.05	50.53	41.99	10.2	7.75	20.82	18.9	21.48	14.4	42.78	36.25
31761	103.25	98.8	48.31	38.32	8.44	6.24	13.93	14.4	17.88	16.3	41.99	33.55
MCD1429	90	85.3	46.84	47.19	7	5.8	15.66	14.7	21.85	16.7	36.55	39.76
34451	104.25	98.05	48.46	43.69	9.44	6.99	20.44	13.6	20.41	17.4	28.75	36.07
DW-PVT LM8	103.25	90.05	49.08	42.53	8.14	5.69	19.82	12.6	21.68	18.6	38.28	36.08
Overall adj. mean	106.31	95.48	46.58	42.45	8.6	6.41	21.38	17.4	19.26	16.3	41.08	38.42
CV	2.34	3	5.66	9.38	6.7	10.29	7.28	9.28	6.96	7.12	11.98	5.13
least sign. increase (LSI)	5.42	6.22	5.74	8.59	1.27	1.46	3.46	3.62	2.89	2.53	10.91	4.28

3.3.3. Principal Component Analysis

In Figure 3.2, the PCA revealed that under both locations five of the thirteen principal components were significant with eigenvalue >1 and contributed to 70.1% and 71.3 of the variance under non-stressed and stressed conditions respectively. PC1 accounted for the highest variance (24.8%) followed by PC2 which accounted for 16.2% at non-stressed conditions (Figure 3.2A) and PC1 (21.4%) followed by PC2 which accounted for 16.5% under stressed condition (Figure 3.2B). PC3, PC4, and PC5 accounted for 10.9%, 9.6, and 8.6% variance under non-stressed and, 14.1, 10.8, and 8.5 under stressed conditions respectively.

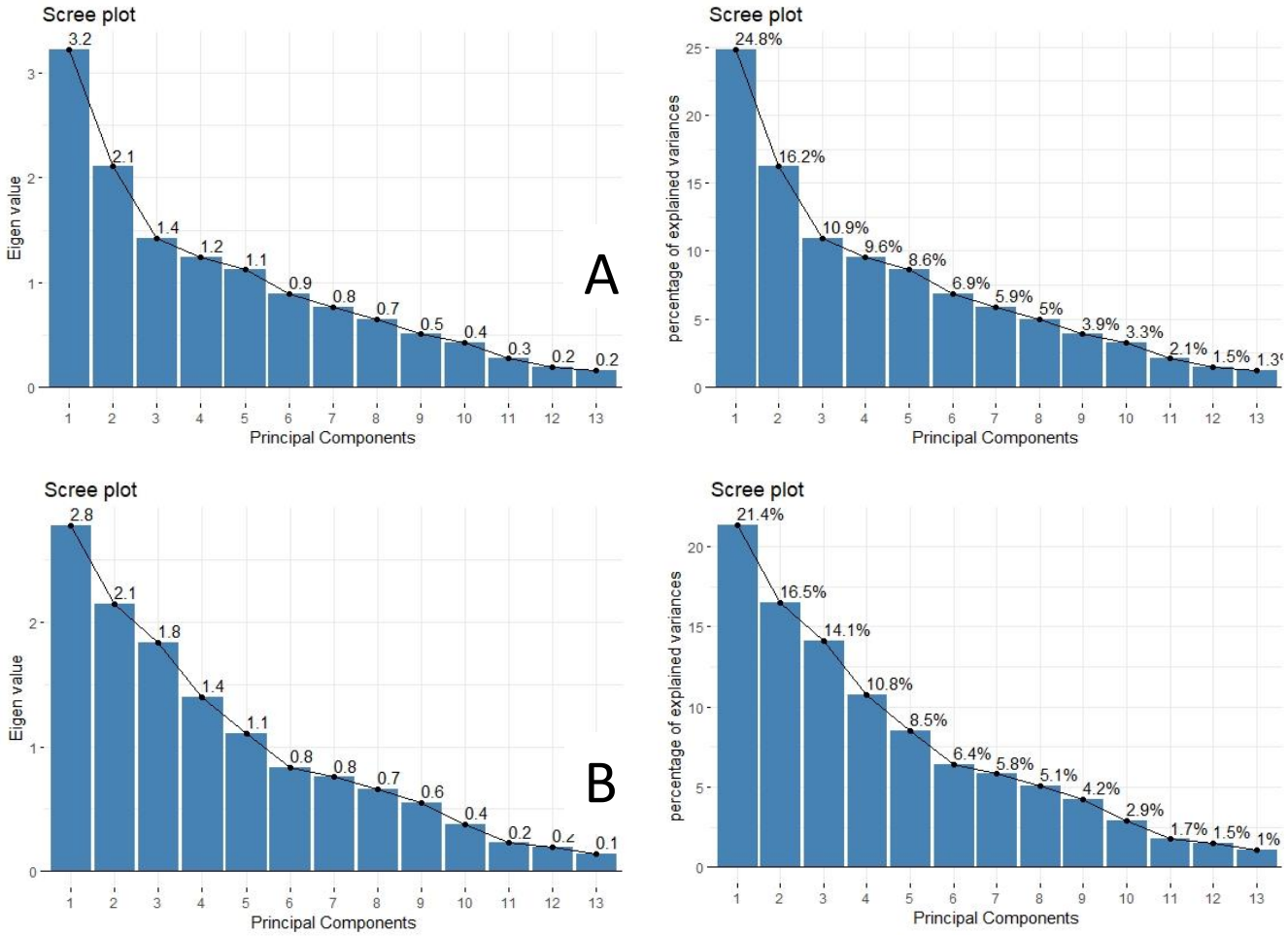

Figure 3.2. Eigenvalues of different principal components and their contribution to the total explained variance for durum wheat landraces grown under non-stressed (A) and stressed conditions (B)

The magnitude and direction of the contribution of different traits in the different principal components are shown in Figure 3.3A and 3.3B for non-stressed and stressed conditions respectively. The characters coming together in different principal components explaining the variability show the tendency to remain together and must be taken into consideration during the exploitation of these characters in the breeding program (Dhakal et al., 2020).

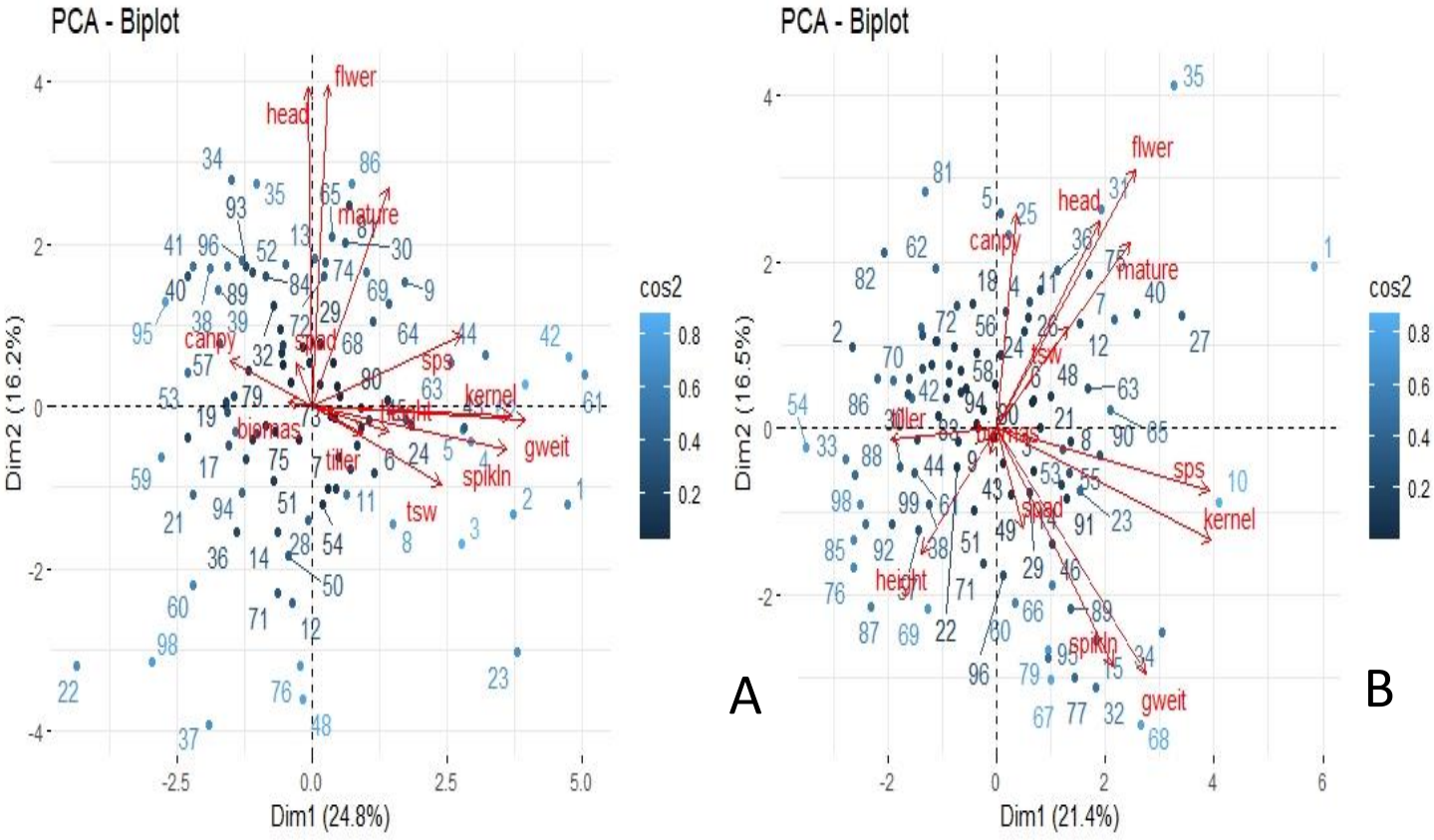

Figure 3.3. The biplot of durum wheat landraces for PC1 and PC2 under non-stressed (A) and stressed (B) conditions. The arrows show the contribution (magnitude and direction) of the trait in PC1 and PC2.

Results from the heatmap of the PCA in Figure 3.4A showed that the first principal component was contributed positively by grain yield, number of kernels per spike, spike length, spikelets per spike, and thousand seed weight under non-stressed condition. The heat map clearly showed that in this condition the PC1 included parameters that were mainly related to grain yield, yield attributes, and grain characteristics. For the stressed condition, in Figure 3.4B, the PC1 contributed positively by grain yield, numbers of kernels per spike, spike length, and spikelets

per spike, and negatively contributed by tiller number. In both conditions, most of the traits positively contributed to PC1 and PC2.

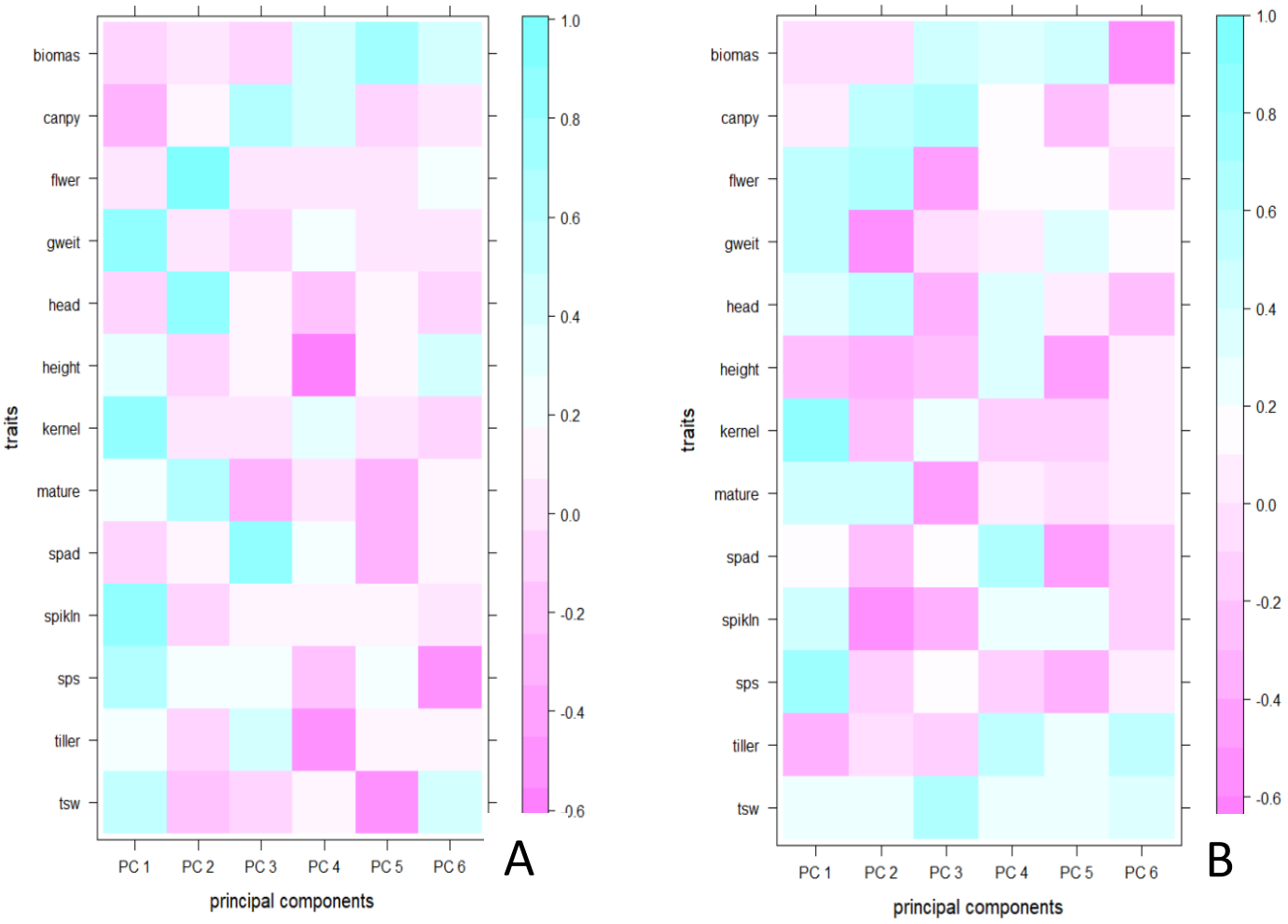

Figure 3.4. Contribution of each trait for the principal component under non-stressed (A) and stressed (B) conditions.

3.3.4. Cluster analysis

The landraces were grouped into six clusters as listed in Table 3.4. Under the non-stressed condition, cluster IV was the largest cluster which included 26 landraces, whereas cluster VI with 24 landraces under stressed condition followed by cluster II which included 21 and 22 landraces under non-stressed and stressed conditions respectively. At non-stressed conditions clusters, I, III, and V had equal 15 landraces and the VI cluster contain the minimum number of

landraces, which is six. At stress conditions, III, V, IV, and I take the next rank with 19, 14,13, and 7 landraces respectively.

Table 3.4. Grouping of the studied durum wheat genotypes based on six clusters for various traits for non-stressed and stressed conditions.

	cluster	No. of landraces	% of landraces	Name of landraces
Non-stressed	I	15	15.31	31831, Ude, Fetan, ETDW/15DZ010, 31778, ETDW/15DZ038, ETDW/15DZ4, 34217, ETDW/15DZ23, 34493, 34415, 34522, MCD3-14, 34560, DZDW170204
	II	21	21.43	31794, 31806, Alemtena, Kuami, MCD3-15, ETDW/15DZ 006, ETDW/15DZ 003, 31789, 31761, MCD12-30, 33235, DW-NVT LM 5, DZDW17002, 33239, 33306, 34295, MCD2-17, 34219, MCD1-32, DZDW17010,8, 34596
	III	15	15.31	31797, ETDW/15DZ 057, 31696, 31755, 31759, 33403, ETDW/15DZ 049, MCD12-2, 34520, 34641, 34625, 34607, DW-NVT LM 6, 34622, 34602
	IV	26	26.53	MCD15-49, MCD4-41, 33405, 33241, ETDW/15DZ #39, 33254, 33205, DW-NVT LM 4, MCD13-42, 34452, DW-NVT LM 11, 34545, 34573, 34510, 34516, ETDW/15DZ #35, 34571, MCD2-29, MCD15-7, MCD4-4, ETDW/15DZ 073, 34500, ETDW/15DZ 043, ETDW/15DZ 014, 34613, 34600
	V	15	15.31	31698, MCD4-15, 34423, 34418, MCD10-11, 34484, 34451, DW-PVT LM 8, ETDW/15DZ 012, 34496, 34620, 34611, 34594, 34632, MCD14-29
	VI	6	6.12	33230, 33300, DZDW17004, 33244, 34481, MCD3-27
Stressed	I	7	7.07	34566, 34573, 34580, 33405, 34571, 34607, 34622
	II	22	22.22	MCD4-15, DW-NVT LM 5, 33300, ETDW/15DZ 049, MCD12-30, 33235, 34500, DZDW17002, 34510, 34481, ETDW/15DZ #35, ETDW/15DZ 073, MCD4-41, ETDW/15DZ #39, 33205, 34613, 31759, MCD4-4, MCD15-49, 33254, 34520, ETDW/15DZ 014

III	19	19.19	DZDW1702,04, 33230, MCD2-29, ETDW/15DZ 003, MCD3-14, MCD3-27, 34545, DZDW17010,8, MCD1-32, DW-NVT LM 6, 34295, ETDW/15DZ 012, 34596, ETDW/15DZ 006, 33403, 33306, 34641, ETDW/15DZ 038, 34415
IV	13	13.13	31831, ETDW/15DZ #4, ETDW/15DZ #23, Ude, Kuami, Fetan, 34522, Alemtena, 34493, 31778, ETDW/15DZ 010, 34217, 31797
V	14	14.14	34452, 33244, 31806, DW-NVT LM 11, 33241, DW-NVT LM 4, 34484, ETDW/15DZ 043, 31761, 34594, 31703, 31755, 34219, MCD13-42
VI	24	24.24	34451, 34516, 34423, 31789, 34611, MCD10-11, 33239, 34620, DW-PVT LM 8, MCD15-7, 31794, 34625, MCD3-15, 34600, 34560, 34496, 31696, ETDW/15DZ 057, MCD12-2, MCD14-29, MCD2-17, 34632, 34418, 34602

Cluster I comprised 15 genotypes, which represented 15.31% of the total genotypes in the non-stressed condition and 7 genotypes (7.07%) in the stressed condition (Table 3.4). In Table 3.5 the data on the mean performance of the traits in each cluster indicated a wide range of mean values among the characters. Under non-stressed condition, the mean value of grain yield (4.42t/ha), plant height (91.33cm), number of kernels per spike (33.89), spike length (11.24cm), spikelets per spike (24.80) and thousand seed weight (48.27g) was found to be maximum. Genotypes with the highest grain yield were included in this cluster. But under stressed condition, only a thousand seed weight (45.27g) was the maximum. Cluster II accounts for 21.43% and 22.22% of the population and includes 21 and 22 genotypes under non-stressed and stressed conditions respectively. In this cluster, the mean values of characters such as canopy temperature (30.07), days to 50% heading (55.46) under non-stressed condition, and biomass weight (700g) under stressed condition were found to be maximum. Cluster III represents 15.31% of the total 98 genotypes and comprises 15 genotypes under non-stressed condition. Under stressed conditions, 19.19% of the total 99 genotypes comprised 19 genotypes The genotypes from this cluster could be picked up for high leaf chlorophyll concentration measurement (50.63) and shortest days to 50% flowering (73.38) under non-stressed condition while under stressed condition for the higher number of spikelets per spike (20.30), number of kernels per spike (28.48), and leaf chlorophyll concentration measurement (46.71).

Cluster IV comprised 26 genotypes that contributed 26.53% of the total accession under non-stressed condition and 13 genotypes contributed 13.3% of the total accession under stressed condition. At non-stressed conditions, most of the genotypes in this cluster have a medium value except days to maturity which take 111.63 which is the maximum. For the stressed condition, this cluster comprised of the cooler canopy (30.21), maximum yield (3.64t/ha), shortest days to 50% flowering (50.21 days), and high spike length (8.32 cm). Under stressed condition, this cluster comprises those with the highest grain yield. Cluster V represents 15.31% of the total 98 genotypes and comprised 15 genotypes under non-stressed conditions as clusters I and III. Those genotypes with low grain yield at non-stressed conditions were incorporated into this cluster. Under stressed conditions, 14.14% of the total 99 genotypes comprised 14 genotypes. The shortest days to maturity (98.48) under non-stressed condition and the highest number of tillers (16.45) under stressed condition were observed in this cluster. Also, the lowest yield (1.56, 0.78) and biomass (762.48, 433.97) were observed under non-stressed and stressed conditions respectively. The sixth cluster accounts for 6.12% and 24.24% of the population and includes 6 and 24 genotypes under non-stressed and stressed conditions respectively. Under non-stressed condition biomass yield have the maximum value in this cluster while shorter days (55.85) for 50% flowering, longest plant height (86.69cm), and shorter days of maturity (91.69), were observed under stressed condition. Also, those genotypes with lower yield potentials were categorized in this cluster.

Table 3.5. Mean performance of genotypes in respective clusters for different traits under non-stressed and stressed conditions.

	Non-stressed						Stressed					
	C1	C2	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6
BY	758.88	814.47	896.81	766.83	762.48	961.86	532.88	700.95	613.59	602.41	433.97	646.79
CT	30.57	30.07	35.24	31.2	32.84	33.68	39.22	33.9	32.73	30.21	32.38	33.42
DTF	73.57	73.54	73.38	79.67	65.82	82.64	72.16	65.73	62.85	59.99	67.09	55.85
GY	4.42	2.62	2.62	2.44	1.56	1.58	1.94	1.58	1.83	3.64	0.78	1.31
DTH	58.87	55.46	56.72	64.29	53.97	70.06	55.64	51.68	52.28	50.21	55.64	47.41
PH	91.33	82.16	90.35	82.79	84.11	73.76	81.13	76.64	79.88	86.44	85.98	86.69
KPS	33.89	26.34	26.07	24.6	22.41	21.92	29.63	20.73	28.48	28.23	15.88	17.85
DTM	107.63	107.13	104.98	111.63	98.45	100.08	101.84	97.23	96.13	93.8	96.76	91.69
LCC	47.45	45.42	50.63	44	47.28	47.82	44.13	38.2	46.71	41.8	41.41	43.45
SL	11.24	8.68	8.14	7.89	7.75	8.14	6.15	6.01	6.74	8.32	5.67	6.04

SPS	24.8	19.33	20.97	21.62	20.91	21.19	21.63	16	20.3	19.54	15.16	15.22
NT	20.35	19.67	17.69	17.97	20.81	20.8	13.78	14.79	14.76	15.9	16.45	16.52
TSW	48.27	46.51	40.28	39.16	36.04	27.08	45.27	39.43	37.77	36.93	35.74	38.38

The intracluster and intercluster distance *(*D^2) presented in Table 3.6 indicated that the intracluster distance was maximum in cluster I (D^2 = 7.68) and (D^2 = 8.19) under non-stressed and stressed conditions respectively, followed by cluster V (D^2 = 7.74) under non-stressed and cluster III (D^2 = 7.06) under stressed condition. Cluster VI has the smallest intracluster distance with (D^2 = 5.29) and (D^2 = 5.32) under non-stressed and stressed conditions respectively. The low intracluster distance indicated that the landraces in the clusters were closely related due to the heterogeneous nature of the landrace within a cluster and the presence of less genetic diversity within the cluster (Aman et al., 2020). Also, the intercluster distance was found to be maximum between clusters I and IV (D^2 = 11.02) followed by clusters I and III (D^2 = 9.80) under non-stressed condition, and clusters I and II (D^2 = 10.33) followed by cluster I and VI (D^2 = 10.19) under stressed condition. The minimum inter-cluster distance was obtained between cluster II and IV (D^2 = 6.97) under non-stressed and between cluster II and IV (D^2 = 6.99) under stressed condition which is an indication of the presence of less genetic diversity among this cluster.

Table 3.6. Intracluster (bold diagonal) and intercluster distance among 6 clusters under non-stressed condition (right) and stressed condition (left)

	c1	c2	c3	c4	c5	c6
c1	**7.687**					
c2	8.679	**5.813**				
c3	9.801	7.826	**7.270**			
c4	8.734	6.979	8.403	**6.936**		
c5	11.026	8.482	9.423	8.726	**7.746**	
c6	9.534	7.454	8.080	7.619	8.032	**5.291**

	c1	c2	c3	c4	c5	c6
c1	**8.195**					
c2	10.331	**6.952**				
c3	8.373	8.512	**7.069**			
c4	8.986	8.786	7.736	**6.487**		
c5	8.756	7.660	8.202	8.679	**5.771**	
c6	10.192	7.595	8.011	8.534	6.994	**5.328**

3.3.5. Estimation of variability parameters

The genetic Variability Analysis of each trait in the experiment is shown in Table 3.7. The partition of genotypic variation is higher than the environmental variation for all traits at non-stressed conditions, except for canopy temperature. Under stressed condition, except for leaf

chlorophyll concentration measurements and canopy temperature measurements, there is a higher partition of genotypic variation. An argumentative result reported by Ahmad et al. (2008) indicates the presence of high level of the genotypic variance than the environmental variance for most of the traits under the study such as days to heading, day to maturity, spikelets per spike, grains per spike, spike weight, thousand kernel weight, spike length, plant height, and biological yield. In connection to their coefficient of variation, the GCV values were greater than ECV values in all studied traits except canopy temperature under non-stressed condition and canopy temperature and leaf chlorophyll concentration measurement under stressed condition. This indicated that the environmental effect was small for the expression of most characters other than the aforesaid characters, which also supports the findings of Kumar et al. (2018b).

For the genotypic and phenotypic coefficient of variation analysis, there is a low to medium variation except for days to maturity, leaf chlorophyll concentration measurement, and canopy temperature measurement in both conditions and days to flowering under non-stressed condition and thousands of seed weight in the stressed condition. In line with this study Demelash et al. (2013) and Dave et al. (2021) also observed that moderate (10-20%) genotypic coefficients of variation for the number of grains per spike, number of effective tillers per plant, and thousand seed weight and lower (<10%) genotypic coefficient of variation for days to maturity. Also Nikkhahkouchaksaraei and Martirosyan (2017), and Alemu et al. (2020c) recorded higher PCV and GCV values for the number of thousand seed weights, kernels per spike, and grain yield.

The traits studied in the present investigation (Table 3.7) expressed moderate to high heritability in a broad sense (h^2b) estimates ranging from 32.84 to 97.87 percent. Among the traits evaluated under non-stressed condition except for leaf chlorophyll concentration measurement (58.22%), and canopy temp. measurements (43.91%) which showed moderate h^2b, all others showed higher h^2b (>60%). Similarly, under stressed condition except for canopy temperature measurements (32.84%) and leaf chlorophyll concentration measurement (39.73%) which showed moderate h^2b, while all others showed high h^2b (>60%). A similar result was reported on high h^2b estimates for days to heading, thousand seed weights, days to heading, the number of spikelets per spike, days to maturity, spike length, and grain yield (Mohammed et al., 2011,

Hailu et al., 2016, Zemede et al., 2019, Alemu et al., 2020c). It is known that if the h^2b of a character is very high, it is less influenced by the environment than characters that are regulated by additive gene action, and direct selection is expected to show improvement in such traits. The plant breeder, therefore, may make his selection safely based on the phenotypic expression of these traits in the individual plant by adopting simple selection methods (Acquaah, 2009).

High expected genetic advance in percent of mean (Jeyabalasingh et al.) estimates was obtained for most of the traits in both non-stressed and stressed conditions. The intermediate to high estimate of h^2b and relatively high estimate of GAM were observed in days to heading, the number of tillers, spike length, kernels per spike, grain yield at non-stressed conditions, and in the number of tillers, spike length, kernels per spike, spikelets per spike, biomass yield, and grain yield under stressed condition. This result is in line with Bayisa et al. (2020) and Dave et al. (2021) in which high h^2b and GAM were reported for spikelets per spike, kernels per spike, and grain yield. This suggested most likely that h^2b is due to additive genetic effects and selection could be effective in early segregating generations for these traits and the possibility of improving durum wheat grain yield through direct selection for grain yield-related traits(Abinasa et al., 2011, Dagnaw et al., 2022, Dragov et al., 2022).

Table 3.7. Genetic Variability Analysis for the thirteen yield and yield-related traits of durum wheat varieties under non-stressed and stressed conditions in 2020/21

Env		DTH	NT	PH	DTM	SL	DTF	SPS	TSW	KPS	BY	GY	LCC	CT
Non stressed	**Mean**	59.18	19.26	84.77	106.31	8.6	74.52	21.38	41.08	26.12	806.99	2.62	46.58	31.88
	Min	43.1	11.67	58.93	67.68	6.1	51.6	10.31	21.4	17.29	487.44	0.68	33.6	26.23
	Max	77.35	24.53	106.56	119.5	13.04	92.6	28.68	59.62	48.19	1092.94	6.13	59.92	41.38
	PV	57.31	6.85	148.84	60.87	2	55.12	7.33	60.59	25.09	16924.1	1.13	16.52	9.71
	GV	48.44	5.1	89.27	54.73	1.67	38.39	4.82	35.66	17.76	10062.85	1.01	9.62	4.26
	EV	8.87	1.75	59.56	6.14	0.34	16.72	2.51	24.94	7.33	6861.25	0.12	6.9	5.45
	GCV	11.76	11.72	11.15	6.96	15.01	8.31	10.27	14.54	16.13	12.43	38.27	6.66	6.48
	PCV	12.79	13.59	14.39	7.34	16.45	9.96	12.66	18.95	19.17	16.12	40.45	8.72	9.77
	ECV	5.03	6.87	9.1	2.33	6.74	5.49	7.41	12.16	10.36	10.26	13.08	5.64	7.32
	h^2b	84.52	74.42	59.98	89.91	83.2	69.66	65.77	58.85	70.79	59.46	89.54	58.22	43.91
	GA	13.2	4.02	15.1	14.47	2.43	10.67	3.67	9.45	7.31	159.58	1.96	4.88	2.82
	GAM	22.3	20.86	17.81	13.61	28.24	14.32	17.18	23	28	19.77	74.71	10.48	8.86
Stressed	**Mean**	51.46	16.33	81.92	95.48	6.41	62.73	17.38	38.42	22.44	605.45	1.75	42.45	33.23
	Min	36.75	6.8	65.23	81.55	4.48	46.55	9.89	28.14	8.39	194.74	0.11	31.8	27.92
	Max	65.75	21.05	102.45	116.55	10.34	85.05	26.36	51.58	39.79	1008.44	5.47	53.89	44.93
	PV	33.91	63.09	93.17	34.61	1.55	53.64	10.52	11.22	40	43909.99	0.95	23.06	8.79
	GV	26.61	61.75	70.26	26.49	1.1	40.42	7.79	7.39	30.2	29569.66	0.85	7.57	3.49
	EV	7.3	1.34	22.91	8.13	0.44	13.22	2.73	3.84	9.79	14340.33	0.09	15.49	5.3
	GCV	10.02	48.13	10.23	5.39	16.37	10.14	16.06	7.07	24.49	28.4	52.97	6.48	5.62
	PCV	11.32	48.65	11.78	6.16	19.39	11.68	18.67	8.72	28.18	34.61	55.82	11.31	8.92
	ECV	5.25	7.1	5.84	2.99	10.39	5.8	9.52	5.1	13.94	19.78	17.62	9.27	6.93
	h^2b	78.47	97.87	75.41	76.53	71.27	75.36	74.01	65.81	75.52	67.34	90.04	32.84	39.73
	GA	9.43	16.04	15.02	9.29	1.83	11.39	4.95	4.55	9.85	291.11	1.81	3.25	2.43
	GAM	18.32	98.22	18.33	9.73	28.51	18.15	28.5	11.84	43.9	48.08	103.68	7.66	7.31

PV, GV and EV: Phenotypic, genotypic and environmental variance, GCV, PCV and ECV: coefficient of genotypic, phenotypic and environmental variation, h2b: heritability in a broad sense, GA: genetic advance and GAM: genetic advance in percent mean. Env: Environment.

3.3.5. Correlation coefficients

The correlation coefficient indicated in Tables 3.8 and 3.9 shows a significant positive correlation between days to head with days to flowering (0.41, 0.679) and maturity (0.33, 0.25) in both conditions separated by commas respectively. The number of tillers is negatively correlated with all traits studied except biomass yield, leaf chlorophyll concentration, and canopy temperature in the non-stressed condition, but positively correlated with all traits except plant height and grain yield in the stressed condition. Grain yield was significantly and positively correlated with spike length (0.55, 0.74), spikelets per spike (0.51, 0.54), and kernel per spike (0.42, 0.53) under both conditions.

Table 3.8. The correlation coefficient between yield component traits and grain yield in durum wheat at non-stressed conditions.

	DTH	TN	PH	DTF	DTM	SL	SPS	KPS	TSW	BY	GYLD	LCC	CT
DTH	1												
TN	0.12	1											
PH	-0.03	-0.08	1										
DTF	0.41	-0.68	0.10	1									
DTM	0.33	-0.55	0.05	0.77	1								
SL	-0.01	-0.10	0.12	0.08	0.06	1							
SPS	0.23	-0.05	0.18	0.11	0.17	0.37	1						
KPS	0.01	-0.23	-0.08	0.21	0.27	0.23	0.25	1					
TSW	-0.19	-0.20	0.13	0.11	0.15	0.35	0.05	0.26	1				
BY	-0.02	0.18	-0.05	-0.06	-0.14	-0.01	-0.07	0.07	-0.15	1			
GYLD	-0.08	-0.24	0.21	0.18	0.23	0.55	0.52	0.43	0.40	0.02	1		
LCC	0.10	-0.05	-0.02	0.11	-0.04	0.13	0.00	0.00	0.02	-0.08	-0.07	1	
CT	0.06	0.09	-0.13	-0.03	-0.11	-0.21	-0.16	-0.08	-0.18	0.09	-0.25	0.40	1
Mean	59.18	20.42	84.77	73.36	106.31	8.6	21.38	26.12	41.08	806.99	2.62	46.58	31.88
Stdev	7.46	8.82	12.07	10.52	7.75	1.47	3	5.28	8.2	141.87	1.17	4.54	3.44

Grain yield is moderately correlated with thousand seed weights (0.402) in the non-stressed condition but low correlation (0.053) in the stressed condition. The positive correlation coefficients of grain yield with most of the traits imply that improving one or more of the traits could result in a high grain yield for durum wheat. Days to heading (-0.077, -0.023) in both conditions, tiller number (-0.235) under non-stressed condition, and days to flowering (-0.011)

and maturity (-0.027) under stressed condition have a negative correlation with grain yield. Demelash et al. (2013) and Mecha et al. (2017) obtained similar results of a highly significant positive correlation of grain yield with most of the yield-attributed traits but negatively correlated with days to head and days to maturity in bread wheat. The negative association of grain yield with days to heading in both conditions and days to flowering and maturity, especially in the water-stressed condition, suggests that early heading and maturing genotypes would give a high grain yield (Al-Karaki, 2012).

Table 3.9. The correlation coefficient between yield component traits and grain yield in durum wheat under stressed condition.

	DTH	TN	PH	DTF	DTM	SL	SPS	KPS	TSW	BY	GYLD	LCC	canopy
DTH	1												
TN	0.13	1											
PH	-0.14	0.12	1										
DTF	0.68	0.11	-0.01	1									
DTM	0.26	0.23	0.16	0.55	1								
SL	0.04	0.03	0.11	0.12	0.06	1							
SPS	0.09	0.04	0.06	0.10	0.04	0.47	1						
KPS	0.16	-0.18	0.01	0.19	-0.01	0.46	0.43	1					
TSW	0.12	0.18	-0.10	0.11	0.07	-0.03	0.06	0.02	1				
BY	0.02	0.07	-0.07	-0.16	-0.13	0.07	-0.10	-0.08	0.14	1			
GYLD	-0.02	0.01	0.12	-0.01	-0.03	0.75	0.54	0.53	0.05	0.10	1		
LCC	0.16	0.06	0.08	-0.02	0.11	0.28	0.21	0.17	0.24	0.12	0.22	1	
CT	0.02	-0.10	-0.14	0.09	0.03	-0.29	-0.14	-0.12	0.43	0.02	-0.32	0.09	1
Mean	56.182	15.626	82.799	67.901	103.16	6.421	21.44	20.08	38.37	610.376	72.414	42.441	33.211
Stdev	6.132	2.764	6.765	7.288	5.979	1.262	2.765	6.099	3.297	211.855	41.938	4.764	2.946

BY: Biomass yield, CT: canopy temperature measurement, DTF: days to 50% flowering, GY: grain yield per plot, DTH: days to heading, PH: plant height, KPS: kennels per spike, DTM: Days to maturity, LCC: leaf chlorophyll concentration, SL: spike length, SPS: the number of spikelets per spike, NT: number of tillers, and TSW: thousand seed weight

3.4. CONCLUSION AND RECOMMENDATIONS

This study revealed considerable genetic variability among Ethiopian durum wheat landraces across both drought-stressed and non-stressed environments, showing their potential for drought-resilient breeding. Significant differences were observed for key agronomic traits, with high heritability and genetic advance in yield-contributing traits such as tiller number, spike length, kernel number per spike, and grain yield, especially under stress conditions. These findings confirm that genetic factors predominantly influence trait expression under both conditions, making selection effective. Principal component and cluster analyses further clarified the genetic structure of the landraces, enabling the identification of promising parental lines for hybridization. The wide inter- and intra-cluster distances suggest the existence of genetically diverse materials suitable for enhancing drought tolerance and productivity. A total of seven landraces out yield the checks namely, ETDW/15DZ4, 34493, ETDW/15DZ23, 34522, MCD3-14, 34217, and 31831. From those out yielded landraces ETDW/15DZ23, 34522, ETDW/15DZ4, 34493 under non-stressed condition, and ETDW/15DZ4 34493 ETDW/15DZ23 under stressed condition surpass all four checks. The overall results reveal that Ethiopian durum wheat landraces represent a valuable resource for improving adaptation and yield stability in drought-prone environments and should be strategically exploited in breeding programs.

CHAPTER FOUR

DROUGHT TOLERANCE EVALUATION IN ETHIOPIAN DURUM WHEAT (TRITICUM TURGIDUM SUBSP. DURUM) BASED ON STRESS INDICES

Abstract

Drought stress is a critical factor that limits durum wheat production and productivity. Identifying drought-tolerant (Triticum turgidum subsp. durum) varieties is essential for sustainable durum wheat production. Various approaches are used to select and characterize drought-tolerant wheat genotypes. This study evaluated nine drought tolerance indices based on grain yields under both non-stress and stress conditions. Landraces ETDW/15DZ23, 34493, ETDW/15DZ4, 34522, and 34217 exhibited high grain yields under both non-stress and stressed conditions for two cropping seasons. These landraces also showed high values for Stress Tolerance Index (STI), Yield Index (YI) and Yield Stability Index (YSI), making them valuable for wheat breeding in stress-prone environments. A significant positive correlation (r = 0.88) was observed between grain yield under non-stressed (Yp) and stressed (Ys) conditions. Except for Stress Susceptibility Index (SSI) and Tolerance (TOL), which showed a negative correlation, all other indices showed a significant positive correlation with grain yield under Yp and Ys conditions emphasizing the use of stress indices as effective predictors for selecting high-yielding, drought-tolerant genotypes.

Keywords: Durum wheat; Drought tolerance; Grain yield; Non-stressed condition; Stressed condition; Stress indices

4.1. INTRODUCTION

Plants, under different environmental conditions, are constantly exposed to stress, leading to changes in their normal physiological functions. This stress affects all plants, including economically important cereals (Khalili et al., 2018). One significant abiotic stressor is drought, which is a major limiting factor for agricultural productivity. Drought inhibits plant growth by reducing water absorption and nutrient uptake (Upadhyaya et al., 2017). In durum wheat drought

stress triggers a range of metabolic changes including the accumulation of osmoprotectant like proline, alterations in photosynthetic activity, and the modulation of antioxidant enzyme activities (Hossain and Fujita, 2010). Antioxidant enzymes like Catalase (CAT) and Peroxidase (POD) play a crucial role in mitigating abiotic stresses in plants by scavenging Reactive Oxygen Species (ROS). Amino acids such as arginine and aspartic acid are key components of these enzymes, showing positive correlations with enzyme activity (Iwaniuk et al., 2022). These metabolic adjustments help the plant to maintain cellular homeostasis, protect cellular structures, and manage oxidative stress. However, these changes often come at the cost of reduced growth and yield, particularly when drought occurs during critical growth stages (Senapati et al., 2019, Zhang et al., 2018).

Drought stress negatively impacts wheat growth and yield. It affects plant growth and development at different growth stages, leading to crop losses (Akram, 2011). Greater yield reductions occur with drought stress during the reproductive stage compared to vegetative stages. (Zhang et al., 2018, Senapati et al., 2019). Senapati et al., (2019) reported a mean yield advantage of 28% to 37% higher in drought-tolerant varieties compared to drought-sensitive wheat during reproductive stages. Identifying drought-tolerant wheat genotypes involves evaluating various stress tolerance indices. The Stress Tolerance Index (STI) is useful for determining the high yield and stress tolerance potential of genotypes (Fernandez, 1992). Fischer and Maurer (1978) proposed the SSI to measure yield stability, capturing changes in both potential and actual yields in variable environments. Genotypes with lower SSI values exhibit higher levels of drought tolerance. Some breeders are interested in the relative performance of crops due to variations in drought stress severity in field environments over the years. They tend to use the Geometric Mean Productivity (GMP) to identify drought-tolerant genotypes with high yield potential (Rosielle and Hamblin, 1981, Fernandez, 1992).

Rosielle and Hamblin (1981) defined Tolerance (TOL) as the yield difference between non-stress and stress environments, and Mean Productivity (MP) as the average yield of genotypes under stress and non-stressed conditions. Moran, et al., (1994) also used SSI to evaluate drought tolerance in different wheat genotypes, finding significant variation in SSI for genotypes which they ranked accordingly. The Yield Stability Index (YSI), introduced by Bouslama and

Schapaugh (1984), evaluates a given genotype's drought tolerance by calculating the grain yield ratio in stressed environments to non-stressed conditions.

These drought tolerance indices are analyzed using different multivariate statistical approaches to obtain more precise selection indicators for drought tolerance. Many authors have studied the relationships between these indices with grain yield under stress and non-stressed conditions in various crops (Khalili et al., 2016, Semahegn et al., 2020, Zhang et al., 2018). Based on these studies, the STI, GMP, and MP are considered useful indices for screening drought tolerance (Darzi-Ramandi et al., 2016, Ghaffari et al., 2012). Aliakbari et al., (2013) and Khalili et al., (2016) found that the TOL and MP indices are the most appropriate for screening drought-tolerant genotypes. Plant breeders are interested in genotypes that produce high yields under stressed conditions and utilize the outputs from drought tolerance selection indices to identify high-yielding genotypes. Correlation analysis, principal component analysis, and biplot analysis have been used to screen drought-tolerant cultivars (Khalili et al., 2016). Correlation analysis reveals significant associations between each index with grain yield and with each other.

In Ethiopia, durum wheat is primarily grown as a rain-fed crop by smallholder farmers in the highlands. Typically, only one crop is planted during the main rainy season (meher), which starts in June (Dibaba, 2019). The short rainy season (belg), beginning in March, is less predictable in most regions, except for areas like the Bale Zone of Oromia, where rainfall patterns are bimodal (Taffesse et al., 2012, Gizaw and Assegid, 2021). In 2022, Ethiopia’s durum wheat production reached 5.4 million tons. Between 2017 and 2022, production grew at an average annual rate of 3.2%, with fluctuations. Peak production occurred in 2020 at 5.5 million tons, though growth slowed slightly in the following years. By 2022, the harvested area totaled 1.8 million hectares, with an average annual growth of 1.4% since 2017 (IndexBox, 2023).

Ethiopia has a rich diversity of durum wheat landraces, which occupy a significant place in the gene bank due to their varied quality and agro-morphological characteristics (Alemu et al., 2020c, Dagnaw et al., 2022, Dinsa, 2023, Dukamo et al., 2023). Despite being one of the centers of origin and diversity for durum wheat, Ethiopia faces challenges such as changing climatic

conditions (Broccanello et al., 2023, Giorgis et al., 2018) and a lack of improved varieties tailored for different environmental conditions (Belete, 2021, Bergh et al., 2019), leading to poor harvests. These factors contribute to food insecurity and declining food reserves in some regions.

Studies on the genetic diversity of Ethiopian durum wheat landraces in response to drought tolerance are limited. The selection of drought-tolerant durum wheat genotypes is of paramount importance for expanding production into untapped potential areas. Evaluating the effectiveness of drought tolerance indices is essential for screening and identifying genotypes with a good combination of important agronomic traits. This approach aims to improve yields under drought conditions and recommend further detailed studies and cultivation strategies to leverage the diverse genetic resources available in Ethiopia. By focusing on the diversity of Ethiopian durum wheat landraces and their response to moisture stress, this study contributes to the development of robust, drought-tolerant varieties that can thrive in varying environmental conditions, ensuring better harvests and food stability.

4.2. MATERIALS AND METHODS

4.2.1. Description of study area

The study used an augmented block design (Federer, 1956) at two test sites: Debrezeit and Dera during the 2020/21 and 2022/23 cropping seasons. The study area was described similarly to Chapter Three, Section 3.2.1.

4.2.2. Experimental Plant Materials

This study initially included 104 durum wheat genotypes, comprising 100 landraces and four standard checks (Alemtena, Fetan, Kuami, and Ude). However, due to germination viability issues, the analysis was conducted on 99 genotypes during the 2021/22 cropping season and 103 genotypes during the 2022/23 cropping season. The source of experimental seeds and screening method were similar to those described in chapter three, section 3.2.2.

4.2.3. Experimental Design and Field Management

The experiment was laid out in 24 × 5 augmented block designs with an area of 168 m2 (24 × 7m). The space between rows was 20 cm, while the space between blocks was 50 cm for both locations and across the seasons. All other field management practices were the same as in Chapter Three, Section 3.2.3.

4.2.4. Data collection and Analysis

Data on grain yield from drought-stressed and non-stress environments were recorded, and drought tolerance indices were calculated using a toolkit to estimate plant abiotic stress indices (Pour-Aboughadareh et al., 2019) according to the formulae presented in Table 4.1. The analysis of variance, genotypic variance analysis, Pearson correlations, and cluster analysis using Ward's method were carried out using R software.

Table 4.1. Stress tolerance formula and selection patterns

NO	Indices	Formula	Reference
1	Tolerance	$Tol = Y_p - Y_s$	(Rosielle and Hamblin, 1981)
2	Mean Productivity	$MP = \frac{Y_p + Y_s}{2}$	(Rosielle and Hamblin, 1981)
3	Geometric Mean Productivity	$GMP = \sqrt{Y_s \times Y_p}$	(Fernandez, 1992)
4	Harmonic Mean	$HM = \frac{2(Y_s \times Y_p)}{(Y_s + Y_p)}$	(Bidinger et al., 1987),
5	Stress Susceptibility Index	$SSI = \frac{1 - (Y_s/Y_p)}{1 - (\bar{Y}_s/\bar{Y}_p)}$	(Fischer and Maurer, 1978)
6	Stress Tolerance Index	$STI = \frac{(Y_s \times Y_p)}{(\bar{Y}_p)^2}$	(Fernandez, 1992)
7	Yield Index	$YI = \frac{Y_s}{\bar{Y}_s}$	(Gavuzzi et al., 1997)
8	Yield Stability Index	$YSI = \frac{Y_S}{Y_P}$	(Bouslama and Schapaugh, 1984)

9	Relative Susceptibility Index	$RSI = \frac{(Y_s/Y_p)}{(\bar{Y}_s/\bar{Y}_p)}$	(Fischer and Wood, 1979)

4.3. RESULT

4.3.1. Analysis of Variance

The average yield under non-stressed conditions was 2.66 t/ha in 2020/21 and 2.59 t/ha in 2022/23. Under stressed conditions, the yield dropped to 1.91 t/ha in 2020/21 and 1.79 t/ha in 2022/23. This indicates an average reduction in grain yield of approximately 28.23% and 30.9% due to drought stress. Table 4.2 A and Table 4.2 B provides a detailed view of the mean squares obtained from an Analysis of Variance (ANOVA) for various traits, aimed at assessing their significance levels under different sources of variation.

The genotypes studied showed highly significant mean square values for Yp and Ys across both cropping seasons. For the 2020/21 season, the mean square values were 1.3** and 1.19** ($P \leq 0.01$), while for the 2022/23 season, the values were 1.41** and 1.03* ($P \leq 0.01$, $P \leq 0.05$, respectively). The ANOVA results for Yp and Ys in both landraces and check varieties followed a consistent trend across the two cropping seasons, indicating stability in performance under different conditions.

Table 4.2 A. Analysis of variance for grain yield (ton/h) under non-stressed (Yp) and drought-stressed (Ys) conditions and drought tolerance indices of wheat genotypes for the 2020/21 cropping season.

Source of var.	Landraces (EB)	Check genotypes	Landrace vs. Check	Test treatments	Block (ET)	Resid.	adj. mean	CV
df	95	3	1	91	4	12		
Yp	1.3**	2.08**	16.47**	1.08**	1.73**	0.12	2.66	12.26
Ys	1.19**	0.79**	24.44**	0.96**	0.89**	0.09	1.76	15.93
TOL	0.27*	0.39*	0.78*	0.26*	0.33*	0.09	0.9	34.7
MP	1.18**	1.34**	20.27**	0.96**	1.22**	0.08	2.21	12.2
GMP	1.21**	1.29**	21.98**	0.98**	1.2	0.08	2.14	12.49
HM	1.25**	1.24**	23.55**	1**	1.17**	0.08	2.08	12.74
SSI	0.32**	0.11ns	5.69**	0.27**	0.15ns	0.07	1.14	25.08

STI	0.7**	0.96**	8.69**	0.61**	0.85**	0.08	0.71	35.13
YI	0.32**	0.21**	6.55**	0.26**	0.24**	0.03	0.91	15.97
YSI	0.03**	0.01ns	0.54**	0.03**	0.02ns	0.01	0.65	12.36
RSI	0.06**	0.02ns	1.15**	0.05**	0.03ns	0.01	0.94	12.18

Moreover, the indices MP, GMP, TOL, and SSI also showed highly significant mean square values. For the 2020/21 season, landraces recorded values of 1.18**, 1.21**, 0.27*, and 0.32**, respectively, while test genotypes exhibited values of 0.96**, 0.98**, 0.26*, and 0.27**. A similar pattern was observed in the 2022/23 season, where landraces displayed values of 1.07**, 1.07**, 0.6*, and 0.52**, and test genotypes showed 0.84**, 0.82**, 0.6*, and 0.53**, all significant at the ($P \leq 0.01$) level. These results highlight the robustness of these indices in assessing genotype performance under stress conditions across multiple seasons.

Almost all the drought indices showed a highly significant difference among the studied genotypes in both cropping seasons, but checks and blocks showed non-significant differences for most traits. The maximum grain yield was observed in ETDW/15DZ 023 under both non-stressed and stressed conditions for both cropping seasons. The minimum yield was observed in landrace 33244 under both non-stressed and stressed conditions in the 2020/21 cropping season. For the 2022/23 cropping season, the minimum yield was shown in landrace 31703 under non-stressed conditions and in landrace 34566 under stressed conditions, as indicated in Supplementary Tables 5 and 6.

Table 4.2 B. Analysis of variance for grain yield (ton/h) under non-stressed (Yp) and drought-stressed (Ys) conditions and drought tolerance indices of wheat genotypes for the 2022/2023 cropping season.

Source of var.	Landraces (EB)	Check genotypes	Landrace vs. Check	Test treatments	Block (ET)	Resid.	adj. mean	CV
df	102	3	1	98	4	12		
Yp	1.41**	1.81**	22.77**	1.18**	1.25**	0.2	2.59	16.4
Ys	1.03*	0.47ns	25.13**	0.79*	0.78ns	0.32	1.79	28.96
TOL	0.6**	1.11**	0.06ns	0.6**	0.34ns	0.17	0.8	51.48
MP	1.07**	0.85*	23.96**	0.84**	0.93*	0.22	2.19	19.84
GMP	1.07**	0.77ns	25.47**	0.82**	0.93*	0.23	2.12	21.25
HM	1.09**	0.7ns	26.71**	0.83*	0.93*	0.25	2.06	22.64
SSI	0.52**	0.64*	1.71**	0.53**	0.19ns	0.14	0.97	39.84
STI	0.64*	0.66ns	11.66**	0.52*	0.73*	0.19	0.71	52.99

YI	0.27*	0.12ns	6.64**	0.21*	0.21ns	0.08	0.92	29
YSI	0.04**	0.05*	0.14**	0.04**	0.02ns	0.01	0.72	14.77
RSI	0.09**	0.11*	0.28**	0.09**	0.03ns	0.02	1.01	14.67

Where ns P > 0.05; * P <= 0.05; ** P <= 0.01; DF: degree of freedom; Yp grain yield of genotypes under non-stress condition; Ys grain yield of genotypes under drought stress condition; TOL tolerance; MP mean productivity; GMP geometric mean; HM: harmonic mean; STI: stress tolerance index; SSI: stress susceptibility index; YI yield index; YSI yield stability index; RSI relative stress index.

4.3.2. Drought tolerance indices.

In this study, various stress indices such as tolerance (TOL), mean productivity (MP) geometric mean (GMP), harmonic mean (HM), stress susceptibility index (SSI), stress tolerance index, (STI), yield index (YI), yield stability index (YSI) and relative stress index (RSI) were calculated based on yield under non-stressed and drought stressed conditions across two cropping seasons (supplementary Tables 5 and 6). Stress tolerance measures the absolute yield loss due to stress, while SSI quantifies a genotype's susceptibility to stress, with lower values, which is better tolerance. Conversely, higher YSI and RSI values indicate superior stress tolerance.

The highest values for TOL and SSI, and the lowest values for YSI and RSI, were observed in landraces ETDW/15DZ 043, 34295, 34510, and 34625 for the 2020/21 cropping season, and in 31806, 34625, MCD12-30, and DZDW1702,04 for the 2022/23 cropping season. These genotypes were identified as drought-susceptible because they had high grain yield under non-stressed conditions and low yield under drought-stressed conditions, making them suitable for sowing in non-stressed environments.

Landraces ETDW/15DZ 04, 33403, 34493, and 31778 for the 2020/21 season, and 33300, DW-NVT LM 11, 31761, and DZDW17004 for the 2022/23 season, with low TOL and SSI and high YSI and RSI values, were considered more tolerant to drought. These landraces performed less well under both conditions, with the declined values of these indices resulting from minimal yield differential between the two conditions. Therefore, low values do not necessarily mean high performance and genotype grain yield should be taken into consideration.

The STI compares a genotype's stress performance to both its non-stress performance and the mean performance of all genotypes, with higher STI values there was superior stress tolerance. MP averages yield across both conditions, offering an overall performance. GMP and HM provide refined averages that reduce the influence of extreme values and emphasize consistency. Landraces ETDW/15DZ 023, 34493, ETDW/15DZ 04, and 34522 for the 2020/21 season, and ETDW/15DZ 023, 34493, ETDW/15DZ 04, and 34217 for the 2022/23 season, had the highest values for STI, MP, GMP, and HM. These are considered the most stable and productive genotypes under both conditions. Conversely, the lowest values for these stress indices were shown by DW-PVT LM 8, 31761, and 33244, and 34310, 34566, and 31703 for the 2020/21 and 2022/23 cropping seasons, respectively.

Yield index and YSI further evaluate performance under stress, with YI values over 1 was strong performance, as seen in genotypes ETDW/15DZ 023, ETDW/15DZ 04, 34493, and 34217, which had YI values greater than 2.5 for both cropping seasons. Higher YSI values indicate greater stability, and RSI compares performance under stress to favorable conditions, with lower RSI values, better relative performance under stress.

4.3.3. Heritability, phenotypic, and genotypic coefficient of variation

Table 4.3 provides a comprehensive evaluation of various genetic and phenotypic parameters, including genetic variance (GV), phenotypic variance (Prasad et al.), heritability (h^2b), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), and genetic advance (GA) across nine drought tolerance indices for the studied durum wheat genotypes over the 2020/21 and 2022/23 cropping seasons.

In the 2020/21 season, the mean yield under non-stressed conditions Yp was 2.66 t/ha, with a high GV of 0.96 and a PV of 1.08. The GCV and PCV were 36.96% and 39.15%, respectively indicating important genetic diversity. The h^2b was 89.12%, and the GA was 1.91, with a GAPM of 71.98%. Under stressed conditions (Ys), the mean yield was 1.76 t/ha, with a GV of 0.86 and a PV of 0.96. The GCV and PCV were higher at 52.8% and 55.61%, respectively, with a heritability of 90.14% and a GA of 1.82, shows a significant potential for genetic improvement

under stress. TOL showed a mean of 0.9, with moderate heritability (65.53%) and a GA of 0.69. MP, GMP, and HM all exhibited high heritability values above 90%, suggesting that these traits are strongly inherited. SSI and STI had heritability values of 73.53% and 86.54%, respectively, with the STI showing a particularly high GAPM of 186.71%. YI and YSI also showed high heritability, with YI having a GAPM of 103.45%.

Table 4.3. Genetic variability analysis of yield under stressed and non-stressed conditions based on nine drought indices of durum wheat genotypes for 2020/21 and 2022/23 cropping seasons.

year	Trait	Mean	PV	GV	GCV	PCV	h^2b	GA	GAPM
2020/21	Yp	2.66	1.08	0.96	36.96	39.15	89.12	1.91	71.98
	Ys	1.76	0.96	0.86	52.8	55.61	90.14	1.82	103.41
	TOL	0.9	0.26	0.17	46.33	57.23	65.53	0.69	77.36
	MP	2.21	0.96	0.87	42.21	44.19	91.26	1.84	83.19
	GMP	2.14	0.98	0.89	44.09	46.09	91.51	1.86	87.01
	HM	2.08	1	0.92	46.11	48.12	91.82	1.9	91.16
	SSI	1.14	0.27	0.2	38.89	45.35	73.53	0.78	68.79
	STI	0.75	0.61	0.53	97.29	104.58	86.54	1.39	186.71
	YI	0.91	0.26	0.23	52.82	55.65	90.1	0.94	103.45
	YSI	0.65	0.03	0.02	21.35	24.91	73.47	0.24	37.75
	RSI	0.94	0.05	0.04	21.41	24.87	74.14	0.36	38.04
2022/23	Yp	2.59	1.18	0.98	38.28	42.03	82.93	1.86	71.91
	Ys	1.79	0.79	0.47	38.42	49.71	59.73	1.1	61.26
	TOL	0.81	0.6	0.44	81.36	95.62	72.4	1.16	142.83
	MP	2.19	0.84	0.62	35.98	41.79	74.15	1.4	63.92
	GMP	2.12	0.82	0.59	36.17	42.79	71.45	1.34	63.08
	HM	2.06	0.83	0.58	36.9	44.26	69.49	1.31	63.45
	SSI	0.97	0.53	0.39	64.6	74.98	74.23	1.11	114.82
	STI	0.73	0.52	0.33	78.86	98.88	63.62	0.95	129.77
	YI	0.92	0.21	0.12	38.4	49.72	59.63	0.56	61.17
	YSI	0.72	0.04	0.03	24.94	29.11	73.4	0.32	44.08
	RSI	1.01	0.09	0.06	25.09	29.19	73.9	0.45	44.49

GV: Genetic variance; **PV**: phenotypic variance; **h^2b** heritability at broad sense; **PCV**: phenotypic coefficient of variation; **GCV**: genotypic coefficient of variation; and **GA:** genetic advance; **GAPM:** genetic advance as percent mean

In the 2022/23 season, the mean yield under non-stressed conditions (Yp) was slightly lower at 2.59 t/ha, with a GV of 0.98 and a PV of 1.18. The GCV and PCV were 38.28% and 42.03%, respectively, with a heritability of 82.93% and a GA of 1.86. Under stressed conditions (Ys), the mean yield was 1.79 t/ha, with a lower GV of 0.47 and a PV of 0.79. The GCV and PCV

were 38.42% and 49.71%, respectively, with a heritability of 59.73% and a GA of 1.1. The TOL showed a mean of 0.81, with a high GCV of 81.36% and a PCV of 95.62%. The heritability was 72.4%, with a GA of 1.16. The MP, GMP, and HM traits showed high heritability values, though slightly lower than the previous year. The SSI and STI had heritability values of 74.23% and 63.62%, respectively, with the STI showing a GAPM of 129.77%. The YI and YSI traits had heritability values of 59.63% and 73.4%, respectively, with the YI showing a GAPM of 61.17%.

4.3.4 Correlation between grain yield and stress tolerance indices.

Figures 4.2 and 4.3 display Pearson's correlation coefficients of grain yield under non-stress and stressed conditions with various yield-based drought tolerance indices for two cropping seasons. The color intensity indicates the strength and direction of the correlations, ranging from -1 (perfect negative correlation) to 1 (perfect positive correlation). For the 2020/21 cropping season, yield under non-stressed conditions (Yp) shows strong positive correlations with MP, GMP, HM, and the STI, with coefficients of 0.97, 0.95, 0.93, and 0.90, respectively. These indices were reliable indicators of performance under non-stressed conditions. Yp also has a moderate positive correlation with yield under stressed conditions (Ys), at 0.88, which shows some consistency in performance across conditions. In contrast, Yp has low correlations with TOL at 0.34, SSI at -0.27, YSI at 0.27, and RSI at 0.27. These indices are less related to yield under non-stressed conditions.

Yield under stressed conditions (Ys) exhibits robust positive correlations with MP, GMP, HM, and STI, all close to 1.00 (0.97, 0.98, 0.99, and 0.96, respectively). This indicates that these indices effectively identify genotypes that perform well under drought stress. Ys also has a strong positive correlation with Yp, at 0.88. However, Ys showed a low correlation with TOL (0.15), suggesting that TOL may not be a reliable performance indicator under stress. Moderate positive correlations with YSI and SSI (both at 0.67).

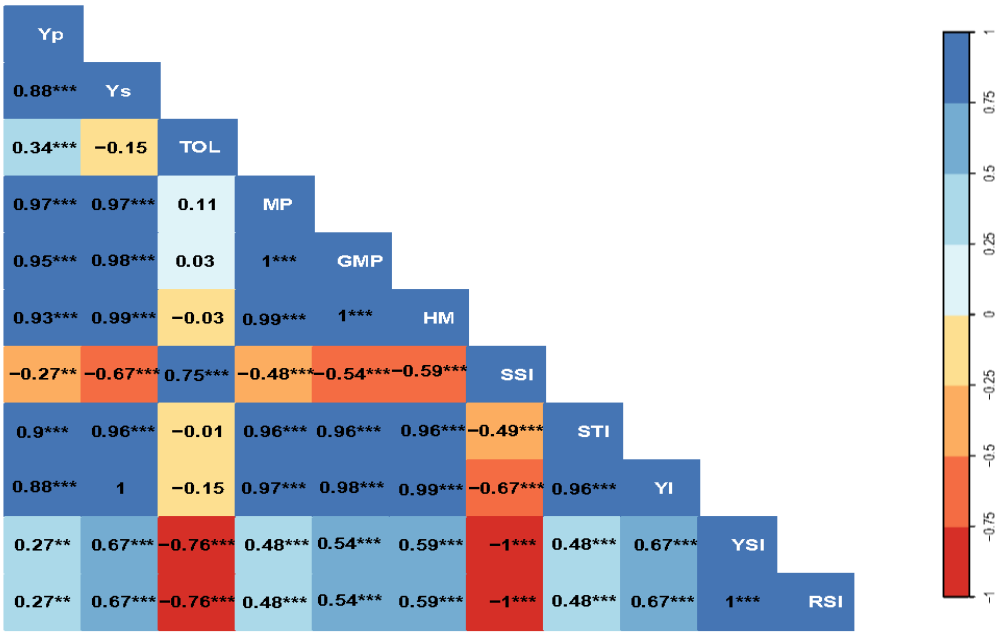

Figure 4.2. Pearson's correlation coefficients between the grain yield of durum wheat genotypes under drought-stressed and non-stressed conditions and yield-based drought tolerance indices for the 2020/21 cropping season.

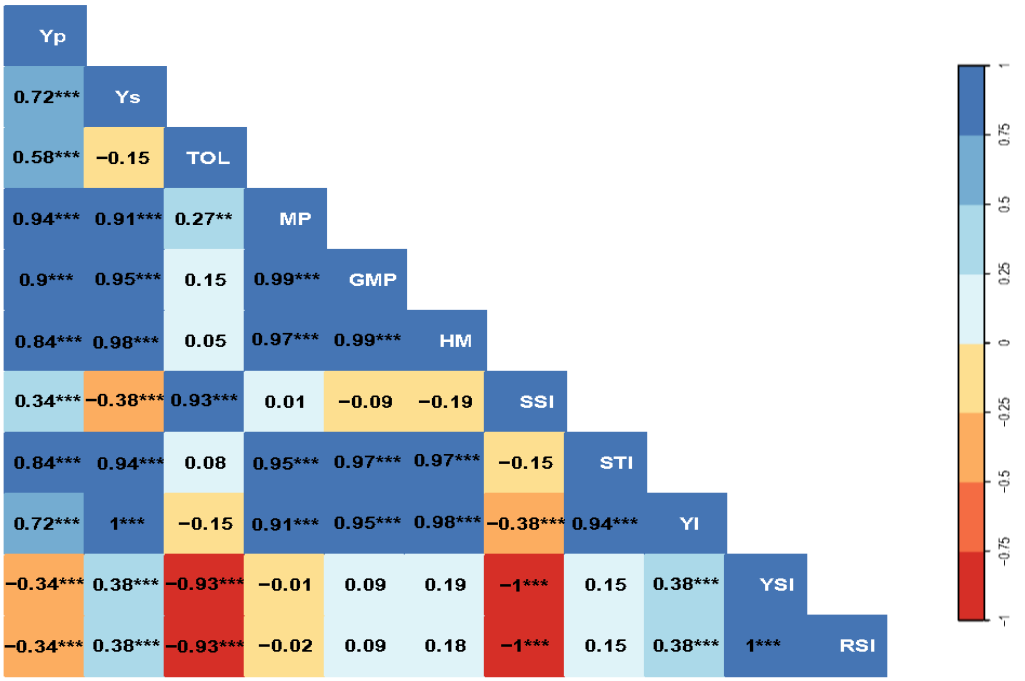

Figure 4.3. Pearson's correlation coefficients between grain yield of durum wheat genotypes under drought-stressed and non-stressed conditions and yield-based drought tolerance indices for the 2022/23 cropping season.

Pearson's correlation coefficients in Figure 4.2 also indicate that MP, GMP, HM, and STI display very high intercorrelations, close to 1.00. It was an indication of their consistency as measures of yield performance under both stressed and non-stressed conditions. These indices also have high positive correlations with Yp and Ys, reinforcing their reliability in evaluating genotypic performance. TOL showed low positive correlations with most indices, except for a moderate positive correlation with SSI (0.76), and a moderate negative correlation with YSI and RSI (-0.67, each), showing that higher TOL values correspond to higher stress susceptibility. This suggests that TOL might be more useful for identifying genotypes with higher yield reductions under stress.

Stress susceptibility index has a moderate negative correlation with Ys (-0.67), and that genotypes with higher SSI values tend to have lower yields under stress. Stress susceptibility index strong positive correlation with TOL (0.76) further supports its role in identifying stress-susceptible genotypes. STI has strong positive correlations with MP, GMP, HM, Ys (0.96, each), and Yp (0.90) demonstrating its utility in assessing stress tolerance and overall yield performance. Yield index and YSI show high and moderate positive correlations with Ys (1.00 and, 0.67 respectively), indicating their potential in evaluating drought tolerance. They have low to moderate correlations with other indices. RSI shows no significant correlation with most indices, except for a strong negative correlation with SSI (-1.00), and a strong positive correlation with YSI (1.00), which indicates its limited utility in assessing yield performance.

In the 2022/23 cropping season, Figure 4.3 indicates strong positive correlations between yield under non-stressed conditions (Yp) and indices like MP, GMP, HM, and the STI, with coefficients of 0.94, 0.89, 0.84, and 0.84, respectively. These correlations suggest that these indices are reliable indicators of performance under non-stressed conditions. Yp also has a moderate positive correlation with yield under stressed conditions (Ys) at 0.72. In contrast, Yp has low to moderate correlations with SSI at 0.34 and TOL at 0.58, respectively. Also, a low negative correlation was indicated with YSI and RSI each at -0.34.

Yield under stressed conditions (Ys) showed very strong positive correlations with MP, GMP, HM, STI, and YI, with coefficients of 0.91, 0.95, 0.98, 0.94, and 1.00, respectively. This

indicates that these indices are effective in identifying genotypes that perform well under drought stress. Ys also has a strong positive correlation with Yp at 0.72. However, Ys shows a low negative correlation with TOL (0.15) and SSI (-0.38) and positive correlations with YSI and SSI (both at 0.38).

The correlation coefficient in Figure 4.3 also indicates that MP, GMP, HM, and STI exhibit very high intercorrelations. These indices also have high positive correlations with Yp and Ys, reinforcing their reliability in evaluating genotypic performance. TOL shows low positive correlations with most indices, except for a strong positive correlation with SSI (0.93), that higher TOL values correspond to higher stress susceptibility. SSI has a moderate negative correlation with Ys (-0.38), showing genotypes with higher SSI values tend to have lower yields under stress. SSI's strong positive correlation with TOL (0.93) further supports its role in identifying stress-susceptible genotypes. STI has strong positive correlations with MP, GMP, HM, Yp, and Ys (0.95, 0.97, 0.97, 0.84, and 0.94, respectively). Yield stability index and RSI show no significant correlation with most indices, except for a strong negative correlation with SSI (-1.00) and TOL (-0.93).

3.6. Cluster Analysis

Based on drought tolerance indices and grain yield under stressed and non-stressed conditions in both cropping seasons, the cluster analysis of the studied durum wheat genotypes classified the genotypes into six distinct clusters as shown in Figure 4.4 and 4.5, respectively. Ward's minimum variance clustering method, utilized for this classification, showed specific patterns in genotype performance. In the 2020/21 season, Cluster 1 included 28 genotypes, accounting for 29.17% of the total landraces. These genotypes had moderate values for most indices, with a relatively low mean yield under stress (Ys) of 1.43. MP and GMP were moderate at 1.71 and 1.68, respectively. Stress tolerance index was low at 0.37. These genotypes might not perform exceptionally well under severe drought stress but had moderate resilience. Cluster 2, with 13 genotypes (13.54% of landraces), showed high values for most indices. The Ys was significantly higher at 2.59, and both MP and GMP were also high at 2.85 and 2.83, respectively. The STI was the highest among all clusters at 1.04, an indication of strong drought tolerance and high productivity under stress (Table 4.4.)

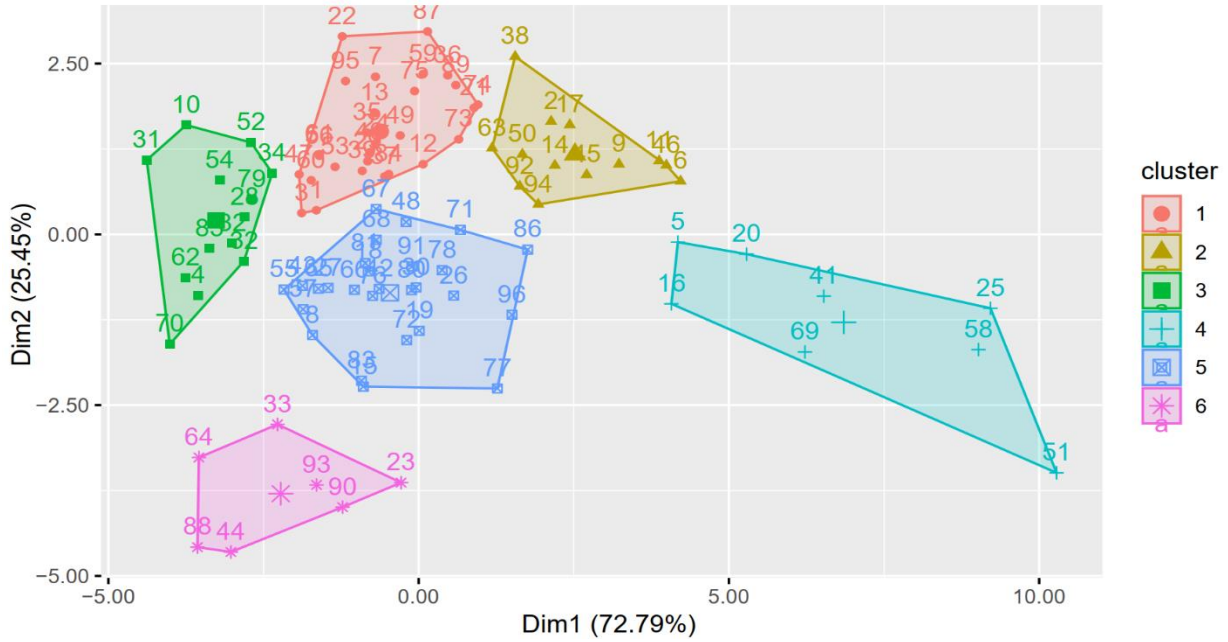

Figure 4.4. Clustering of durum wheat genotypes using drought tolerance indices for the 2020/21 cropping season

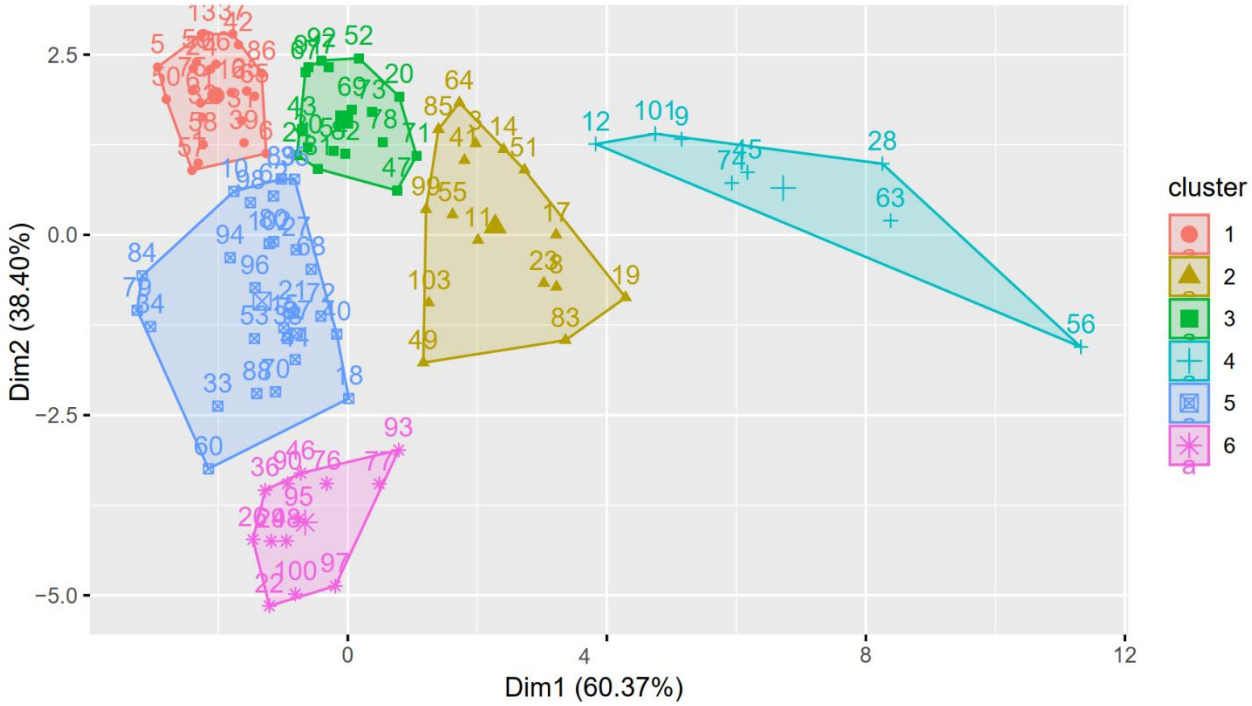

Figure 4.5. Clustering of durum wheat genotypes using drought tolerance indices for the 2022/23 cropping season

Another 13 genotypes (13.54% of landraces) were grouped in Cluster 3, displaying low values for yield and other indices. Ys was the lowest at 0.69, and the STI was also minimal at 0.13, which showed their poor performance under drought conditions. Cluster 4, a smaller cluster containing 8 genotypes (8.33% of landraces), had the highest values for all indices. Ys was exceptionally high at 4.26, and the MP and GMP were high at 4.68 and 4.66, respectively. This cluster had the highest SSI and STI, indicating that these genotypes performed very well under stress but were also highly susceptible. Cluster 5 comprised 27 genotypes (28.13% of landraces) with moderate to high values for most indices. Ys was moderate at 1.64, and the MP and GMP were also moderate at 2.24 and 2.15, respectively. The STI was moderate at 0.61, indicating reasonable performance under stressed conditions. Cluster 6, the smallest cluster with 7 genotypes (7.29% of landraces), had high TOL and SSI values but moderate stress Indices values. Yield under stress conditions was 1.15, and STI was 0.51, suggesting moderate drought tolerance and susceptibility.

In the 2022/23 season, Cluster 1 had 22 genotypes (21.36% of landraces) with low values for most indices. Ys was low at 1.16, and the MP and GMP were also low at 1.24, with a low STI of 0.21, indicating poor performance under stressed conditions. Cluster 2 included 16 genotypes (15.53% of landraces) and showed high values for yield and other indices. Ys was high at 2.60, and the MP and GMP were also high at 2.99 and 2.96, respectively. The STI was high at 1.19, which is strong drought tolerance. Cluster 3, with 17 genotypes (16.50% of landraces), exhibited moderate values for stress Indices. Ys was 1.89, and MP and GMP were around 2.02. The STI was moderate at 0.54, suggesting average performance under stress.

Table 4.4. Mean performance of yield under non-stressed and stressed conditions and drought tolerance indies in respective clusters for the 2020/21 and 2022/23season

year	cluster	Ys	TOL	MP	GMP	HM	SSI	STI	YI	YSI	RSI
2020/21	1	1.434	0.547	1.71	1.683	1.659	0.895	0.37	0.743	0.723	1.048
	2	2.587	0.514	2.847	2.831	2.818	0.533	1.043	1.34	0.834	1.209
	3	0.693	0.749	1.07	0.995	0.929	1.65	0.134	0.358	0.488	0.708
	4	4.264	0.836	4.685	4.661	4.64	0.536	2.876	2.209	0.834	1.209
	5	1.642	1.194	2.241	2.155	2.074	1.364	0.609	0.85	0.576	0.835

	6	1.15	2.236	2.271	1.961	1.701	2.143	0.509	0.596	0.334	0.484
2022/23	1	1.164	0.165	1.245	1.241	1.238	0.417	0.21	0.596	0.88	1.238
	2	2.6	0.788	2.993	2.958	2.927	0.77	1.191	1.334	0.778	1.094
	3	1.886	0.264	2.017	2.012	2.006	0.411	0.545	0.968	0.881	1.24
	4	4.176	0.502	4.425	4.418	4.407	0.341	2.686	2.143	0.902	1.268
	5	1.314	0.966	1.796	1.721	1.653	1.436	0.408	0.674	0.585	0.822
	6	1.248	2.433	2.463	2.134	1.848	2.283	0.612	0.641	0.342	0.479

Cluster 4, a smaller cluster of 8 genotypes (7.77% of landraces), had the highest values for stress Indices, similar to Cluster 4 of the 2020/21 season. Ys was 4.18, and MP and GMP were 4.42, with a high STI of 2.69, which is exceptional performance under stress but high susceptibility. Cluster 5, the largest cluster with 27 genotypes (26.20% of landraces), had moderate values for most indices. Ys was 1.31, MP and GMP were around 1.80, and STI was 0.41, indicating moderate performance under stress. Cluster 6 included 13 genotypes (12.62% of landraces) with high TOL and SSI values but moderate stress index values. Ys was 1.25, and STI was 0.61, showing moderate drought tolerance and susceptibility.

4.4. DISCUSSION

In the study by Dukamo et al. (2023), significant differences were observed among durum wheat landraces for various agromorphological and phenological traits and their responses to different environmental conditions. The findings revealed that these landraces exhibited considerable variation in traits, influenced by other sources of environmental stress. The overall adjusted means across the two environmental conditions confirmed genotype-specific responses, with several landraces outperforming the checks under both conditions. Specifically, seven landraces ETDW/15DZ4, 34493, ETDW/15DZ23, 34522, MCD3-14, 34217, and 31831 showed superior grain yields compared to the checks. Among these, ETDW/15DZ23, 34522, ETDW/15DZ4, and 34493 excelled under non-stressed conditions, while ETDW/15DZ4, 34493, and ETDW/15DZ23 surpassed all four checks under stressed conditions.

This study employed grain yield and mean grain yield to screen for stress indices, allowing for the analysis of drought tolerance among the durum wheat genotypes. Several drought-tolerant, high-performing landraces were identified, demonstrating their potential for breeding programs

to enhance resilience in wheat production. We observed a 30.9-33.83% decrease in mean grain yield due to drought stress, indicating that water shortages significantly reduce the grain yield of wheat landraces. This negative correlation between grain yield and drought stress poses significant challenges for plant breeders in maintaining high yields. Previous studies have reported similar decreases in cereal crop yields due to drought stress. Rashidi et al., (2011) noted wheat yield reductions ranging from 13% to 76%, averaging 55%, which is slightly higher than our findings. Hooshmandi (2019) documented a mean reduction of 34.03% in wheat yield due to stress, closely aligning with our 30.79% mean reduction. Amare et al. (2019) and Habtamu et al. (2016) also reported that grain yield under non-stressed conditions was approximately 30.6% higher than under stressed conditions, showing the significant impact of drought stress on yield performance.

Different wheat-growing areas experience varied stress levels depending on the local environment. Under late-sown conditions, wheat genotypes face drought stress at the anthesis and post-anthesis stages, experiencing temperatures approximately 3–4°C higher than normally sown genotypes. Studies by Dadbakhsh et al. (2011), Farshadfar et al. (2012), Dixon et al. (2008), and Bennani et al. (2017) support these findings, showing significant yield losses due to drought in various cereal crops. The stability of crop performance under stressed conditions is a critical indicator of resilience. Research on other crop species also shows severe yield reductions during reproductive stages under stressed conditions. Farshadfar et al. (2012), Mathew et al. (2019), and Semahegn et al. (2020) reported 40-47% yield losses in bread wheat, while Nadeem et al. (2019) and Nayyar et al. (2005) revealed yield reductions of up to 70% in chickpeas due to drought stress. These consistent findings emphasize the urgent need to develop drought-tolerant cultivars to sustain agricultural productivity under adverse conditions.

Various stress tolerance indices are effectively utilized to identify landraces that are resilient under stressed conditions. In research on durum wheat, the Stress Tolerance Index (STI) has been used to select genotypes that can endure high temperatures (Aberkane et al., 2021). Smaller values of Tolerance (TOL) are preferred for selecting stress-tolerant genotypes, as larger values indicate greater susceptibility to stress. The results show that tolerance increases as SSI and TOL values decrease. However, reports by Thiry et al. (2016) and Khayatnezhad et al. (2010)

indicate that these indices alone cannot differentiate between genotypes that perform well under both stressed and non-stressed conditions. When selecting genotypes based on TOL and SSI, those with lower yield in non-stressed conditions but higher yield under stress is often favored (Kumar et al., 2023, Thiry et al., 2016).

The landraces ETDW/15DZ23, 34493, ETDW/15DZ4, 34522, 34423, and 34217 exhibited the highest drought tolerance, performing well under stress and non-stressed conditions in both cropping seasons. Their grain yields were statistically comparable to the best-performing genotypes in both conditions and surpassed the best drought-stress check, Alemtena. Conversely, landraces 33244, 31761, DW-PVT LM 8, and 24500 were the most sensitive to drought stress. The highest values for TOL and SSI were observed in landraces ETDW/15DZ 043, 34295, 31806, and 34625. These landraces produced high grain yields under non-stressed conditions but low yields under stress, classifying them as drought-sensitive. In contrast, landraces ETDW/15DZ 04, 33403, 33300, and DW-NVT LM 11 exhibited the lowest TOL and SSI values, which indicates minimal yield differences between the two conditions. Low TOL and high grain yield should be considered for selecting high-yielding genotypes. Similar findings by Ayed et al. (2021) and Kumar et al. (2023) in durum wheat confirmed that genotypes with low TOL values exhibited superior performance under stress conditions. Their research emphasized the importance of selecting high-yielding genotypes capable of thriving in diverse environments. Both studies showed that TOL and yield must be considered together for an effective selection strategy. Similarly, the study by Khayatnezhad (2012) on durum wheat evaluated stress indices like TOL, SSI, and STI, concluding that lower TOL values were linked to improved stress tolerance. However, the study also highlighted the need to select genotypes with high grain yield to ensure productivity under both stressed and non-stressed conditions.

A genotype's superior performance in both non-stressed and stressed environments is reflected by higher values of STI, MP, and GMP. Genotypes selected based on STI tend to exhibit higher grain yield and better stress tolerance. While selections based on MP generally improve the average performance of genotypes in both stress and non-stressed conditions, they do not effectively distinguish between stress-tolerant and high-yielding genotypes. MP tends to favor genotypes with higher yield potential but lower stress tolerance. A high STI value indicates

robust drought tolerance, whereas a high MP value suggests good performance in both conditions. Genotypes with high values of both MP and STI, coupled with low SSI values, are considered drought-tolerant (Hooshmandi, 2019). In our studies, landraces such as ETDW/15DZ 023, 34493, ETDW/15DZ 04, 34522, and 34217 exhibited the highest values for HM, GMP, and STI. This suggests that these landraces showed greater productivity under stress conditions compared to the others. As distinguished in Dukamo et al. (2023), these landraces also outperformed the check variety used in the study in most agromorphological and phenological traits. This indicates the need for further investigation into these high-yielding landraces to assess their potential for use in breeding programs or targeted cultivation in specific environmental conditions. Their superior performance under stress makes them promising candidates for enhancing crop resilience and productivity.

Similar results were presented by Kamrani et al. (2018) and Basavaraj et al. (2021), who suggested that higher-yielding and drought-tolerant genotypes could be selected based on high values of GMP, and STI. Our findings also align with those of Mevlut and Sait (2011) and Ilker et al. (2011), who recommend using these indices for selecting high-yielding wheat genotypes under various conditions. Khalili et al., (2016) also endorse STI as the best index for selecting high-yielding varieties in both stress and non-stressed conditions. To determine the most desirable drought-tolerant criteria, we calculated correlation coefficients between Yp, Ys, and other quantitative indices of drought tolerance. Studying these correlations helps determine the overall linear association between different attributes (Talebi et al., 2009). A positive correlation between Yp and Ys has been reported in several studies, confirming the potential of certain indices to identify high-yielding genotypes under both non-stressed and stressed conditions (Mevlut and Sait, 2011, Gholinezhad et al., 2014, Nanda et al., 2018, Pour-Aboughadareh et al., 2019).

Based on drought tolerance indices like MP, GMP, HM, and STI, all studied wheat genotypes were grouped into six clusters. The highest values of MP, GMP, HM, and STI were found in genotypes of cluster II, followed by those in cluster IV in both cropping seasons. The minimum values of the indices and yield under stressed and non-stressed conditions were exhibited by genotypes in cluster III for the 2020/21 cropping season and cluster I for the 2022/23 cropping

season. This clustering pattern aligns with findings by Aliakbari et al., (2013) and Khalili et al., (2016), shows these genotypes as strong candidates for breeding programs aimed at enhancing drought tolerance. Naghavi and Khalili (2017) categorized eight maize genotypes into three classes using stress tolerance indices such as MP, GMP, and STI. They found that genotypes with high values of these indices were stress-tolerant. Similarly, Thanaa et al. (2019) classified all studied genotypes into five clusters based on their performance and degree of stress tolerance. They concluded that genotypes with high values of MP, GMP, HM, STI, and YSI are the best-performing and most stress-tolerant. Consequently, genotypes with high values of MP, GMP, HM, STI, and YSI could be used as parents in breeding programs aimed at developing stress-tolerant genotypes.

4.5. CONCLUSION AND RECOMMENDATION

In conclusion, the tested durum wheat genotypes exhibited a wide range of variation in grain yield under both non-stressed and drought conditions, with significant reductions observed due to drought stress. The correlation between stress tolerance indices and yield in both conditions identified the most suitable indicators for screening drought-tolerant cultivars. Specifically, STI, MP, GMP, and HM were found to be the most reliable yield-based indices for identifying high-performing genotypes under both conditions. The genotypes ETDW/15DZ23, 34493, ETDW/15DZ4, 34522, and 34217 showed the highest drought tolerance, while clusters II and IV contained genotypes with notable performance under drought conditions. The correlation coefficient analysis revealed a strong positive association of STI, MP, GMP, and HM with Yp and Ys, while TOL and SSI were negatively correlated with Ys. These findings emphasize the value of using stress indices as effective predictors for selecting high-yielding, drought-tolerant genotypes. Further physiological and biochemical screening of these genotypes can enhance their utilization as genetic resources in agricultural improvement programs.

CHAPTER FIVE

ASSESSMENT OF DROUGHT STRESS ADAPTIVE TRAITS IN DURUM WHEAT (*T. TURGIDUM SUBSP. DURUM*) LANDRACES USING AGROMORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS

Abstract

Drought is a significant abiotic stress affecting wheat productivity under changing climate conditions. Various agronomic, physiological, and molecular approaches have been employed, with conventional and modern breeding strategies, particularly effective for developing drought-adapted wheat varieties. Growing drought-tolerant genotypes offers a sustainable solution to enhance productivity under variable environmental conditions. This study aimed to screen durum wheat landraces by evaluating their morpho-physiological and biochemical responses. Twenty landraces and four checks were grown in pots under non-stressed (70% pot capacity) and stressed (35% pot capacity) conditions, arranged in a completely randomized design (CRD) with three replicates. Data on yield, yield-related traits, and physio-biochemical attributes were recorded. ANOVA results indicated significant effects of genotype and stress condition on all studied parameters, with drought stress significantly impacting the morpho-physiological and biochemical characteristics of all tested durum wheat genotypes. Traits such as earlier heading dates, optimum tiller production, and a short grain filling period were identified as crucial for tolerating drought impacts. The study also highlighted the role of high proline content and Relative leaf water content (RLWC) in sustaining metabolic processes under drought conditions. A comprehensive analysis of phenotypic and genotypic correlations and path coefficient analysis provided a valuable understanding of these traits' direct and indirect effects on grain yield. Landraces ETDW/15DZ023, ETDW/15DZ04, 34217, 34522, and 31831 maintained high yields under non-stressed and stressed conditions. Also, the landraces showed greater drought tolerance than the other tested genotypes, emphasizing their potential for use in breeding programs to enhance drought resilience in durum wheat.

Key words: Drought tolerance; physiological trait; proline content; durum wheat; greenhouse; stress-adaptative

5.1. INTRODUCTION

The wheat genus, *Triticum*, a member of the Poaceae family (previously known as Gramineae), encompasses some of the most crucial crops for human consumption. Among them, bread wheat (*Triticum aestivum*, AABBDD) and durum wheat (*Triticum turgidum subsp*. *durum* or *Triticum durum Desf*., AABB) stand out for their global significance. Wheat, as one of the world's most

vital cereal crops, was cultivated on over 219.2 million hectares in 2022, yielding an impressive 808.44 million tons of grain (FAOSTAT, 2024). Durum wheat, though representing only 5% to 8% of global wheat production, was grown on approximately 16.1 million hectares, underscoring its importance in specific regions (FAOSTAT, 2024). In Ethiopia, wheat plays a significant role in the agricultural landscape, covering about 1.89 million hectares and producing 5.78 million tons in 2021, according to CSA/ESS (2021) data. Notably, around 40% of Ethiopia's wheat production is durum wheat, valued for its hardness, high protein content, and unique quality traits (Bergh et al., 2019). Ethiopia contributes 0.6% to the world's durum wheat supply, making it one of the leading producers in Sub-Saharan Africa (Tidiane Sall et al., 2019).

Plants, particularly economically important cereals, are constantly exposed to various stressors under both natural and agricultural conditions, leading to alterations in their normal physiological processes (Khalili et al., 2018). Among these stressors, drought is a major abiotic challenge, severely limiting agricultural productivity by reducing water absorption and nutrient uptake, thus stunting plant growth (Upadhyaya et al., 2017). In Sub-Saharan Africa, drought poses a significant threat, with frequent occurrences that have devastating impacts on both the population and the economy. The region's extreme dependence on rainfall, combined with poor soil moisture retention, renders nearly 60% of Sub-Saharan Africa highly vulnerable to drought conditions (Gan et al., 2016).

Wheat, being one of the most important cereal crops globally, faces negative impacts on growth and yield due to drought stress. It is estimated that for wheat, there is a yield loss of around 4.1% to 6.4% per degree increase in temperature due to climate change, equivalent to a quarter of all global wheat trade (Asseng et al., 2017). Adoption of drought-tolerant genotypes is a sustainable approach to mitigate the impacts of marginal rainfall and prolonged dry spells on wheat production and productivity. Developing high-yielding wheat cultivars under drought conditions is a crucial goal of breeding programs (Leilah and Al-Khateeb, 2005). Phenotyping is a key criterion for screening breeding materials based on drought adaptive and constitutive morpho-physiological characteristics, including yield and its components (Monneveux et al., 2012). Utilizing controlled water application with various physiological parameters like stomatal conductance, canopy temperature measurement, chlorophyll content measurement, and

leaf Relative leaf water content offers effective germplasm screening with a good combination of agronomically important traits for improving yields under target drought conditions (Mwadzingeni et al., 2016b)

The developmental response of plants to drought stress is manifested through enhanced root growth and suppressed shoot growth, resulting in an increased root-to-shoot ratio (Xu et al., 2013). Studies suggest that during drought stress, an increase in abscisic acid (ABA) levels promotes root growth while simultaneously inhibiting shoot growth, leading to an increase of up to 50% in wheat root: shoot ratio. Deep root systems contribute to greater yield potential under drought conditions, as it is crucial for crops to extract water from a larger soil volume for yield stability under depleting soil moisture in rainfed production systems (Pask and Reynolds, 2013). The positive contribution of root depth to drought avoidance is facilitated by the ability to access moisture from deeper soil layers, enhancing photosynthesis and grain filling under drought conditions. Crop varieties that extract moisture from deeper zones (60–120 cm) maintain higher stomatal conductance and can sustain cooler canopy temperatures (Pask and Reynolds, 2013). Stomatal closure in response to stress leads to decreased leaf water potential, reduced carbon assimilation, oxidative stress, and increased canopy temperature. The stacking of deep root biomass and transpiration efficiency traits in wheat varieties enhances protection from drought stress (Kulkarni et al., 2017).

Drought stress also affects photosynthetic activity by reducing chlorophyll content and damaging the photosystem II reaction sites (Cowley and Luckett, 2011). Both traits are valuable for wheat breeding. Chlorophyll fluorescence is useful for evaluating yield performance under rainfed conditions when measured during the grain-filling period. Fv/Fm is identified as a suitable trait for screening tolerance to high temperatures (Baker and Rosenqvist, 2004). Chlorophyll content has shown a correlation with grain yield and thousand kernel weights under drought stresses. The normalized difference vegetation index (NDVI) at the vegetative stage is highly correlated with grain yield and biomass yield, showing significant genotypic variation that allows for efficient selection. Additionally, the grain filling rate (GFR) is highly correlated with grain yield and biomass yield under drought stress (Gholinezhad et al., 2014).

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Different biochemical analyses have long been proposed as a useful complementary strategy for selecting drought-tolerant genotypes in plant breeding (Mwadzingeni et al., 2016b). Proline, an amino acid that accumulates widely in plants under environmental stresses, has been associated with various osmo-protective roles such as osmotic adjustments, stabilizing subcellular structures, scavenging free radicals, and buffering (Zadehbagheri et al., 2014). Proline accumulates faster and is found in higher proportions in drought-tolerant genotypes than drought-sensitive genotypes under drought stress, making it a valuable trait in breeding for drought tolerance. Exploring proline content under severe stress in a diverse pool of genotypes at critical growth stages and describing its correlation with yield and yield components provide useful information for rapid germplasm screening in breeding for drought tolerance (Mwadzingeni et al., 2016a)

Ethiopia is rich in diversity of durum wheat landraces, each possessing different qualities and agro-morphological characteristics. Despite the diversity, poor yield and recurring insecurity in some regions have led to food instability and declining food reserves across the country. Therefore, there is a need to select genotypes with a good combination of agronomically important traits that collectively contribute to improved yields under drought conditions (Tardieu, 2012). Studying genetic diversity in a crop species is essential for its improvement as it forms the basis for understanding the genetic structure and enhancing quantitative traits like drought tolerance. Evaluating genetic diversity levels among adapted and elite germplasm can provide valuable understanding into the genetic variation among segregating progeny for the development of pure-line cultivars (Slafer et al., 2015, Wang et al., 2017).Therefore, the present study aimed to assess selected Ethiopian Durum wheat landraces using agromorphological, physiological parameters and proline content under drought stressed and non-stressed conditions to determine their stress-adaptive traits, drought tolerance ability, and potential for future breeding efforts.

5.2. MATERIALS AND METHODS

5.2.1 Experimental Plant Materials

In this study, 20 top-performing durum wheat landraces and 4 check varieties were evaluated under greenhouse conditions using a completely randomized design with three replications. These materials were selected based on the results from field experiment characterization in Chapter 3 and screening using stress tolerance indices in Chapter 4.

5.2.2. Experimental Design and Crop Establishment

A pot experiment was conducted using durum wheat landraces to evaluate their morphological, physiological, and biochemical characteristics. The selected varieties were planted in 5-liter pots (22 cm top diameter, 16 cm bottom diameter, 18 cm height) filled with 4 kg of air-dried, sieved soil. Topsoil was collected from the top 30 cm of land at Hawassa University's main campus, and a composite of 288 kg was prepared. This soil was air-dried for one week to ensure uniform weight, then saturated with water and left to equilibrate overnight. Six seeds were sown in each pot, and the plants were grown in a rainout shelter that replicated natural conditions in terms of daylight duration and air circulation. The experiment was arranged in a completely randomized design (CRD) with three replications, under both non-stressed and stressed conditions. Non-stressed conditions were maintained at 70% pot capacity water content, while drought stress was induced by limiting watering to 35% pot capacity from the heading stage onward. Pot capacity was determined according to the methods by Imakumbili et al. (2021) and Ogbaga et al. (2014). After 20 days of emergence, the wheat was thinned to four plants per pot. Fertilizers were applied at the sowing date based on recommended rates for wheat, with each pot receiving 0.28 g of nitrogen (N) from urea and 0.12 g of phosphorus (P) from triple super phosphate, equivalent to 70 kg N and 30 kg P per hectare. An additional amount of nitrogen was supplied during the tillering stage.

5.2.3 Data Collection

Data on phenological, yield, and yield-related traits were collected at different developmental stages of the wheat plant. Days to heading (DTH) will be calculated as the number of days

between the sowing date and when 50% of all the shoots in a plot have fully emerged spikes. The grain filling period (GFP) is the interval between flowering (anthesis) and physiological maturity. The number of tillers (TN) will be recorded by counting the total tillers at physiological maturity, and the spike length (SL) will be measured in cm. Root biomass to shoot biomass ratio (RBSB) was calculated by dividing the dry weight of root biomass by the dry weight of shoot biomass. The rooting length to plant height ratio (RLPH) was calculated by dividing the total root length by the corresponding plant height for each genotype. Leaf area was calculated by multiplying leaf length and maximum width by a correction factor of 0.75, and the number of leaves was calculated by counting all fully emerged leaves on the main stem at the time of data collection. Finally, grain yield (GY) will be determined as the weight (grams) of the grain from a pot and converted to tons/ha. The following parameters were also measured.

5.2.3.1 **Stomatal Aperture**

Stomata number and width were measured following the protocol proposed by Xu and Zhou (2008). A thin layer of transparent nail polish was uniformly applied to the lower surface of fresh, intact leaves and left to dry for 10 minutes to capture the epidermal imprint. Once dried, the nail polish layer was carefully peeled off using transparent tape and attached to a microscope slide. Stomata counts and width measurements were conducted using an Automated Upright Leica Microscope DM5000 B equipped with a 40x magnification lens and a digital Leica DFC425/DFC425C image processing camera.

5.2.3.2 **Canopy Temperature Measurement**

A hand-held Infrared Thermometer (IRT) with a resolution of 0.1°C was used for the canopy temperature measurement. Measurements were taken at an angle of 30° to the horizontal plane, one meter away from the edge of the plot, and approximately 50 cm above the crop. The temperature sensing area was about 15–30 cm in diameter to ensure only sunlit leaves were in view of the infrared temperature sensor. Measurements were taken when the sky was clear and there was little wind during the late morning. The measurements using IRT were taken two times at two different stages of development, during pre-heading time and grain-filling time with a 6 day interval between each measurement to give a reasonably heritable estimate of trait expression (Pask et al., 2012)

5.2.3.3. **Chlorophyll Concentration Index (CCI) Reading**

Chlorophyll concentration index (CCI) reading took place using randomly selected flag leaves from different plants within the pot. CCI measurement can be quickly, and non-destructively measured using a hand-held optical meter Minolta SPAD-502 chlorophyll meter. Calibration of the SPAD-502 chlorophyll meter took place before each measurement and the accuracy of measurements was checked by taking multiple readings from the same leaf and comparing the readings (Pask et al., 2012).

5.2.3.4. **Determination of Leaf Chlorophyll Concentration**.

Leaves were placed in bags sealed with aluminum foil and immediately transported to the plant cell laboratory. A 0.5 g sample of fresh leaf was placed in 15 ml tubes containing 95% (v/v) ethanol and homogenized using a pestle and mortar. The homogenized mixture was centrifuged in the dark for 15 minutes at room temperature at 10,000 rpm. The supernatant was collected, and 0.5 mL of each concentration level was analyzed in triplicate for chlorophyll-a and chlorophyll-b using a spectrophotometer UV-2450 (Hitachi, Tokyo, Japan) at absorbances of 664 nm and 648 nm, respectively. The following equations were used for the quantification of chlorophyll-a and chlorophyll-b (Lichtenthaler and Buschmann, 2001).

$$\text{Ch a}\left(\frac{\mu g}{ml}\right) = 13.36\ A_{664} - 5.19\ A_{648}$$

$$\text{Ch b}\left(\frac{\mu g}{ml}\right) = 27.43\ A_{648} - 5.19\ A_{664}$$

$$\text{Total Chl}\left(\frac{\mu g}{ml}\right) = \text{chl a} + \text{chl b}$$

where A: absorbance, Ch a: chlorophyll a, and Ch b: chlorophyll b.

5.2.3.5 **Determination of Leaf Chlorophyll Fluorescence**.

The efficiency of photosystem II (Fv/Fm) was measured from the flag leaf of three randomly selected plants per pot. Measurements were taken between 4:00 AM and 6:00 PM using a Handy-PEA fluorimeter (Hansatech, Kings Lynn, UK) following the methodology outlined by

Strasser et al. (2004). Leaves were dark-adapted for 30 minutes using a leaf clip before measurement. Light was then provided by an array of three high-intensity light-emitting diodes set to 1500 μmol·m^{-2}·s^{-1} to ensure that photosynthesis was saturated during the measurements.

5.2.3.6. **Relative Leaf Water Content (RLWC)**

Three fully expanded leaves were collected from representative plants and 5 leaf disks (9 mm in diameter) were collected from each leaf and immediately weighed (leaf fresh weight). The samples were then hydrated to full turgidity in a closed 15 ml tube with double distilled water for 24 hours at room temperature (25°C). Afterward, the water droplet left on the surface of the leaf disc was carefully removed with tissue paper and reweighed to obtain fully turgid mass (leaf turgid weight). To attain a consistent dry mass, samples were oven dried for 24 hours at 75°C (leaf dry weight). Finally, we calculated the relative leaf water content as follows (Turner, 1981).

$$\text{RLWC (\%)} = \left[\frac{(\text{leaf fresh weight} - \text{leaf dry weight})}{(\text{leaf turgid weight} - \text{leaf dry weight})}\right] \times 100$$

5.2.3.7. **Determination of Proline Concentration**

Fresh leaf samples (50 mg) were placed in one ml of ethanol and allowed to sit overnight at 4°C. The next day, the samples were centrifuged at 14,000 g for 5 minutes. A reaction mix (100 μl) containing 1% ninhydrin (w/v), 60% glacial acetic acid (v/v), and 20% ethanol (v/v) was added to each sample, which was then heated at 95°C for 20 minutes. After cooling to room temperature, the supernatant was centrifuged for one minute at 2,500 rpm. The optical density was determined using a UV-Vis spectrophotometer at 520 nm. Proline concentration was calculated based on fresh weight using an L-proline standard curve. The proline concentration was determined from a standard curve and calculated on a fresh weight basis expressed as μg/g of leaf fresh weight according to Bates et al. (1973).

5.2.4. Data Analysis

The phenotypic data were analyzed using different packages in R. Combined analysis of variance, Pearson's correlation coefficients (r), and Principal Component Analysis (PCA) were

performed to indicate the relationships among studied genotypes based on recorded traits under drought stress and non-stressed conditions and PCA biplots were plotted.

5.3. RESULT

5.3.1. Analysis of variance

The combined analysis of variances (ANOVA) presents the analysis for 24 durum wheat landraces across two different environmental conditions, drought-stressed and non-stressed, as given in Table 5.1. A highly significant difference ($P<0.001$) was observed among genotypes, locations, and the genotype-to-location interaction effects on grain yield and other related traits. Chl_a, Chl_a+b, and SMW were not significantly affected by the genotype interaction by location.

Table 5.1 Combined analysis of variances for morphological, physiological, and biochemical parameters of durum wheat landraces tested at greenhouse conditions

Parameter	TRT	LOC	TRT:LOC	Residuals	cv
Df	23	1	23	96	
Chl_a	0.017 ***	1.184 ***	0.011 .	0.007	5.73
Chl_b	0.006 **	0.488 ***	0.006 **	0.002	9.95
Chl_a+b	0.03 ***	3.192 ***	0.017 .	0.011	5.31
DTH	51.41 ***	702.25 ***	17.61 ***	1.9	2.42
PC	0.257 ***	4.984 ***	0.106 **	0.046	18.42
RLWC	187.4 ***	8833.7 ***	109.2 **	50.9	9.98
GFP	25.43 ***	733.51 ***	12.91 ***	3.47	4.09
LN	0.809 ***	1.361 *	0.552 ***	0.209	8.63
CT	4.182 ***	59.998 ***	4.625 ***	0.751	3.52
CCI	28.32 ***	471.22 ***	10.4 ***	3.69	3.74
TN	3.106 ***	71.417 ***	0.739 *	0.42	12.93
SMW	9.2 *	4699.6 ***	4.5	5.1	19.73
SMN	2.065 ***	73.674 ***	1.384 **	0.611	14.34
LA	26.49 ***	370.4 ***	9.54 ***	2.31	9.83

SL	3.373 ***	78.131 ***	1.435 ***	0.457	9.71
RBSB	0.011 ***	0.608 ***	0.011 ***	0.002	16.94
Fv.Fm	0.002 ***	0.009 ***	0.001 *	0	2.41
RLPH	0.017 ***	0.186 ***	0.018 ***	0.001	6.31
GY	4.437***	194.885***	1.217***	0.182	11.75

Where TRT: treatments (landrace and checks); LOC: location (stress condition); TRT:LOC: treatment location interaction; DTH: Days to heading; GFP: grain filling period, Leaf number; canopy temperature, CCI: Chlorophyll concentration index reading; Tiller number; LA: leaf area; SL: spike length; RBSB: root-to-shoot biomass ratio RLPL: root-to-shoot length; RLWC: Relative leaf water content; PC: Proline content; Chl_a: Chlorophyll a; Chl_b: Chlorophyll b; Chl_a+b: total chlorophyll; Fv/Fm, SMW: stomata width; SMN: stomata number and GY: grain yield

For chlorophyll content, all three measures (Chl_a, Chl_b, Chl_a+b) showed significant effects for treatments and locations, with some interactions also being significant. Days to heading exhibited significant effects for treatments, locations and the interaction between treatments and locations. Proline content was significantly affected by treatments, locations, and their interaction. Relative leaf water content (RLWC) showed significant differences across treatments.

The GFP was significantly influenced by treatments, locations, and their interaction. Both LN and CT exhibited significant effects across all factors. CCI and TN were significantly impacted by treatments, locations, and their interaction. SMN and SMW displayed significant differences across all factors. LA and SL were significantly influenced by treatments, locations, and their interaction. RBSB and RLPH varied significantly across all factors. The Fv/Fm ratio showed significant variation, and grain yield was significantly affected by treatments, locations, and their interaction (Table 5.1).

5.3.2. Effects of Drought on Agronomic, phenological, physiological and yield related traits

Days to 50% heading and Grain filling period

The early days to 50% heading were observed in landrace 34217 (51.33 days after sowing) followed by 31778 (55.67 days), 34571 (56.33 days), and ETDW/15DZ04 (56.67 days after sowing) under non-stressed conditions (Table 5.2). The data in Table 5.2 and 5.3 showed that under stressed condition there was a notable decline of 50% heading in genotypes such as 34522 (-14 days), 34295 (-11 days), and ETDW/15DZ 038 (-8 days). Genotypes such as Alemtena, 31831, 34217, and 34415 revealed no change in days to 50% heading under non-stressed and stressed conditions. The table also showed that the shortest grain filling period was observed in MCD2-17 (42.67 days) followed by 34607 (45.33 days) and DZDW170108 (47.33 days). However, the late were noted as ETDW/15DZ 038 (51.67 days), 34217 (51 days), 31831 and Ude (50 days) under non-stressed conditions. The data in the results also indicated that most of the landraces reached early maturity under drought stressed conditions. 31797 had a GFP of (37.33 days), 34295 (37.67 days), ETDW/15DZ 038 (39 days) and MCD2-17 (40 days). ETDW/15DZ 038 and 34295 showed decreased days -12.67 and -11 days, respectively, as a greater decline in GFP. The minimum reduction was noted in Kuami and 31778 (-1.67 days), and MCD3-14 (-2 days).

Table 5.2. Mean separation for non-stressed environmental condition

VAR	DTH	GFP	LN	LA	TN	SL	CT	PC	RLWC
Kuami	59 d	48.67 b-d	5.33 c-f	20.61 bc	4.64 hi	6.6 g	23.23 e-h	0.93 gh	81.01 b-e
ETDW/15DZ010	58.33 de	47.67 c-e	5 e-g	16.12 e-g	5.19 f-h	8 c-e	23.2 e-h	0.89 g-i	74.42 de
31778	58.33 de	46.67 de	4.57 g	13.41 h	5.25 f-h	9.85 a	24.93 b-d	0.86 hi	77.67 c-e
Alemtena	55.67 f	48.67 b-d	5 e-g	14.23 gh	4.75 g-i	6.9 e-g	23.93 d-g	1.11 b-d	80.66 b-e
ETDW/15DZ 038	64.33 ab	51.67 a	6.1 ab	17.52 de	5.42 d-h	8.84 a-c	24.7 b-e	0.89 g-i	77.91 c-e
Ude	57.33 d-f	50 a-c	5.43 b-f	16.2 e-g	5.5 c-h	7.99 c-e	24.16 c-f	0.87 hi	82.11 a-e
31797	63.33 bc	46.33 de	4.9 e-g	17.53 de	6.61 a-c	7.82 c-f	26.93 a	1.08 b-d	74.7 de
31831	57.33 d-f	50 a-c	6 a-c	13.74 h	6.14 a-f	8.14 cd	24.18 c-f	0.82 i	83.39 a-d
ETDW/15DZ04	56.67 ef	46.67 de	5.9 a-c	16.39 e-g	4.75 g-i	7.25 d-g	22.79 f-h	1.04 d-f	89.42 ab
33403	58 de	47.33 c-e	5.53 b-e	15.58 e-h	6.25 a-f	9.29 ab	23.63 d-h	0.93 gh	73.57 d-f
34217	51.33 g	51 ab	5.53 b-e	16.45 e-g	4.64 hi	6.47 g	23.75 d-h	1.05 c-e	82.35 a-e
34295	66.33 a	48.67 b-d	5.1 d-g	14.89 f-h	6.83 ab	8.61 bc	25.5 a-c	0.93 gh	74.79 de
MCD2-17	63.33 bc	42.67 f	5.43 b-f	16.82 ef	7.08 a	8.03 cd	24.8 b-d	0.86 hi	71.35 ef
34415	57 d-f	46.33 de	5.53 b-e	14.44 gh	6.42 a-e	8.2 b-d	25.8 ab	0.86 hi	74.2 d-f
ETDW/15DZ023	57.67 d-f	46.33 de	6.23 a	22.81 ab	4.86 g-i	6.75 fg	22.99 f-h	1.14 bc	93.07 a
34493	57.33 d-f	48.33 b-d	5.77 a-d	15.63 e-h	6.42 a-e	6.54 g	22.77 f-h	0.97 e-g	88.1 a-c

ETDW/15DZ049	57 d-f	49 a-d	5 e-g	14.83 f-h	5.33 e-h	6.87 fg	22.4 h	1.15 ab	83.9 a-d
34522	63.67 bc	46.67 de	4.97 e-g	21.62 a-c	6.14 a-f	6.21 g	23 f-h	0.97 e-g	79.12 b-e
34571	56.33 ef	48.67 b-d	5.1 d-g	14.49 f-h	6.5 a-d	9.9 a	24.87 b-d	1.16 ab	73.66 d-f
MCD3-14	57.67 d-f	46.67 de	5.2 d-g	20.66 bc	3.83 i	6.66 g	22.48 gh	1.15 a-c	87.84 a-c
DW-NVT LM 5	57 d-f	49.67 a-c	5.57 a-e	19.68 cd	5.83 b-g	8.57 bc	24.07 c-f	0.94 f-h	77.98 c-e
Fetan	62.33 bc	48 c-e	5.9 a-c	15.02 f-h	5.55 c-h	8.06 cd	22.52 gh	1.15 a-c	84.95 a-d
34607	62 c	45.33 ef	4.8 fg	17.41 de	6.78 ab	6.46 g	25.03 b-d	0.56 j	62.82 f
DZDW170108	62.67 bc	47.33 c-e	5.43 b-f	23.08 a	6.42 a-e	6.63 g	23.57 d-h	1.24 a	74.77 de
MSE	1.75	3.31	0.18	2.05	0.5	0.46	0.86	0	48.55
LSD	2.17	2.98	0.7	2.35	1.16	1.11	1.52	0.1	11.44
Cv	2.24	3.8	7.9	8.4	12.37	8.83	3.87	6.09	8.78

Table 5.2. Contd.

VAR	CCI	SMW	SMN	Chl_a	Chl_b	Chl_a+b	RBSB	RLPH	Fv.Fm	GY
Kuami	53.35 a-d	18.53 a-c	6 a-d	1.52 b-g	0.54 b-e	2.07 c-f	0.46 ab	0.41 lm	0.78 ef	4.91 d-h
ETDW/15DZ010	54.04 a-c	16.2 c-e	6.67 a-c	1.51 c-g	0.56 b-e	2.07 b-f	0.43 a-d	0.56 c-e	0.82 ab	5.12 d-g
31778	54.03 a-c	17.84 a-d	5.67 b-e	1.54 b-f	0.56 b-e	2.1 b-e	0.45 a-c	0.47 i-k	0.8 b-f	3.32 j-l
Alemtena	50.89 cd	18.14 a-d	6.67 a-c	1.49 c-g	0.55 b-e	2.04 c-f	0.33 e-j	0.61 bc	0.81 a-d	4.84 e-h
ETDW/15DZ 038	51.78 b-d	19.75 a	6.67 a-c	1.46 d-g	0.56 b-e	2.02 c-f	0.39 a-g	0.48 h-j	0.81 b-e	3.15 kl
Ude	53.16 a-d	16.62 b-e	6 a-d	1.52 b-g	0.53 c-e	2.05 c-f	0.25 j-l	0.6 b-d	0.81 b-e	5.6 cd
31797	52.9 a-d	14.82 e	7.33 a	1.43 fg	0.54 b-e	1.98 ef	0.41 a-e	0.51 g-i	0.78 c-f	2.84 l
31831	51.81 b-d	16.24 c-e	6.33 a-d	1.54 b-f	0.59 bc	2.13 bc	0.3 g-k	0.56 de	0.78 ef	5.63 cd
ETDW/15DZ04	55.93 a	14.7 e	7 ab	1.63 ab	0.59 bc	2.22 b	0.38 b-h	0.63 b	0.78 c-f	6.53 b
33403	52.48 b-d	17.71 a-d	6 a-d	1.53 b-f	0.57 b-e	2.1 b-e	0.44 a-c	0.43 k-m	0.81 b-e	3.98 ij
34217	54.46 ab	17.24 a-e	6 a-d	1.59 bc	0.53 de	2.11 b-e	0.23 kl	0.48 h-j	0.77 f	5.25 d-g
34295	52.4 b-d	19.57 a	5.33 c-e	1.44 e-g	0.54 c-e	1.97 ef	0.2 l	0.47 i-k	0.81 b-e	3.88 i-k
MCD2-17	54.58 ab	17.49 a-e	5 de	1.49 c-g	0.57 b-e	2.06 c-f	0.45 a-c	0.62 b	0.8 b-e	4.28 hi
34415	52.01 b-d	16.25 c-e	5 de	1.49 c-g	0.54 c-e	2.03 c-f	0.31 f-k	0.51 f-i	0.8 b-e	4.34 hi
ETDW/15DZ023	53.91 a-c	15.95 c-e	6.67 a-c	1.71 a	0.67 a	2.39 a	0.31 g-k	0.52 e-h	0.8 b-f	7.72 a
34493	53.21 a-d	16.51 b-e	7 ab	1.56 b-d	0.57 b-d	2.13 b-d	0.34 d-i	0.54 e-g	0.81 a-c	6.2 bc
ETDW/15DZ049	51 cd	17.31 a-e	6.67 a-c	1.54 b-f	0.56 b-e	2.1 b-e	0.4 a-f	0.44 kl	0.82 ab	4.59 f-i
34522	50.14 d	15.55 de	6.33 a-d	1.53 b-g	0.54 b-e	2.07 b-f	0.47 a	0.46 jk	0.81 a-c	5.33 d-f
34571	54.84 ab	16.52 b-e	6.33 a-d	1.42 g	0.52 de	1.93 f	0.37 c-i	0.74 a	0.81 a-c	3.31 j-l
MCD3-14	53.14 a-d	16.07 c-e	6.33 a-d	1.55 b-e	0.6 b	2.15 bc	0.29 i-l	0.49 h-j	0.82 ab	6.14 bc

DW-NVT LM 5	53.76 a-c	18.64 a-c	6.33 a-d	1.5 c-g	0.55 b-e	2.04 c-f	0.38 a-h	0.38 m	0.84 a	4.57 g-i
Fetan	53.97 a-c	16.48 c-e	6 a-d	1.53 b-g	0.55 b-e	2.08 b-f	0.38 a-h	0.56 d-f	0.77 f	5.46 c-e
34607	54.57 ab	19.32 ab	6.33 a-d	1.48 c-g	0.53 c-e	2.01 c-f	0.29 h-k	0.63 b	0.78 d-f	4.17 hi
DZDW170108	53.02 a-d	18.49 a-c	4.33 e	1.47 d-g	0.51 e	1.98 d-f	0.36 c-i	0.46 jk	0.81 a-d	4.04 ij
MSE	4.23	2.99	0.79	0	0	0.01	0	0	0	0.209
LSD	3.37	2.84	1.46	0.11	0.06	0.15	0.09	0.05	0.03	0.751
Cv	3.87	10.08	14.43	4.44	6.66	4.48	15.23	5.36	2.45	9.54

DTH: Days to heading; GFP: grain filling period; LN: leaf number; LA: leaf area; TN: Tiller number; SL: spike length; CT: canopy temperature; PC: Proline content; RLWC: Relative leaf water content; MSE: mean square error; LSD: least significant difference; CV: coefficient of variation.

Flag Leaf Area and Leaf number

The maximum flag leaf area was observed under non-stressed condition in DZDW170108 (23.08) followed by ETDW/15DZ023 (22.81 cm^2) and 34522 (21.62 cm^2), while a narrow flag leaf area was observed in 31778 (13.41 cm^2), 31831 (13.74 cm^2) and 34415 (14.44 cm^2) (Table 5.2). The greater decrease in flag leaf area was shown in ETDW/15DZ023 (-8.76), DW-NVT LM 5 (-8.54), and 34522 (-8.05) as compared to others under drought stressed conditions. Genotypes such as ETDW/15DZ049 (-1.19), 34607 (-1.01), Alemtena (-0.4), and 34295 (-0.36) were less affected under drought stressed conditions (Table 5.3). All the wheat genotypes showed a wide range of variation for leaf number ranged from 4.57-6.23 (in non-stressed condition) and 4-6 (in stressed condition). The genotype ETDW/15DZ023 showed the highest number of leaves (6.23) and the genotype Alemtena showed the lowest number of leaves (4.57) under non-stressed conditions. The genotypes 31778 and 34295 also showed the highest number of leaves (6) and the genotype 34571 showed the lowest number of leaves (4) in stressed conditions. The total mean of leaf number is (5.12) under non-stressed conditions and (5) under stressed conditions (Table 5.2).

Number of tillers

All the wheat genotypes showed a wide range of variation for the number of tillers, i.e., 3.83 - 7.08 (under non-stressed conditions) (Table 5.2) and 3.17-5.92 (under stressed conditions) (Table 5.3). The genotype MCD2-17 showed the highest number of tillers (7.08) and the

genotype MCD3-14 showed the lowest number of tillers (3.83) under non-stressed conditions (Table 5.2). The genotypes 34295 and 34607 showed the highest number of tillers (5.92 and 5.83, respectively) and the genotype 34493 showed the lowest number of tillers (3.17) in stressed conditions (Table 5.3). The mean of the number of tillers was (5.71) under non-stressed conditions and (4.3) under stressed conditions.

Spike length

A wide range of variation was also shown by all the genotypes with the range (6.21-9.9 cm) in non-stressed conditions (Table 5.2) and (4.95-7.55 cm) in stressed conditions (Table 5.3). The genotype 34571 showed the maximum spike length (9.9 cm) followed by Alemtena (9.85 cm), and the genotype 34522 showed the smallest spike length (6.21 cm) in non-stressed conditions (Table 5.2). The genotype 34295 showed the maximum (7.55 cm) and the genotype Kuami showed the minimum spike length (4.95 cm) in stressed conditions (Table 5.3). The total mean of spike length (7. 7cm) under non-stressed condition and (6.2 cm) under stressed conditions.

Proline content

Proline accumulation plays adaptive roles in plant stress tolerance. The accumulation of proline has been advocated as a parameter of selection for stress tolerance. In this study, it showed a wide range of proline content (i.e., 0.56 to 1.24 μg g^{-1} fresh wt. under non-stressed conditions and 0.9 to 2.29 μg g^{-1} fresh wt. under stressed conditions). The highest proline content observed in genotype DZDW170108 (1.24 μg g^{-1} fresh wt.) and the lowest amount of proline accumulated in genotype 34607 (0.56 μg g^{-1} fresh wt.) under non-stressed conditions (Table 5.2). ETDW/15DZ023 showed the highest proline content (2.29 μg g^{-1} fresh wt.) and genotype Ude and 34607 showed lowest the proline content (0.9 and 0.92 μg g^{-1} fresh wt., respectively) under stressed conditions (Table 5.3). The total mean of proline content was 0.98 μg g^{-1} fresh wt. under non-stressed conditions (Table 5.2) and 1.35 μg g^{-1} under stressed conditions (Table 5.3).

Canopy temperature (CT)

The results in Tables 5.2 showed that under non-stressed conditions the highest canopy temperature was observed in 31797 (26.93), followed by 34415 (25.8), 34295 (25.5), and 34607 (25.033). The lowest canopy temperature was observed in ETDW/15DZ049 (22.4), followed by MCD3-14 (22.48) and Fetan (22.52). Under drought stressed conditions (Table 5.3) the highest

canopy temperature was observed on 34217 (27.27) followed by ETDW/15DZ010 (26.73), ETDW/15DZ04 (26.67) and ETDW/15DZ023 (26.63), while 31778 (21.5) and 34295 (23.5) were among the coolest canopy temperatures during drought stressed conditions. The total mean canopy temperature was (23.97) under non-stressed conditions and (25.26) under stressed conditions.

Relative leaf water content (RLWC)

All the wheat genotypes showed a wide range of Relative leaf water content ranging from 62.82% to 93.07% under non-stressed conditions (Table 5.2) and from 44.91% to 75.18% under stressed conditions (Table 5.3). The highest value of Relative leaf water content was observed in genotype ETDW/15DZ023 (93.06%), and the lowest value of Relative leaf water content was observed in genotype 34607 (62.82%) under non-stressed conditions (Table 5.2). Genotype 34217 showed the highest value (75.18%) and genotype MCD3-147 showed the lowest value (44.91%) under stressed conditions (Table 5.3). The total mean of Relative leaf water content was 79.32% under non-stressed conditions and 63.66% under stressed conditions.

Chlorophyll Concentration Index (CCI) reading

The results of the chlorophyll concentration index indicated that under stressed conditions less concentration was recorded in wheat genotypes. It ranged from 50.14 to 55.93 under non-stressed conditions and from 38.77 to 53.16 under stressed conditions (Table 5.3). The chlorophyll index observed in genotype ETDW/15DZ04 was 55.93, and the genotype 34522 showed a lower chlorophyll index reading with a value of 50.14 in non-stressed conditions (Table 5.2). The genotype 34571 showed the highest value of chlorophyll index (53.16) followed by 34607 and ETDW/15DZ04 with chlorophyll index values of 52.78 and 52.43, respectively. The genotype ETDW/15DZ 038 showed a lower value of chlorophyll concentration index (38.77) followed by 34522 with a value of 43.78 under stressed conditions (Table 5.3). The total mean of the chlorophyll concentration index was *53.14* under non-stressed conditions and 49.52 under stressed conditions.

Stomata characteristics

The stomata width showed a high variability among genotypes in the studied genotypes across the two conditions. Under non-stressed conditions, the total mean stomatal width was 17.164 μm, whereas 5.739 μm was recorded under stressed conditions. The widest stomata were observed in ETDW/15DZ 038 which was 19.75 μm, followed by 34295 with 19.57 μm, and DW-NVT LM 5, 18.64 μm under non-stressed conditions. While narrow stomatal width was observed in ETDW/15DZ04, with a value of 14.7 μm followed by 31797 with a value of 14.82 μm (Table 5.2). Under stressed conditions, the stomatal width of 34415 was the largest (9.19) compared to other genotypes. The next widest open stomata width was found in genotypes ETDW/15DZ 038 with 8.19 μm and 33403 with 7.42 μm. The data in Tables 5.2 and 5.3 also showed that under stressed conditions, there was a notable closure or decline in the width of stomata in genotypes 34295 (-14.21 μm) followed by ETDW/15DZ023 (-13.8 μm) and 31778 (-13.71 μm). The variability of stomata number differed from the width both under non-stressed and stressed conditions as revealed in Tables 5.2 and 5.3. Under non-stressed condition the stomata number ranged from 4.33 to 7.33 and from 3.33 to 6 for stressed conditions. The highest number of stomata was observed in genotype 31797 with 8.33 followed by ETDW/15DZ04 and 34493 with each scoring 7 stomata under non-stressed conditions. under non-stressed conditions 31831 and 34493 scored highest with 6 stomata each and the lowest number was observed in 31797 and ETDW/15DZ 038, each scoring 3.33, the lowest under stressed conditions.

Table 5.3. Mean separation for drought stressed environmental condition

VAR	DTH	GFP	LN	LA	TN	SL	CT	PC	RLWC
Kuami	56 c-e	47 ab	5.67 ab	16.91 ab	3.5 h-j	4.95 h	25.6 b-f	1.2 c-g	73.76 ab
ETDW/15DZ010	57 b-e	44 b-e	4.33 de	14.09 c-g	4.5 c-g	6.77 a-d	26.73 ab	0.98 fg	60.58 c-h
31778	57.67 b-d	43 d-f	4.33 de	13.01 e-h	4.14 c-i	6.49 a-g	25.63 b-f	1.5 c-e	59.62 d-h
Alemtena	52 gh	47 ab	6 a	12.29 e-h	4.17 c-i	6.19 c-g	21.5 i	1.42 c-f	65.59 a-f
ETDW/15DZ 038	56 c-e	39 gh	5 b-d	16.05 b-d	4.47 c-g	6.32 b-g	24.47 f-h	1.14 d-g	64.76 a-g
Ude	50 hi	47.67 a	5.67 ab	13.82 c-g	4.33 c-h	5.65 e-h	26.23 a-e	0.9 g	66.87 a-f
31797	57.67 b-d	37.33 h	5 b-d	11.89 f-h	4.67 b-e	7.22 a-c	26.03 a-e	1.28 c-g	64.85 a-g
31831	56.33 b-e	47.33 a	5 b-d	12.31 e-h	4.17 c-i	6.36 b-g	25.5 b-f	1.52 c-e	72.27 a-c
ETDW/15DZ04	53.33 fg	44 b-e	5.67 ab	13.43 d-h	3.61 f-j	6.7 a-e	26.67 a-c	1.27 c-g	56.03 f-i
33403	56 c-e	45 a-d	5.33 a-c	12.77 e-h	4.83 bc	5.84 d-h	26.23 a-e	1.33 c-g	63.29 a-h
34217	50 hi	47.33 a	5.67 ab	13.77 d-g	3.75 e-j	5.78 d-h	27.27 a	1.28 c-g	75.18 a
34295	55.33 d-f	37.67 h	6 a	14.53 b-e	5.92 a	7.55 a	23.5 h	1.1 e-g	53.55 g-i
MCD2-17	61 a	40 f-h	5 b-d	14.39 b-f	5.58 ab	6.17 c-g	24.33 f-h	1.19 c-g	57.76 e-h

34415	55.67 d-f	43.67 c-e	5 b-d	11.64 gh	4.5 c-g	6.64 a-f	25.37 c-g	1.32 c-g	65.28 a-g
ETDW/15DZ023	55.67 d-f	42.67 d-f	5.33 a-c	14.05 c-g	4.5 c-g	5.73 d-h	26.63 a-d	2.29 a	66.74 a-f
34493	55 ef	44 b-e	5 b-d	12.52 e-h	3.17 j	5.59 f-h	25.23 e-g	1.2 c-g	72.69 ab
ETDW/15DZ049	55 ef	46.67 a-c	5.33 a-c	13.64 d-h	4 c-j	6.1 d-g	24.4 f-h	2.02 ab	68.78 a-e
34522	49.33 i	41 e-g	5.33 a-c	13.57 d-h	4.75 b-d	5.68 d-h	25.07 e-g	1.35 c-g	71.04 a-d
34571	50 hi	42.67 d-f	4 e	12.66 e-h	4.56 c-f	6.27 b-g	26.13 a-e	1.27 c-g	65.99 a-f
MCD3-14	50.33 hi	44.67 a-d	5.33 a-c	19.44 a	3.31 ij	6.08 d-g	25.33 d-g	1.39 c-g	44.91 i
DW-NVT LM 5	50.67 hi	42.67 d-f	4.67 c-e	11.14 h	3.83 d-j	6.38 b-g	24.4 f-h	1.65 bc	61.98 b-h
Fetan	58.33 bc	41.33 e-g	5.33 a-c	11.97 e-h	3.58 g-j	7.32 ab	24.1 gh	1.36 c-g	63.71 a-h
34607	58.67 ab	42 d-g	5 b-d	16.4 bc	5.83 a	5.42 gh	25.37 c-g	0.92 g	52.25 hi
DZDW170108	57 b-e	42.33 d-f	5.67 ab	15.92 b-d	3.67 f-j	6.1 d-g	24.47 f-h	1.59 b-d	60.31 c-h
MSE	2.04	3.64	0.24	2.56	0.34	0.45	0.64	0.09	53.25
LSD	2.35	3.13	0.8	2.63	0.96	1.1	1.31	0.49	11.98
Cv	2.61	4.4	9.35	11.56	13.54	10.81	3.17	22.04	11.46

Table 5.3 Contd.

VAR	CCI	SMW	SMN	Chl_a	Chl_b	Chl_a+b	RBSB	RLPH	Fv.Fm	GY
Kuami	47.38 h	5.94 a-c	5.33 a-c	1.25 b-e	0.42 b-f	1.66 cd	0.23 c-h	0.47 b-d	0.77 d-g	2.27 f-i
ETDW/15DZ010	51.83 a-d	6.56 a-c	5.67 ab	1.4 a-c	0.42 b-f	1.82 a-c	0.22 c-h	0.4 f-h	0.8 a-d	2.97 c-e
31778	51.28 a-e	7.06 ab	3.67 ef	1.4 a	0.37 ef	1.78 a-d	0.19 e-h	0.37 g-i	0.78 b-f	2.85 c-f
Alemtena	44.32 i	4.43 bc	4.67 b-e	1.31 a-d	0.38 d-f	1.69 b-d	0.23 c-h	0.4 f-h	0.75 g	2.2 g-j
ETDW/15DZ 038	38.77 j	8.19 ab	3.33 f	1.38 a-c	0.49 a-c	1.87 ab	0.17 gh	0.41 f-h	0.79 a-d	2 h-k
Ude	51.05 a-f	4.37 bc	5.67 ab	1.35 a-c	0.49 a-c	1.84 a-c	0.25 b-f	0.5 bc	0.81 ab	2.6 d-h
31797	50.05 b-h	6.46 a-c	3.33 f	1.3 a-d	0.37 ef	1.67 cd	0.25 b-f	0.58 a	0.76 e-g	2.11 g-j
31831	50.86 a-f	6.24 a-c	6 a	1.4 ab	0.51 ab	1.91 a	0.22 c-h	0.49 b-d	0.8 a-d	3.07 b-d
ETDW/15DZ04	52.43 a-c	4.17 bc	5.33 a-c	1.4 ab	0.46 a-e	1.86 ab	0.22 c-h	0.43 d-f	0.79 a-e	3.95 a
33403	51.85 a-d	7.42 ab	4.33 c-f	1.31 a-d	0.46 a-e	1.77 a-d	0.31 ab	0.45 c-f	0.76 e-g	2.41 e-i
34217	49.3 d-h	3.83 bc	4.33 c-f	1.39 a-c	0.49 a-c	1.88 a	0.22 c-h	0.47 b-e	0.75 fg	3.02 c-e
34295	48.7 e-h	5.36 a-c	4.33 c-f	1.42 a	0.4 c-f	1.83 a-c	0.33 a	0.58 a	0.79 a-e	0.88 l
MCD2-17	49.61 c-h	6.46 a-c	4.33 c-f	1.41 a	0.32 f	1.73 a-d	0.22 c-h	0.4 f-h	0.78 b-f	1.95 h-k
34415	49.62 c-h	9.19 a	4.67 b-e	1.34 a-c	0.48 a-d	1.82 a-c	0.17 gh	0.41 e-g	0.78 b-f	2.15 g-j
ETDW/15DZ023	52.32 a-c	2.15 c	5 a-d	1.39 a-c	0.43 a-e	1.81 a-c	0.26 a-d	0.58 a	0.77 c-g	3.68 ab
34493	50.52 a-g	4.16 bc	6 a	1.36 a-c	0.52 a	1.88 a	0.2 d-h	0.4 f-h	0.8 a-c	2.68 d-g
ETDW/15DZ049	47.68 gh	4.76 bc	5 a-d	1.31 a-d	0.43 a-e	1.74 a-d	0.26 a-d	0.36 hi	0.79 a-d	2.5 d-h

34522	43.78 i	5.16 a-c	5.67 ab	1.38 a-c	0.38 d-f	1.76 a-d	0.24 c-g	0.5 bc	0.82 a	3.45 a-c
34571	53.16 a	6.56 ab	3.67 ef	1.19 de	0.48 a-d	1.66 cd	0.28 a-c	0.46 b-e	0.82 a	2.43 d-i
MCD3-14	50.62 a-f	4.83 a-c	4.33 c-f	1.14 e	0.49 a-c	1.63 d	0.18 f-h	0.33 i	0.79 a-d	2.28 f-i
DW-NVT LM 5	51.39 a-e	6.74 ab	5.33 a-c	1.24 c-e	0.39 d-f	1.63 d	0.23 c-h	0.45 c-f	0.81 ab	1.85 i-k
Fetan	48.22 f-h	4.02 bc	5.33 a-c	1.35 a-c	0.5 a-c	1.84 a-c	0.26 a-e	0.51 b	0.81 ab	3 c-e
34607	52.78 ab	6.63 ab	4.33 c-f	1.37 a-c	0.43 a-e	1.8 a-d	0.16 h	0.45 c-f	0.75 g	1.46 kl
DZDW170108	51.04 a-f	7.04 ab	4 d-f	1.31 a-d	0.47 a-d	1.79 a-d	0.19 d-h	0.47 b-e	0.77 d-g	1.56 jk
MSE	3.16	7.22	0.43	0.01	0	0.01	0	0	0	0.155
LSD	2.92	4.41	1.08	0.15	0.1	0.18	0.07	0.05	0.03	0.646
CV	3.59	46.81	13.85	7.03	13.53	6.24	19.41	7.36	2.36	15.93

CCI: Chlorophyll concentration index reading; SMW: stomata width; SMN: stomata number; Chl_a: Chlorophyll a; Chl_b: Chlorophyll b; Chl_a+b: total chlorophyll; RBSB: root-to-shoot biomass ratio RLPL: root-to-shoot length; Fv/Fm: Chlorophyll fluorescence; and GY: grain yield; MSE: mean square error; LSD: least significant difference; CV: coefficient of variation

Chlorophyll (Chl_a, Chl_b, and Chl_a+b)

In the current study, under non-stressed conditions, ETDW/15DZ023 (1.713 mg g^{-1} FW) recorded the maximum chlorophyll a content, whereas landrace 34571 (1.417 mg g^{-1} FW showed the minimum chlorophyll a content (Table 5.2). Under drought stress, 34295 (1.423 mg g^{-1} FW) had the highest chlorophyll a content, whereas MCD3-14 (1.143mg g^{-1} FW) recorded the lowest chlorophyll a content among the genotypes (Table 5.3). Data showed that under non-stressed conditions, ETDW/15DZ023 (0.673 mg g^{-1} FW) again recorded the maximum chlorophyll b content, in contrast to DZDW170108 (0.51 mg g^{-1} FW), which recorded the minimum chlorophyll b content. Under stressed conditions, 34493 (0.523 mg g^{-1} FW) had the highest chlorophyll b content, in contrast to MCD2-17 (0.323 mg g^{-1} FW), in which the minimum was recorded. Under non-stressed conditions, total chlorophyll was maximized in 31831 (2.387 mg g^{-1} FW), in contrast to 34571 (1.93 mg g^{-1} FW), where the minimum yield was recorded. Under stressed conditions, 31831 recorded the maximum total chlorophyll (1.93 mg g^{-1} FW), in contrast to MCD3-14 and DW-NVT LM 5 (1.63 mg g^{-1} FW), in which the minimum was recorded.

The ratio of plant height to root length (RLPH) and ratio of root biomass to shoot biomass (RBSB)

The ratio of root length to plant height (RLPH) is a useful indicator of drought stress in wheat. A higher ratio suggests that the plant is allocating more resources to below growth, which may be a sign of stress tolerance. RLPH under non-stressed conditions showed a wide range of variation from 0.383 for DW-NVTLM5 to 0.743 for 34571 (Table 5.2). Under stressed conditions the minimum and maximum value for RLPH decreased to the range of 0.33 to 0.58 for MCD3-14 and ETDW/15DZ023, respectively (Table 5.3). The total mean of RLPH was 0.524 under non-stressed conditions and 0.452 under drought stressed conditions. The root biomass to shoot biomass ratio under non-stressed conditions showed a wide range of variation from 0.203 (34295) to 0.467 (34522). Under stressed conditions the minimum and maximum value for RBSB decreased to the range of 0.17 in ETDW/15DZ 038 to 0.313 in 33403. The highest decrease in RBSB was observed in 34522 (-0.227), followed by Kuami (-0.233) and ETDW/15DZ 038 (-0.217). The total mean of RBSB was 0.359 under non-stressed conditions and .0.229 under drought stressed conditions (Table 5.4).

Chlorophyll fluorescence (Fv/Fm)

Chlorophyll fluorescence showed significant differences among cultivars under non-stressed and drought stressed conditions (Tables 5.2 and 5.3). The ranges for chlorophyll fluorescence were from 0.77 to 0.84 under non-stressed conditions and 0.75 to 0.817 under stressed conditions. The highest chlorophyll fluorescence was observed in genotype DW-NVT LM 5, which was 0.84, and the lowest amount was in genotypes 34217 and Fetan, with 0.77 under non-stressed conditions, while 34522 and 34571 showed the highest chlorophyll fluorescence (0.817), and genotypes 31778 and 34607 showed the lowest (0.75) under stressed conditions.

Grain yield

The results in Tables 5.2 and 5.3 highlighted that under non-stressed conditions the highest yield was observed in ETDW/15DZ023 (7.72 ton/ha), compared to ETDW/15DZ04 (6.53 ton/ha), 34493 (6.2 ton/ha), MCD3-14 (6.14 ton/ha), and 31831 (5.63 ton/ha). The varieties such as ETDW/15DZ010, 34217 and 34522 still showed high grain yield compared to the remaining landraces. The highest decline in yield under stressed conditions was observed in genotype

ETDW/15DZ023 (-4.04 ton/ha) and MCD3-14 (-3.86 ton/ha), 34493 (-3.52 ton/ha) and Ude (-3 ton/ha) compared with non-stressed conditions. Greater grain yield was noted in ETDW/15DZ04 (3.95 ton/ha) and ETDW/15DZ023 (3.68 ton/ha) compared with 34522 (3.45 ton/ha), 34217 (3.02 ton/ha), Fetan (3 ton/ha) and ETDW/15DZ010 (2.97 ton/ha) under drought stressed conditions. Insignificant yield loss was observed in 31778 (-0.47 ton/ha), 31797 (-0.73 ton/ha), 34571 (-0.88 ton/ha) under stressed conditions compared with non-stressed conditions.

5.3.3. Genetic Variability analysis

The genetic variability analysis indicates the presence of higher variability between the traits studied (Table 5.4). Under non-stressed conditions, Genotypic Coefficient of Variance (GCV) values varied from 1.766% (for Fv/Fm) to 23.815% (for GY), while Phenotypic Coefficient of Variance (PCV) ranged from 3.059% (for Fv/Fm) to 25.7% (for GY). Environmental coefficient of Variance (ECV) value also varied from 1.969% (for DTH) to 15.489% (for RBSB). Heritability value at broad sense (H_b^2) ranged from 11.1% (for CCI) to 93.8% (for PC). Also, genetic advance (GA) and genetic advance as percentage of mean (Jeyabalasingh et al.) shows a range of variability from 0.017 and 0.934% (for Fv/Fm and CCI, respectively) to 7.765 (for RLWC) and 45.452% (for GY), respectively.

Table 5.4. Genetic Variability Analysis for the eleven traits of durum wheat varieties at non-stressed and drought stressed conditions

	Non-stressed condition							Stressed condition						
	ECV	**GCV**	**PCV**	H_b^2	**GA**	**GAM**	**Mean**	**ECV**	**GCV**	**PCV**	H_b^2	**GA**	**GAM**	**Mean**
DTH	1.969	5.815	6.139	89.7	6.713	11.346	59.167	2.354	5.823	6.281	86	6.089	11.121	54.75
GFP	3.529	3.532	4.993	50.1	2.463	5.148	47.847	4.435	6.422	7.805	67.7	4.717	10.886	43.333
LN	7.517	6.921	10.218	45.9	0.52	9.657	5.389	8.812	8.396	12.171	47.6	0.62	11.93	5.194
CT	3.889	4.349	5.834	55.6	1.6	6.677	23.967	3.069	4.616	5.543	69.3	2	7.919	25.258
CCI	3.867	1.364	4.101	11.1	0.496	0.934	53.142	3.647	6.348	7.321	75.2	5.616	11.34	49.524
TN	12.396	13.258	18.15	53.4	1.14	19.949	5.714	13.736	15.116	20.426	54.8	0.992	23.045	4.306
LA	8.448	16.108	18.189	78.4	5.01	29.387	17.048	11.609	12.337	16.94	53	2.562	18.509	13.841
SL	9.01	13.413	16.159	68.9	1.765	22.937	7.694	10.929	7.599	13.311	32.6	0.556	8.938	6.221
RBSB	15.489	18.713	24.319	59.2	0.106	29.654	0.359	19.629	14.517	24.37	35.5	0.041	17.814	0.229
RLPH	5.357	15.852	16.745	89.6	0.162	30.914	0.524	6.941	14.335	15.951	80.8	0.12	26.544	0.452
RLWC	8.353	7.03	10.917	41.5	7.397	9.325	79.322	11.216	9.446	14.664	41.5	7.98	12.536	63.658
PC	3.92	15.287	15.788	93.8	0.299	30.492	0.981	22.154	19.283	29.371	43.1	0.353	26.076	1.353
Fv/Fm	2.479	1.766	3.059	33.3	0.017	2.098	0.801	2.25	2.206	3.12	50	0.025	3.21	0.785
Chl_a	4.477	3.292	5.547	35.2	0.061	4.022	1.519	4.798	4.669	6.687	48.8	0.09	6.713	1.338
Chl_b	6.541	4.745	8.02	35	0.032	5.774	0.558	13.795	9.065	16.499	30.2	0.045	10.266	0.441
Chl_a+b	4.464	3.604	5.738	39.4	0.097	4.661	2.077	5.116	3.729	6.335	34.7	0.08	4.52	1.779
SMW	9.953	5.888	11.564	25.9	1.06	6.176	17.164	9.892	6.219	11.685	28.3	1.17	6.819	5.739
SMN	14.229	7.88	16.265	23.5	0.485	7.863	6.167	13.56	15.216	20.381	55.7	1.108	23.401	4.736
GY	9.675	23.815	25.706	85.8	2.181	45.452	4.799	15.018	27.448	31.288	77	1.226	49.603	2.472

ECV: Environmental Coefficient of Variance; GCV: Genotypic Coefficient of Variance; PCV: Phenotypic Coefficient of Variance; hBS: Heritability (Broad Sense); GA: Genetic Advance; GAM: Genetic Advance as percentage of mean. DTH: Days to heading; GFP: grain filling period, Leaf number; canopy temperature, CCI: leaf chlorophyll concentration measurement, Tiller number; LA: leaf area; SL: spike length; RBSB: root-to-shoot biomass ratio RLPL: root-to-shoot length; RLWC: Relative leaf water content; PC: Proline content; Chl_a: Chlorophyll a; Chl_b: Chlorophyll b; Chl_a+b: total chlorophyll; Fv/Fm, SMW: stomata width; SMN: stomata number and GY: grain yield

Under stress conditions, a similar trend as non-stressed conditions, but with significant variation was observed. GCV values varied from 2.206% (for Fv/Fm) to 27.448% (for GY), while PCV ranged from 3.12% (for Fv/Fm) to 31.288% (for GY). ECV value also varied from 2.25% (for Fv/Fm) to 22.15 % (for PC). H_b^2 ranged from 28.3% (for SMW) to 86 % (for DTH). Also, GA and GAM show a range of variability from 0.025 and 3.21% for (for Fv/Fm) to 7.98 (for RLWC) and 49.603 % (for GY), respectively (Table 5.4).

5.3.4. Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was performed on 19 traits using the singular value decomposition approach. A total of 19 principal components were calculated, of which seven had eigenvalues greater than 1 and were retained for further analysis in accordance with Kaiser's rule. Under non-stress conditions, the highest variation was explained by the first principal component (PC1), which accounted for 33.64% of the total variation with an eigenvalue of 2.53. The subsequent components, PC2, PC3, PC4, PC5, PC6, and PC7, explained 11.7%, 10.37%, 7.95%, 6.56%, 5.59%, and 5.38% of the variation, respectively as indicated in Table 5.5 below. PC1 showed positive correlations with traits such as CT (0.2914), TN (0.2733), and SMW (0.1953). However, it had negative correlations with traits like Chl_a (-0.3633), RLWC (-0.3552), and GY (-0.3596), showing that these traits contributed inversely to this component.

PC2 exhibited strong positive correlations with RLPH (0.5163) and CCI (0.4213), along with moderate correlations with CT (0.2101) and SL (0.1925). Negative correlations were observed with traits such as SMW (-0.2558), LA (-0.355), and DTH (-0.1415). In contrast, PC3 highlighted a significant positive correlation with the GFP (0.6184), followed by moderate correlations with SL (0.1991) and SMN (0.1549). However, it showed negative correlations with DTH (-0.3768), LA (-0.3883), and RBSB (-0.3132), suggesting these traits had a contrary influence on this component (Table 5.5).

PC4 was positively correlated with SMW (0.393), LN (0.3323), and LA (0.1511), but negatively correlated with traits such as RBSB (-0.4947) and SMN (-0.3826). Similarly, PC5 showed strong positive correlations with SL (0.5106) and Chl_b (0.4029), while negatively correlating with RLPH (-0.2537) and PC (-0.2917). PC6 emphasized positive correlations with PC (0.4616)

and CCI (0.4329), while PC7 was positively associated with CCI (0.3359) and SMW (0.3216), but negatively correlated with TN (-0.3684), DTH (-0.3655), and LN (-0.3553).

Table 5.5. loading of each trait for the principal component at non-stressed conditions.

Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7
DTH	0.193	-0.142	-0.377	0.130	0.181	-0.104	-0.366
GFP	-0.032	-0.208	0.618	0.108	0.073	-0.045	-0.079
LN	-0.213	0.051	0.074	0.332	0.353	0.258	-0.355
CT	0.291	0.210	0.007	0.079	0.120	-0.118	-0.145
CCI	-0.050	0.421	-0.106	0.094	-0.141	0.433	0.336
TN	0.273	0.128	-0.215	0.103	0.141	0.015	-0.368
LA	-0.120	-0.355	-0.388	0.151	-0.172	0.111	-0.036
SL	0.191	0.193	0.199	-0.194	0.511	0.386	0.054
RBSB	0.048	-0.112	-0.313	-0.495	0.229	0.035	0.249
RLPH	0.025	0.516	0.020	-0.102	-0.254	0.085	-0.246
RLWC	-0.355	-0.067	0.146	-0.062	0.046	0.069	-0.192
PC	-0.147	-0.224	0.073	-0.289	-0.292	0.462	-0.358
Fv_Fm	0.059	-0.317	0.016	-0.313	0.164	0.267	-0.014
Chl_a	-0.363	0.073	-0.084	0.101	0.107	-0.012	0.191
Chl_b	-0.303	0.082	-0.177	-0.027	0.403	-0.065	-0.053
Chl_a+b	-0.363	0.081	-0.123	0.060	0.222	-0.032	0.113
SMW	0.195	-0.256	0.123	0.393	0.150	0.049	0.322
SMN	-0.161	0.110	0.155	-0.383	0.097	-0.502	-0.110
GY	-0.360	0.055	-0.067	0.140	-0.086	-0.036	-0.070
Eigen value	6.4	2.2	2	1.5	1.2	1.1	1
% of variance	33.64	11.703	10.367	7.948	6.562	5.591	5.384
cumm % of vari	33.64	45.343	55.71	63.658	70.22	75.812	81.195

Under stressed condition, the first principal component (PC1) explained the highest variation, accounting for 20.58% of the total variability with an eigenvalue of 1.98. The subsequent components, PC2, PC3, PC4, PC5, PC6, and PC7 explained variations of 14.56% (eigenvalue 1.39), 11.11% (eigenvalue 1.05), 10.63% (eigenvalue 1.01), 8.49% (eigenvalue 0.81), 7.03% (eigenvalue 0.67), and 6.06% (eigenvalue 0.58), respectively as indicated in Table 5.6.

PC1 showed positive correlations with traits such as GY (0.3768), GFP (0.3738), SMN (0.3501), RLWC (0.2929), Chl_b (0.2786), and CT (0.1982), among others, while it had negative correlations with Chl_a (-0.0159), LA (-0.0799), SL (-0.216), and TN (-0.3422). Similarly, PC2 exhibited strong positive correlations with Chl_a (0.4589), RLPH (0.4167), and Chl_a+b (0.3529), while negatively correlating with GFP (-0.2013), LA (-0.3334), and SMW (-0.1374).

PC3 was positively correlated with Chl_a+b (0.3787), CT (0.3104), and SMW (0.306), but showed negative correlations with LN (-0.3419), RBSB (-0.426), and RLPH (-0.2388).

Table 5.6. loading of each trait for the principal component under stressed conditions.

Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7
DTH	-0.250	0.173	0.262	0.119	-0.095	-0.305	0.240
GFP	0.374	-0.201	0.000	0.121	-0.080	-0.143	-0.117
LN	0.075	0.074	-0.342	0.520	-0.075	0.193	0.075
CT	0.198	0.053	0.310	-0.217	-0.464	0.035	-0.179
CCI	0.063	0.016	0.130	-0.343	-0.544	0.048	0.133
TN	-0.342	0.241	0.016	0.036	-0.157	0.026	-0.250
LA	-0.080	-0.333	0.048	0.314	-0.252	0.280	0.129
SL	-0.216	0.271	-0.007	-0.228	0.215	0.223	0.348
RBSB	0.006	0.287	-0.426	-0.191	-0.122	0.165	-0.197
RLPH	-0.029	0.417	-0.239	-0.030	-0.231	0.156	-0.215
RLWC	0.293	0.161	-0.060	0.043	0.202	-0.354	-0.478
PC	0.136	0.018	-0.282	-0.120	-0.042	-0.432	0.415
Fv.Fm	0.143	0.032	0.056	-0.383	0.402	0.346	0.066
Chl_a	-0.016	0.459	0.260	0.266	0.041	-0.124	0.107
Chl_b	0.279	-0.064	0.246	0.075	0.045	0.390	-0.065
Chl_a+b	0.162	0.353	0.379	0.276	0.064	0.140	0.051
SMW	-0.291	-0.137	0.306	-0.123	0.190	-0.135	-0.333
SMN	0.350	0.124	0.002	0.016	0.123	0.088	0.073
GY	0.377	0.149	0.093	-0.123	-0.044	-0.145	0.221
Eigen value	3.8	2.8	2.1	2	1.6	1.3	1
% of variance	20.577	14.557	11.114	10.633	8.491	7.028	6.061
cumu % of vari	20.577	35.134	46.248	56.881	65.372	72.400	78.461

PC1- PC7: principal components 1 to 7; DTH: Days to heading; GFP: grain filling period; LN: leaf number; LA: leaf area; TN: Tiller number; SL: spike length; CT: canopy temperature; PC: Proline content; RLWC: Relative leaf water content; CCI: Chlorophyll concentration index reading; SMW: stomata width; SMN: stomata number; Chl_a: Chlorophyll a; Chl_b: Chlorophyll b; Chl_a+b: total chlorophyll; RBSB: root-to-shoot biomass ratio RLPL: root-to-shoot length; Fv/Fm: Chlorophyll fluorescence; and GY: grain yield.

PC4 highlighted positive correlations with LN (0.5202), LA (0.3143), and Chl_a+b (0.276), but negative associations with GY (-0.1234), CCI (-0.3434), and CT (-0.2171). PC5 emphasized positive correlations with Fv/Fm (0.4024), SL (0.2151), and RLWC (0.2023), but negatively correlated with CCI (-0.5441), CT (-0.4637), and TN (-0.1571). Meanwhile, PC6 showed positive relationships with Chl_b (0.3899), Fv/Fm (0.3463), and LA (0.2796), while negatively correlating with RLWC (-0.3543), PC (-0.4323), and GFP (-0.1425). Lastly, PC7 was positively

associated with (PC, 0.4147), SL (0.3484), and DTH (0.2404), but negatively correlated with RLWC (-0.478), SMW (-0.3325), and RLPH (-0.2153) (Table 5.6).

5.3.5. Principal Component Biplot Analysis

The relationships between the different variables and genotypes with respective principal components are illustrated by the principal component biplots in Figures 5.1 and 5.2 for the non-stressed and stressed conditions, respectively. Smaller angles between dimension vectors in the same direction indicate a high correlation of the variable traits in terms of discriminating genotypes. Genotypes excelling in a particular trait are plotted closer to the vector line and further in the direction of that particular vector, often at the vertices of the convex hull. Under non-stressed conditions, the genotypes were sparsely distributed along the two principal components, with genotypes 31831, ETDW/15DZ04, ETDW/15DZ023, 34493, and MCD3-14 g more inclined in the direction of GY, SMN, RLWC, LN and Chl_a, Chl_b and total chlorophyll (Figure 5.1). The local checks Fetan and Ude, along with other landraces such as 34217 and 31831, clustered together in the direction of early heading and a short grain-filling period.

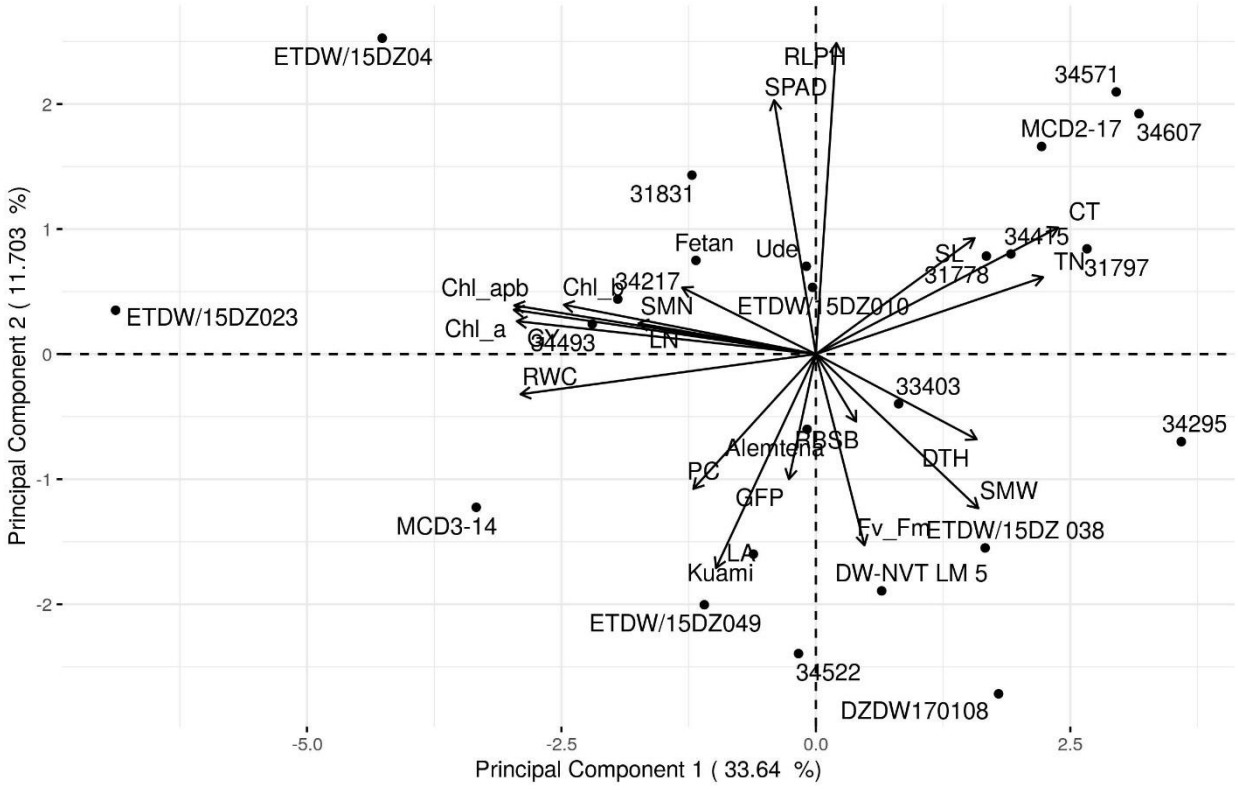

Figure 5.1. The PCA biplot of durum wheat landraces for PC1 and PC2 at non-stressed conditions. (The arrows show the contribution (magnitude and direction) of the trait in PC1 and PC2.)

Additionally, it is indicated that several traits, such as SMN, GY, Chl_a, Chl_b, Chl_a+b, RLWC, CT, and LN, make high contributions to PC1. These traits are crucial in distinguishing the wheat landraces along the first dimension, with high values clustering in the direction of their respective arrows. For PC2, traits like DTH, GFP, SMW and RBSB show significant contributions, making them more effective in distinguishing the wheat landraces along the second dimension.

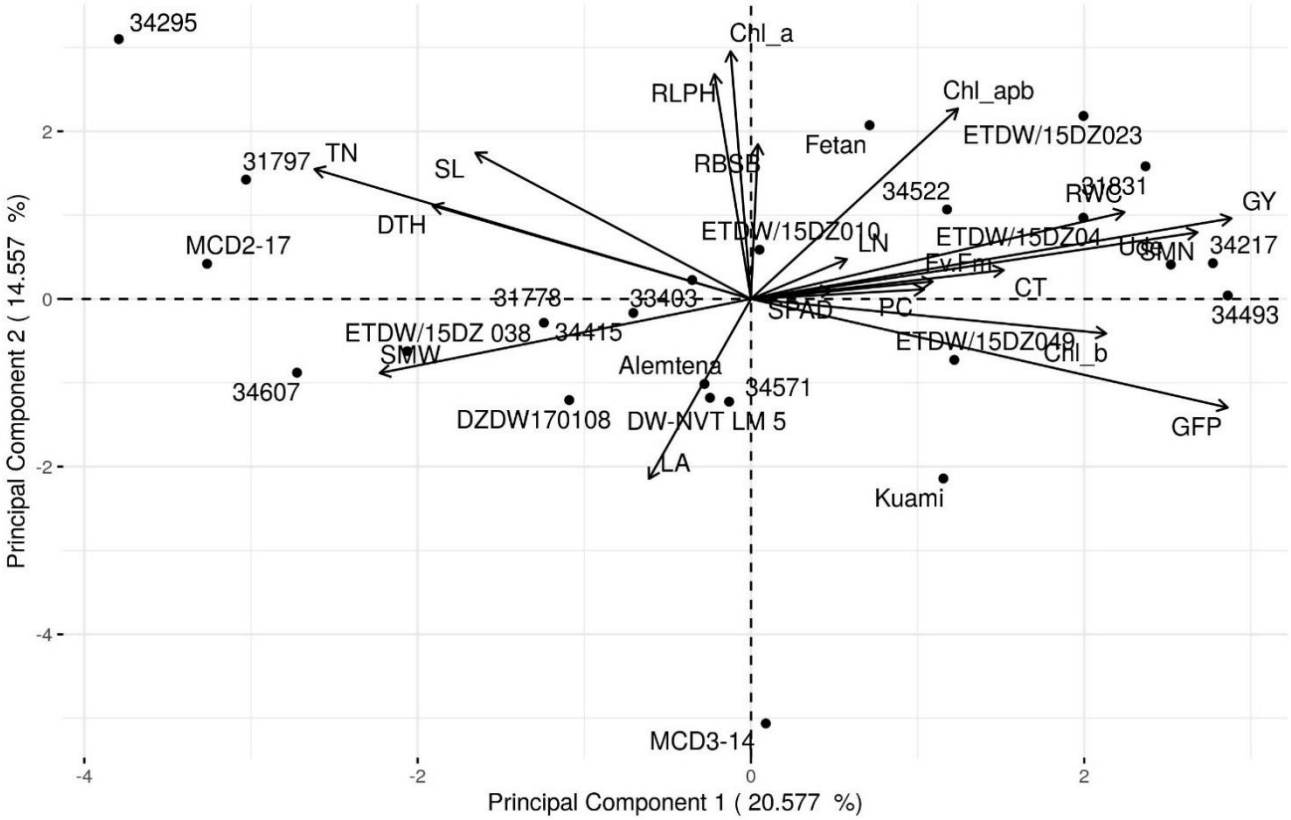

Figure 5.2. The biplot of durum wheat landraces for PC1 and PC2 under stressed conditions. (The arrows show the contribution (magnitude and direction) of the trait in PC1 and PC2)

Under stressed conditions, most genotypes were scattered on the positive side of the first principal component, with genotypes ETDW/15DZ04, 34217, 34522, and 34571excelling in

yield. This was mainly due to their water retention ability, cool canopy, and optimal values for other yield components (Figure 5.2). The biplot graph of the two principal components reveals that PC1 which accounts for 20% of the variance, is primarily influenced by SMN, GY and Fv/Fm. Additionally, GFP also shows considerable contributions to PC1. PC2, explaining 15.3% of the variance, is mainly influenced by DTH RSBR, and RLPH. LN and CCI also show significant contributions to PC2.

5.3.6. Cluster analysis

The cluster analysis was performed using Tocher's method and the Mahalanobis D^2 distance. Under non-stressed conditions, as indicated in Table 5.8, Cluster 1 contained the highest number of genotypes, with 13, followed by Cluster 2, which included 6 genotypes. Clusters 4, 5, and 6 each had the fewest genotypes, with just 1 genotype per cluster. Cluster 4 stands out with the highest grain yield (5.99 t/ha) and superior water retention, as indicated by the highest Relative leaf water content (86.38%). Genotypes in this cluster also exhibit early heading (56.89 days) and small stomata width (16.43 μm), this cluster has a well-balanced combination of physiological and agronomic traits and make it highly promising for breeding programs aimed at improving yield potential under non-stress conditions (Table 5.7).

Table 5.7 Mean performance of genotypes in respective clusters for different traits under non-stressed condition

Trait	C- I	C- II	C- III	C- IV	C- V	C- VI
DTH	59.08	64.83	62.67	56.89	56.33	62.00
GFP	48.05	47.50	47.33	47.89	48.67	45.33
LN	5.40	5.00	5.43	5.63	5.10	4.80
CT	23.91	26.22	23.57	23.08	24.87	25.03
CCI	52.72	52.65	53.02	53.72	54.84	54.57
TN	5.82	6.72	6.42	4.73	6.50	6.78
LA	16.63	16.21	23.08	17.59	14.49	17.41
SL	7.93	8.22	6.63	7.02	9.90	6.46
RBSB	0.39	0.31	0.36	0.32	0.37	0.29
RLPH	0.50	0.49	0.46	0.55	0.74	0.63
RLWC	78.83	74.75	74.77	86.38	73.66	62.82
PC	0.92	1.01	1.24	1.10	1.16	0.56
Fv_Fm	0.81	0.80	0.81	0.79	0.81	0.78
Chl_a	1.52	1.44	1.47	1.58	1.42	1.48
Chl_b	0.56	0.54	0.51	0.58	0.52	0.53

SMW	17.28	17.20	18.49	16.43	16.52	19.32
SMN	6.13	6.33	4.33	6.44	6.33	6.33
GY	4.69	3.59	4.17	5.99	3.31	4.16

Cluster 5 and Cluster 6 also display unique traits. Cluster 5 is notable for its long spike length (9.90 cm) and high CCI value (54.84), indicative of efficient photosynthetic capacity. However, it has the lowest leaf area (14.49) and grain yield (3.31 t.ha). In contrast, Cluster 6 is characterized by the widest stomatal opening (19.32 μm) and highest tiller number (6.78), reflecting strong vegetative growth. However, its low Relative leaf water content (62.82%) and grain filling period (45.33 days) may limit grain yield (4.16 g).

Table 5.8. Grouping of the studied durum wheat genotypes based on their respective clusters for various traits under non-stressed and stressed conditions.

Cluster		No of genotypes	Name of genotypes
I	Non-stressed	13	ETDW/15DZ010, ETDW/15DZ 038, Ude, Kuami , 31831, ETDW/15DZ04, 33403, 34295, 34415, 34522, MCD3-14, DW-NVT LM 5, 34607
II		6	31778, ETDW/15DZ023, 34571, MCD2-17, DZDW170108, 31797
III		2	Alemtena, Fetan
IV		1	34217
V		1	34493
VI		1	ETDW/15DZ049
I	Stressed	12	ETDW/15DZ 038, Ude, 31831, ETDW/15DZ010, ETDW/15DZ04, 34295, ETDW/15DZ023, 34493, ETDW/15DZ049, 34522, 34607, DZDW170108,
II		4	33403, 34217, DW-NVT LM 5, 31778
III		2	Fetan , 31797
IV		2	34571, MCD2-17
V		1	Kuami
VI		1	Alemtena
VII		1	34415
VIII		1	MCD3-14

Clusters 1, 2, and 3 represent intermediate performance levels with varying strengths. Cluster 1 shows early heading (59.08 days) and moderate grain yield (4.69 t/ha), balancing maturity and productivity. Cluster 2, with the latest heading (64.83 days) and moderate tiller number (6.72), prioritizes vegetative growth but has a relatively low yield (3.59 t/ha). Cluster 3 is unique for its large leaf area (23.08 cm^2) and high proline content (1.24), suggesting potential stress

responsiveness, although its grain yield (4.17 t/ha) and spike length (6.63 cm) are relatively low. These clusters provide valuable options for selecting genotypes suited to specific breeding goals, such as stress tolerance with balanced yield performance.

Under stressed conditions, Cluster 1 contained the highest number of genotypes, with 12, followed by Cluster 2, which had 4 genotypes. Clusters 5, 7, 8, and 6 each had the fewest genotypes, with only 1 genotype per cluster (Table 5.8). Cluster 1 stands out with a high Relative leaf water content (RLWC) of 65.80% and one of the highest grain yields (2.90 t/ha), coupled with moderate proline content (1.79) and stomatal width (4.31 μm) as indicated in Table 5.9. These traits indicate good stress tolerance and productivity. Similarly, Cluster 2 also showed high RLWC (69.77%) and favorable photosynthetic efficiency (Fv/Fm of 0.80), maintaining a relatively high grain yield (2.88 t/ha). Its traits suggest enhanced water use efficiency under stress.

Table 5.9 Mean performance of genotypes in respective clusters for different traits under stressed conditions

Trait	C- I	C- II	C- III	C- IV	C- V	C- VI	C- VII	C- VIII
DTH	56.67	49.83	50.67	51.17	56.75	56.00	56.00	55.33
GFP	40.00	44.67	42.67	45.83	43.61	39.00	47.00	37.67
LN	5.17	5.17	4.67	5.67	5.08	5.00	5.67	6.00
CT	26.33	26.18	24.40	23.42	25.34	24.47	25.60	23.50
CCI	51.19	49.32	51.39	47.47	50.64	38.77	47.38	48.70
TN	4.58	4.35	3.83	3.74	4.30	4.47	3.50	5.92
LA	12.97	13.45	11.14	15.86	13.51	16.05	16.91	14.53
SL	6.48	5.84	6.38	6.13	6.29	6.32	4.95	7.55
RBSB	0.26	0.25	0.23	0.21	0.22	0.17	0.23	0.33
RLPH	0.58	0.48	0.45	0.36	0.43	0.41	0.47	0.58
RLWC	65.80	69.77	61.98	55.25	62.71	64.76	73.76	53.55
PC	1.79	1.20	1.65	1.41	1.35	1.14	1.20	1.10
Fv.Fm	0.77	0.80	0.81	0.77	0.78	0.79	0.77	0.79
Chl_a	1.35	1.33	1.24	1.23	1.36	1.38	1.25	1.42
Chl_b	0.40	0.46	0.39	0.44	0.45	0.49	0.42	0.40
SMW	4.31	4.98	6.74	4.63	6.14	8.19	5.94	5.36
SMN	4.17	4.83	5.33	4.50	4.89	3.33	5.33	4.33
GY	2.90	2.88	1.85	2.24	2.55	2.00	2.27	0.88

Cluster 4 and Cluster 6 show high leaf area (15.86 and 16.05 cm^2, respectively) and spike length (6.13 and 6.32 cm, respectively), with Cluster 6 further distinguished by the highest stomata width (8.19 µm). These clusters, however, have moderate to low grain yields (2.24 and 2.0 t/ha, respectively). Cluster 7, with the highest RLWC (73.76%) and an extended grain-filling period (47 days), indicates strong stress resilience, although its grain yield (2.27 t/ha) is moderate, suggesting room for improvement in yield-related traits (Table 5.9).

In contrast, Cluster 8 is characterized by the lowest grain yield (0.88 t/ha), RLWC (53.55%), and stomatal width (5.36 µm), along with high spike length (7.55 cm). Clusters 3 and 5 show intermediate traits, with moderate grain yield (1.85 and 2.55 t/ha, respectively) and balanced performance in RLWC, proline content, and photosynthetic traits.

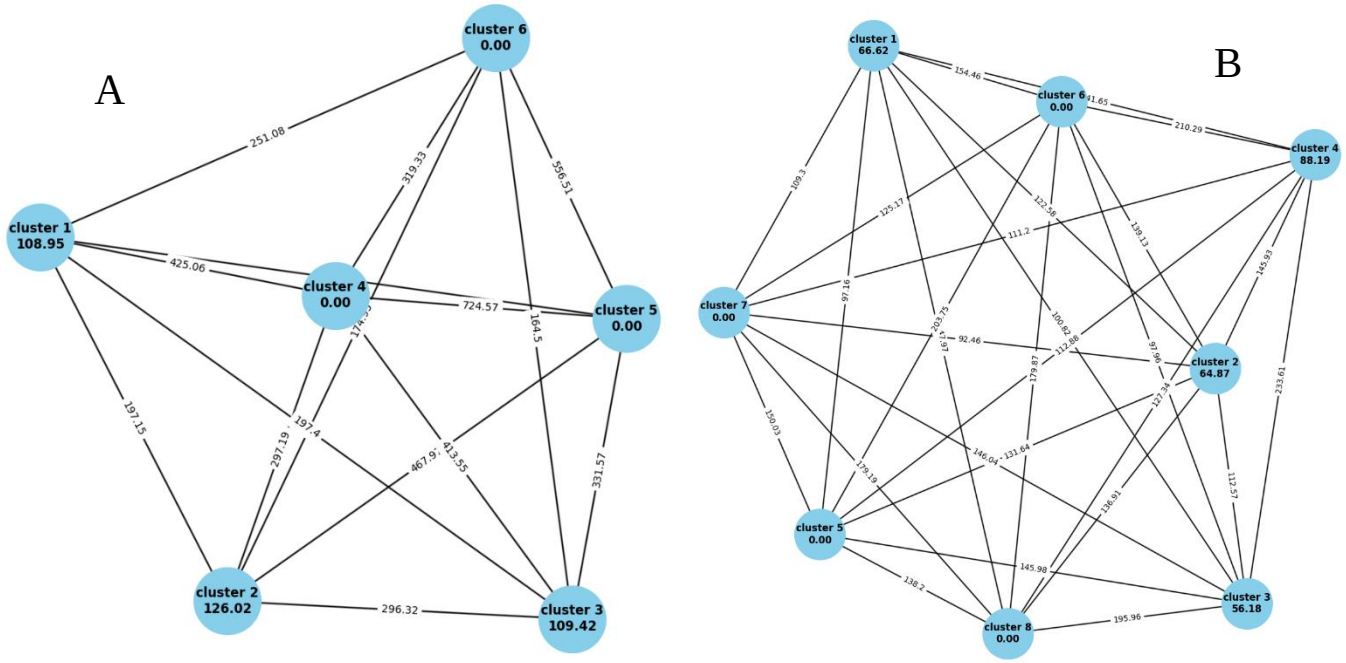

Figure 5.3. Cluster diagram indicating intracluster (edge weight) and intercluster distance (lines) among 6 cluster under non-stressed (A) and 8 clusters under stressed conditions (B).

The intra-cluster distances (edge weight) and inter-cluster distances (line) of studied genotypes under non stressed and stressed condition is shown in Figure 5.3A and 5.3B respectively. Under non stressed condition, Figure 5.3A Cluster 2 exhibited the highest intra-cluster distance (126.0), showing the greatest variability within its group. This was followed by Cluster 3 (109.4)

and Cluster 1 (109.0), which also showed notable but slightly lower variability. Clusters 4, 5, and 6 show no variability within, as they only have one representative each. Regarding inter-cluster distances, the highest diversity was observed between Clusters 4 and 5 (724.6), followed by Clusters 5 and 6 (556.5). These high values suggest significant genetic divergence between these clusters. Conversely, the shortest inter-cluster distance was between Clusters 3 and 6 (164.5).

Under stressed condition, in Figure 5.3B cluster 4 exhibits the highest intra-cluster distances value at 88.19, an indication of presence of more genetic variation within this group than others. This is followed by Cluster 1, 2 and 3 with an intra-cluster distance of 66.62, 64.87 and 56.18 respectively. Notably, Cluster 5 to 8 exhibits an intra-cluster distance of 0.00 as they have only one representative. For the inter-cluster distances, the greatest divergence is observed between Cluster 3 and Cluster 4, with a distance of 233.61, showing considerable differentiation in traits between these clusters. Conversely, the smallest inter-cluster distance is found between Cluster 2 and Cluster 7 (92.46).

5.3.7. Correlation analysis

Under stressed conditions, significant phenotypic correlations were observed among various traits in Ethiopian durum wheat landraces. DTH exhibited a negative correlation with GY of -0.24. TN showed a significant negative correlation with GY of -0.38, and the GFP positively correlated with GY at 0.32. CT had a strong positive correlation with GY at 0.41, whereas LA was negatively and significantly correlated with GY at -0.18. RLWC showed a positive and significant correlation with GY at 0.3. PC was positively and significantly correlated with GY at 0.33. For chlorophyll measurements, including Chl_a, Chl_b, and Chl_a+b, showed weak correlations. The Fv/Fm had a positive correlation with GY. SMN and SMW positively and negatively correlated with GY at 0.43 and -0.28, respectively, as shown in Figure 5.4A.

At the genotypic level, Figure5.4A, DTH again showed a significant negative correlation with GY at -0.23 and the GFP maintained a positive correlation at 0.4. CT positively and significantly correlated with GY at 0.58, as well as with Chl_b at 0.46 and CCI at 0.54. Conversely, CCI readings showed a positive correlation with GY at -0.13. LA had weak correlations, while

RLWC was significantly positively correlated with GY (0.46). PC showed a positive correlation. Chlorophyll components (Chl_a, Chl_b, Chl_a+b) showed weak correlations with GY. Fv/Fm showed a positive correlation, and SMW exhibited a strong negative correlation with GY 0.99.

Under non-stressed conditions in Ethiopian durum wheat landraces revealed significant phenotypic correlations with grain yield in Figure 5.4B. DTH showed a negative correlation of -0.37, and GFP exhibited a small negative correlation of -0.04. CT correlated negatively at -0.59, but specific chlorophyll components (Chl_a, Chl_b, Chl_a+b) showed positive correlations ranging from 0.46 to 0.58. RLWC showed a strong positive correlation with GY at 0.62, and SMW and SMN also correlated positively and negatively with yield at 0.-31 and 0.2, respectively.

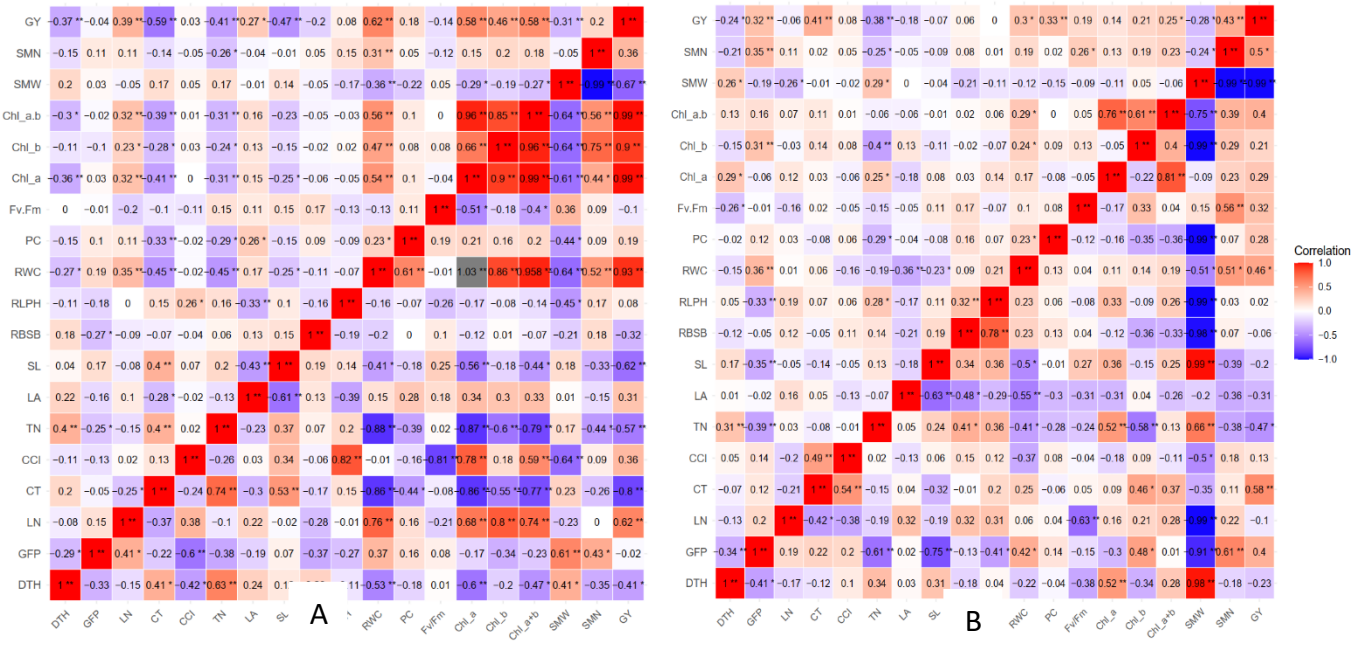

Figure 5.4. Genotypic (below diagonal) and phenotypic (above diagonal) correlation coefficients of 19 traits for the tested durum wheat genotypes under non stress condition (A) and stressed condition (B).

Under non-stressed conditions in Ethiopian durum wheat landraces revealed significant phenotypic correlations with grain yield in Figure 5.4B. DTH showed a negative correlation of -0.37, and GFP exhibited a small negative correlation of -0.04. CT correlated negatively at -

0.59, but specific chlorophyll components (Chl_a, Chl_b, Chl_a+b) showed positive correlations ranging from 0.46 to 0.58. RLWC showed a strong positive correlation with GY at 0.62, and SMW and SMN also correlated positively and negatively with yield at 0.-31 and 0.2, respectively.

At the genotypic level under non-stressed conditions, DTH exhibited a slightly stronger negative correlation at -0.41, and the GFP maintained a negative correlation of -0.02. CCI readings showed a positive correlation at 0.36, but specific chlorophyll components (Chl_a, Chl_b, Chl_a+b) maintained strong positive correlations ranging from 0.9 to 0.99). RLWC retained a strong positive correlation at 0.90, and stomatal traits, SMW at -0.67 and SMN at 0.36, also showed robust negative and positive correlations with GY, respectively as indicated in Figure5.4B.

5.3.8. Path coefficient analysis

The genotypic path analysis under non-stressed conditions in Table 5.10 indicates traits like DTH, GFP, LN, CT, CCI readings, and others showed varying degrees of direct and indirect effects on GY. DTH showed a significant negative correlation with GY (r = -0.4094), but had a positive direct effect, while indirect effects varied across traits, such as TN (0.375) and CT (-0.517). Similarly, GFP exhibited a non-significant negative correlation (r = -0.015), with indirect effects either amplifying or mitigating its influence on GY.

Significant positive and negative correlations were observed between various traits and GY. Relative leaf water content (r = 0.926) and Chl_a (r = 0.99) had strong positive correlations but negative direct effects, emphasizing the role of their indirect pathways. In contrast, CT (r = -0.796) and TN (r = -0.572) had significant negative correlations, with varying direct and indirect influences. CT exerted a negative direct effect (-1.266) but positive indirect effects through traits like TN (0.44) and RBSB (0.176). Similarly, CCI (r = 0.358) had a positive direct effect on GY (0.455), while indirect effects through DTH (-0.152) and RLPH (-0.497) counteracted its contribution (Table 5.10).

From the Table 5.11 phenotypic path analysis under non-stressed conditions, DTH showed a significant negative correlation with GY (r = -0.366) and a direct effect of -0.208, with positive indirect effects through traits like GFP (0.046) and LA (0.043) and negative effects via traits such as RLWC (-0.075) and CT (-0.065). Similarly, CT had a negative correlation with GY (r = -0.594) and a direct effect of -0.324, but positive indirect effects through PC (0.048 and RLPH (0.021). LN positively correlated with GY (r = 0.394), with a direct effect of 0.122 and positive indirect effects through RLWC (0.096) and CT (0.079). Conversely, TN and SL negatively impacted GY, with direct effects of -0.0371 and -0.093, respectively.

RLWC had the highest positive direct effect on GY (0.276) and significant positive indirect effects through CT (0.146) and Chl_a+b (0.109). Chlorophyll-related traits also contributed significantly; Chl_a, though having a negative direct effect (-0.028), exhibited strong positive indirect effects through RLWC (0.148) and CT (0.131). Chl_a+b showed a strong positive direct effect (0.195) and indirect contributions via RLWC (0.154) and CT (0.126) (Table 5.11). Despite some traits, such as SMW and SMN, having minor negative direct effects on GY, their indirect contributions through other traits highlight the involved balance of physiological and morphological factors that influence GY under non-stressed conditions.

Under stressed conditions, genotypic path analysis depicted in Table 5.12, DTH showed a negative, non-significant correlation with GY, with a direct negative effect and mixed indirect effects, notably positive via Chl_a and SL, but negative through traits like Chl_b and TN. GFP had a positive direct effect on GY, supported by indirect positive effects via Chl_b and TN but hindered by traits like SL and Chl_a. LN and CT presented contrasting impacts; LN had a negative direct effect but was indirectly boosted by Chl_a, whereas CT showed a significant positive correlation and direct impact, with support from indirect contributors such as Chl_b and Chl_a. CCI readings and TN had a negative direct impact, but TN exhibited some compensatory positive indirect effects through Chl_a and SL.

Table 5.10. Estimate of direct (bold and diagonal) and indirect effects (off diagonal) at genotypic level for durum wheat genotypes tested at non-stressed condition.

	DTH	GFP	LN	CT	CCI	TN	LA	RBSB	RLPH	RLWC	PC	Fv/Fm	Chl_a	Chl_b	Chl_a+b	SMW	SMN
DTH	**0.359**	0.090	0.009	-0.517	-0.192	0.375	-0.031	-0.239	0.064	0.086	0.017	0.004	-0.240	0.004	0.070	-0.081	-0.197
GFP	-0.120	**-0.270**	-0.025	0.277	-0.273	-0.224	0.025	0.381	0.166	-0.061	-0.015	0.024	-0.067	0.007	0.034	-0.122	0.241
LN	-0.053	-0.110	**-0.061**	0.469	0.173	-0.063	-0.028	0.294	0.004	-0.123	-0.015	-0.062	0.275	-0.018	-0.110	0.047	-0.003
CT	0.147	0.059	0.023	**-1.266**	-0.109	0.440	0.040	0.176	-0.092	0.140	0.042	-0.023	-0.346	0.012	0.115	-0.046	-0.146
CCI	-0.152	0.162	-0.023	0.304	**0.455**	-0.154	-0.004	0.063	-0.497	0.002	0.015	-0.237	0.315	-0.004	-0.088	0.127	0.050
TN	0.225	0.101	0.006	-0.933	-0.117	**0.598**	0.031	-0.074	-0.122	0.143	0.037	0.005	-0.349	0.013	0.118	-0.034	-0.247
LA	0.085	0.052	-0.013	0.381	0.015	-0.139	**-0.131**	-0.130	0.239	-0.025	-0.026	0.052	0.137	-0.007	-0.050	-0.002	-0.086
RBSB	0.083	0.099	0.017	0.215	-0.027	0.043	-0.016	**-1.036**	0.116	0.033	0.000	0.029	-0.046	0.000	0.011	0.041	0.102
RLPH	-0.038	0.074	0.000	-0.192	0.371	0.119	0.052	0.197	**-0.609**	0.025	0.007	-0.076	-0.068	0.002	0.021	0.090	0.093
RLWC	-0.191	-0.101	-0.046	1.093	-0.006	-0.525	-0.020	0.211	0.096	**-0.162**	-0.057	-0.002	0.415	-0.019	-0.149	0.128	0.291
PC	-0.065	-0.043	-0.010	0.561	-0.071	-0.233	-0.037	0.002	0.044	-0.098	**-0.095**	0.057	0.084	-0.004	-0.029	0.088	0.047
Fv/Fm	0.005	-0.022	0.013	0.099	-0.370	0.011	-0.024	-0.102	0.158	0.001	-0.018	**0.291**	-0.204	0.004	0.060	-0.071	0.052
Chl_a	-0.214	0.045	-0.042	1.089	0.356	-0.518	-0.045	0.120	0.103	-0.168	-0.020	-0.148	**0.402**	-0.020	-0.147	0.123	0.245
Chl_b	-0.073	0.091	-0.049	0.695	0.080	-0.359	-0.039	-0.013	0.046	-0.140	-0.015	-0.053	0.364	**-0.022**	-0.143	0.129	0.416
Chl_a+b	-0.169	0.062	-0.045	0.975	0.267	-0.474	-0.044	0.076	0.085	-0.162	-0.019	-0.118	0.398	-0.021	**-0.149**	0.128	0.311
SMW	0.146	-0.164	0.014	-0.289	-0.289	0.101	-0.001	0.213	0.275	0.104	0.042	0.104	-0.247	0.014	0.095	**-0.200**	-0.601
SMN	-0.127	-0.117	0.000	0.332	0.041	-0.265	0.020	-0.190	-0.101	-0.085	-0.008	0.027	0.177	-0.016	-0.083	0.216	**0.557**
rg	**-0.41 ***	**-0.02**	**0.62 ****	**-0.8 ****	**0.36**	**-0.57 ****	**0.31**	**-0.32**	**0.08**	**0.93 ****	**0.19**	**-0.1**	**0.99 ****	**0.9 ****	**0.99 ****	**-0.67 ****	**0.36**

Table 5.11. Estimate of direct (bold and diagonal) and indirect effects (off diagonal) at phenotypic level for durum wheat genotypes tested at non-stressed condition.

	DTH	GFP	LN	CT	CCI	TN	LA	RBSB	RLPH	RLWC	PC	Fv/Fm	Chl_a	Chl_b	Chl_a+b	SMW	SMN
DTH	**-0.208**	0.046	-0.010	-0.065	0.001	-0.015	0.043	-0.032	-0.014	-0.075	0.022	-0.001	0.010	0.003	-0.058	-0.011	0.001
GFP	0.061	**-0.156**	0.018	0.017	0.002	0.009	-0.031	0.046	-0.023	0.052	-0.015	0.000	-0.001	0.003	-0.003	-0.002	-0.001
LN	0.017	-0.023	**0.122**	0.079	0.000	0.006	0.019	0.014	0.000	0.096	-0.016	0.024	-0.009	-0.007	0.062	0.003	-0.001
CT	-0.041	0.008	-0.030	**-0.324**	-0.001	-0.015	-0.054	0.013	0.021	-0.124	0.048	0.009	0.011	0.009	-0.076	-0.009	0.001
CCI	0.023	0.021	0.002	-0.041	**-0.012**	-0.001	-0.004	0.007	0.035	-0.006	0.004	0.012	0.000	-0.001	0.002	-0.003	0.000
TN	-0.083	0.039	-0.019	-0.130	0.000	**-0.037**	-0.025	-0.011	0.022	-0.123	0.042	-0.013	0.008	0.007	-0.060	-0.009	0.001
LA	-0.047	0.025	0.012	0.092	0.000	0.005	**0.192**	-0.021	-0.043	0.048	-0.038	-0.013	-0.004	-0.004	0.031	0.000	0.000
RBSB	-0.039	0.043	-0.010	0.024	0.001	-0.002	0.024	**-0.170**	-0.017	-0.031	-0.011	0.000	0.000	0.000	0.000	0.003	0.000
RLPH	0.022	0.028	0.000	-0.050	-0.003	-0.006	-0.063	0.022	**0.132**	-0.020	0.011	0.000	0.004	0.000	0.000	0.009	-0.001
RLWC	0.056	-0.029	0.042	0.146	0.000	0.017	0.033	0.019	-0.010	**0.276**	-0.064	0.014	-0.015	-0.015	0.109	0.020	-0.002
PC	0.031	-0.016	0.013	0.105	0.000	0.011	0.049	-0.013	-0.010	0.119	**-0.148**	0.000	-0.004	-0.004	0.021	0.012	0.000
Fv/Fm	-0.002	0.000	-0.028	0.029	0.001	-0.004	0.023	0.000	0.000	-0.036	0.000	**-0.106**	0.000	0.000	0.000	-0.002	0.000
Chl_a	0.075	-0.005	0.039	0.131	0.000	0.011	0.029	0.000	-0.018	0.148	-0.023	0.000	**-0.028**	-0.016	0.194	0.016	-0.001
Chl_b	0.023	0.016	0.029	0.093	0.000	0.009	0.025	0.000	0.000	0.130	-0.021	0.000	-0.015	**-0.031**	0.182	0.011	-0.001
Chl_a+b	0.062	0.003	0.039	0.126	0.000	0.011	0.030	0.000	0.000	0.154	-0.016	0.000	-0.027	-0.029	**0.195**	0.016	-0.001
SMW	-0.041	-0.004	-0.006	-0.054	-0.001	-0.006	-0.002	0.009	-0.022	-0.098	0.032	-0.004	0.008	0.006	-0.054	**-0.057**	0.000
SMN	0.032	-0.016	0.014	0.046	0.001	0.010	-0.007	-0.008	0.021	0.085	-0.007	0.009	-0.004	-0.006	0.034	0.003	**-0.005**
rp	**-0.37 ****	**-0.04**	**0.39 ****	**-0.59 ****	**0.03**	**-0.41 ****	**0.27 ***	**-0.2**	**0.08**	**0.62 ****	**0.18**	**-0.1**	**0.58 ****	**0.46 ****	**0.58 ****	**-0.31 ****	**0.2**

Table 5.12. Estimate of direct (bold and diagonal) and indirect effects (off diagonal) at genotypic level for durum wheat genotypes tested at drought stressed condition.

	DTH	GFP	LN	CT	CCI	TN	LA	RBSB	RLPH	RLWC	PC	Fv/Fm	Chl_a	Chl_b	Chl_a+b	SMW	SMN
DTH	**-0.239**	-0.812	0.053	-0.101	-0.083	-0.245	0.033	0.012	0.049	0.125	-0.020	-0.180	8.785	-3.273	-4.846	0.283	-0.064
GFP	0.097	**2.004**	-0.060	0.194	-0.166	0.437	0.022	0.009	-0.524	-0.245	0.075	-0.069	-5.102	4.668	-0.196	-0.263	0.219
LN	0.040	0.381	**-0.315**	-0.360	0.322	0.139	0.323	-0.021	0.399	-0.034	0.022	-0.295	2.689	2.032	-4.782	-0.533	0.079
CT	0.028	0.449	0.131	**0.864**	-0.461	0.104	0.045	0.000	0.256	-0.144	-0.029	0.022	1.503	4.475	-6.306	-0.101	0.041
CCI	-0.023	0.391	0.119	0.469	**-0.850**	-0.014	-0.129	-0.010	0.158	0.217	0.040	-0.018	-2.999	0.850	1.945	-0.145	0.065
TN	-0.082	-1.223	0.061	-0.126	-0.016	**-0.716**	0.055	-0.027	0.467	0.238	-0.142	-0.115	8.799	-5.610	-2.317	0.189	-0.137
LA	-0.008	0.044	-0.101	0.039	0.108	-0.039	**1.012**	0.032	-0.379	0.322	-0.156	-0.147	-5.185	0.392	4.536	-0.059	-0.129
RBSB	0.043	-0.267	-0.101	-0.005	-0.128	-0.293	-0.483	**-0.066**	1.006	-0.132	0.066	0.021	-1.992	-3.482	5.693	-0.283	0.024
RLPH	-0.009	-0.814	-0.098	0.172	-0.104	-0.259	-0.297	-0.051	**1.290**	-0.134	0.028	-0.036	5.650	-0.856	-4.471	-0.337	0.012
RLWC	0.052	0.843	-0.018	0.214	0.317	0.293	-0.559	-0.015	0.298	**-0.582**	0.068	0.019	1.920	1.366	-3.323	-0.147	0.183
PC	0.009	0.289	-0.014	-0.049	-0.066	0.197	-0.305	-0.008	0.071	-0.077	**0.516**	-0.054	-2.670	-3.337	6.185	-0.421	0.023
Fv/Fm	0.092	-0.292	0.198	0.040	0.032	0.174	-0.317	-0.003	-0.098	-0.023	-0.060	**0.471**	-2.896	3.162	-0.653	0.043	0.203
Chl_a	-0.124	-0.603	-0.050	0.077	0.150	-0.372	-0.310	0.008	0.430	-0.066	-0.081	-0.080	**16.948**	-2.148	-13.881	-0.025	0.081
Chl_b	0.081	0.970	-0.066	0.401	-0.075	0.417	0.041	0.024	-0.115	-0.082	-0.179	0.155	-3.776	**9.638**	-6.870	-0.317	0.104
Chl_a+b	-0.067	0.023	-0.087	0.316	0.096	-0.096	-0.266	0.022	0.335	-0.112	-0.185	0.018	13.648	3.841	**-17.237**	-0.217	0.140
SMW	-0.235	-1.826	0.582	-0.302	0.426	-0.469	-0.206	0.065	-1.507	0.297	-0.754	0.070	-1.468	-10.602	12.935	**0.289**	-0.622
SMN	0.043	1.213	-0.069	0.098	-0.153	0.271	-0.361	-0.004	0.044	-0.295	0.034	0.265	3.823	2.776	-6.678	-0.497	**0.361**
rg	**-0.23**	**0.4**	**-0.1**	**0.58 ****	**0.13**	**-0.47 ***	**-0.3**	**-0.06**	**0.02**	**0.46 ***	**0.28**	**0.3**	**0.29**	**0.21**	**0.4**	**-0.99 ****	**0.5 ***

Table 5.13. Estimate of direct (bold and diagonal) and indirect effects (off diagonal) at phenotypic level for durum wheat genotypes tested at drought stressed condition.

	DTH	GFP	LN	CT	CCI	TN	LA	RBSB	RLPH	RLWC	PC	Fv/Fm	Chl_a	Chl_b	Chl_a+b	SMW	SMN
DTH	**-0.067**	-0.058	0.015	-0.038	-0.018	-0.044	-0.003	0.003	0.005	0.024	-0.008	-0.001	-0.067	0.043	0.060	-0.022	-0.067
GFP	0.023	**0.172**	-0.024	0.068	-0.046	0.055	0.005	0.001	-0.035	-0.056	0.041	0.000	0.013	-0.086	0.071	0.017	0.111
LN	0.009	0.035	**-0.116**	-0.111	0.066	-0.005	-0.036	-0.003	0.021	-0.002	0.011	-0.001	-0.024	0.006	0.032	0.022	0.034
CT	0.005	0.021	0.024	**0.542**	-0.161	0.011	-0.011	0.001	0.007	-0.009	-0.028	0.000	-0.007	-0.038	0.052	0.001	0.007
CCI	-0.004	0.024	0.023	0.268	**-0.326**	0.002	0.028	-0.002	0.007	0.025	0.020	0.000	0.014	-0.023	0.003	0.002	0.017
TN	-0.021	-0.067	-0.004	-0.042	0.004	**-0.141**	0.016	-0.003	0.028	0.031	-0.100	-0.001	-0.058	0.112	-0.028	-0.025	-0.080
LA	-0.001	-0.004	-0.019	0.027	0.041	0.010	**-0.222**	0.004	-0.018	0.057	-0.014	0.000	0.042	-0.037	-0.028	0.000	-0.015
RBSB	0.008	-0.007	-0.016	-0.028	-0.035	-0.020	0.046	**-0.022**	0.053	-0.014	0.046	0.000	0.000	0.000	0.000	0.019	0.029
RLPH	-0.003	-0.056	-0.023	0.038	-0.021	-0.038	0.037	-0.011	**0.106**	-0.033	0.024	0.000	-0.036	0.000	0.000	0.009	0.005
RLWC	0.010	0.061	-0.001	0.032	0.052	0.027	0.080	-0.002	0.022	**-0.157**	0.080	0.000	-0.040	-0.066	0.134	0.010	0.060
PC	0.002	0.020	-0.004	-0.045	-0.019	0.041	0.009	-0.003	0.007	-0.037	**0.343**	0.000	0.019	-0.019	0.000	0.013	0.007
Fv/Fm	0.018	-0.002	0.022	0.015	0.014	0.026	0.015	0.000	0.000	-0.015	0.000	**0.005**	0.000	0.000	0.000	0.008	0.078
Chl_a	-0.020	-0.010	-0.012	0.017	0.020	-0.036	0.040	0.000	0.016	-0.028	-0.029	0.000	**-0.229**	0.042	0.318	0.010	0.040
Chl_b	0.010	0.054	0.003	0.074	-0.027	0.057	-0.030	0.000	0.000	-0.038	0.024	0.000	0.035	**-0.276**	0.278	-0.004	0.058
Chl_a+b	-0.009	0.027	-0.008	0.062	-0.002	0.009	0.013	0.000	0.000	-0.046	0.000	0.000	-0.160	-0.168	**0.457**	0.005	0.069
SMW	-0.017	-0.033	0.030	-0.008	0.007	-0.041	0.001	0.005	-0.011	0.018	-0.051	0.000	0.026	-0.014	-0.027	**-0.086**	-0.075
SMN	0.014	0.060	-0.013	0.011	-0.018	0.036	0.011	-0.002	0.002	-0.030	0.007	0.001	-0.029	-0.051	0.101	0.021	**0.314**
rp	**-0.24 ***	**0.32 ****	**-0.06**	**0.41 ****	**0.08**	**-0.38 ****	**-0.2**	**0.06**	**0**	**0.3 ***	**0.33 ****	**0.2**	**0.14**	**0.21**	**0.25 ***	**-0.28 ***	**0.43 ****

Traits like LA, SL, and RLPH exhibited varying contributions to GY. LA had a positive direct effect, largely offset by negative indirect influences such as Chl_a and SL. SL had a positive direct impact, complemented by Chl_a and RLPH, though traits like Chl_b and GFP hindered its contribution. RLPH had a strong positive direct effect, supported by traits like Chl_a, though its indirect effects via traits like Chl_a+b were negative. Positive correlations with GY were observed for chlorophyll-related traits (Chl_a, Chl_b, and Chl_a+b), with notable direct effects, though the interaction of negative indirect pathways (e.g., Chl_a+b via DTH) reduced their overall contribution (Table 5.12). SMW and CCI values showed significant negative relationships, while SMN showed a strong positive correlation, driven by both direct effects and positive indirect contributions from Chl_a and GFP.

Phenotypic path analysis results under stressed conditions in table 5.13 indicates several traits showed significant relationships with GY. DTH had a negative and significant correlation (r = -0.237), showing a negative direct effect (-0.067) but positive indirect contributions via traits like Chl_a+b (0.06) and RLWC (0.024). Conversely, traits like CT and GFP positively influenced GY, with direct effects of 0.542 and 0.172, respectively, and considerable positive indirect contributions through related traits. While traits such as TN and CCI had overall negative direct impacts on GY, their indirect interactions often counterbalance some of these effects, focusing the complex trait interaction under stress.

Traits like SMN and Chl_a+b were highly significant contributors to GY, with strong positive direct effects (0.314 and 0.457, respectively) and notable indirect effects through related parameters. While some traits, like LA and SMW, had negative direct effects on GY, their indirect contributions via supportive traits, such as RLWC and CT, mitigated these influences to an extent (Table 5.13). Overall, the phenotypic path analysis revealed that a combination of physiological and morphological traits influenced GY under stressed conditions, with traits like CT, Chl_a+b, GFP, and SMN emerging as pivotal factors for enhancing stress tolerance and productivity.

5.4. DISCUSSION

The study showed significant effects on all traits studied across all sources of variation, including treatments, locations, and their interactions. These results align with Kumar et al. (2023), who found significant genotypic variations in wheat agro-physiological traits under drought conditions. Jemal Abdulkerim et al. (2015) revealed that DTH, GFP, and chlorophyll levels were significantly affected by the main effects of genotypes and locations. Similarly, Blum (2010) indicated that the timing of heading varies significantly with genotype and environment, which is essential for breeding programs targeting specific environments.

In this study, we assessed wheat genotypes for grain yield, agronomic traits, and physiological parameters under both non-stressed and drought-stressed conditions. We evaluated various morphological, phenological, physiological, and yield-related traits to identify the best-performing genotypes in both scenarios. The primary stage for selecting potential genotypes for drought tolerance is understanding variability in genotypic expression (Sallam et al., 2018). Selecting for improved grain yield under both conditions ensures that genotypes maintaining high yields under non-stressed conditions will also perform well under stress. Drought stress typically reduces grain yield due to impaired photosynthesis, reduced kernel number, and weight. Moderate drought during critical growth stages, such as heading and grain filling, can significantly lower yield potential (Dong et al., 2019). Drought stress can also cause changes in phenological development, physiological and biochemical responses of durum wheat, affecting yield through alterations in early maturity, chlorophyll characteristics, water use efficiency, stomatal architecture, and proline content (Dastborhan et al., 2021). Drought impacts yield components such as tiller number, spike length, and individual kernel weight, collectively contributing to lower overall yield (Naderianfar and Heydari Gharae, 2021). However, some durum wheat varieties shows enhanced water use efficiency under drought stress, mitigating yield losses by maintaining higher yields with limited water (Bai et al., 2021).

Drought stress has a negative impact on grain yield and associated agronomic traits of wheat genotypes. Our study shows a yield decrement ranging from 14.11% in landrace 31778 to 77.26% in landrace 34295, consisting with Boussakouran et al. (2019). Studies by Collaku and

Harrison (2002) and Noori (2023) also showed significant reductions in grain yield due to drought stress, with decreases as high as 45% to 50%. Although drought affects wheat performance at all growth stages, it is more critical during flowering and grain-filling phases. Shahryari et al. (2011) reported that drought-induced grain yield reduction was likely due to a shorter grain filling period. However, some genotypes, such as 31831, ETDW/15DZ04, ETDW/15DZ023, and Fetan, maintained high yields under both stressed and optimum conditions, supporting findings reported by Farooq et al. (2014) which shows that genotypes performing well under non-stressed conditions retain high yield under stress. Notably, five genotypes, namely 31831, ETDW/15DZ04, 34217, ETDW/15DZ023, and 34522, outperformed all local checks under stress. This result also aligns with Mwadzingeni et al. (2016b) findings that twenty genotypes from heat and drought nurseries outperformed all local checks.

Drought induces a reduction in photosynthetic pigments and RLWC, leading to decreased plant growth and productivity. In this study, photosynthetic pigments, Chl_a, Chl_b, total chlorophyll, and RLWC showed reduction due to stress. This result aligns with Anjum et al. (2017) who observed a decrease in photosynthetic pigments and RLWC under stress conditions. Variations in RLWC among different genotypes were significant, with higher RLWC observed in drought-tolerant genotypes, supported by studies such as Zegaoui et al. (2017) and Lehari et al. (2019), indicates that higher RLWC helps maintain cell turgor and metabolic processes under drought stress. Similar results were reported by Khakwani et al. (2012) and Khoshro et al. (2013), with RLWC ranging from 69.3% to 81.1%, showing the importance of maintaining higher RLWC for drought tolerance and overall plant health. Our study also indicated that stability in Fv/Fm, is an indication of the absence of loss of the yield of PSII photochemistry due to positive leaf turgor maintenance under drought stress through osmotic adjustment. This finding contrasts with Bogale et al. (2011), who reported that chlorophyll fluorescence parameters were sensitive to water deficit, with reductions observed at tillering and grain-filling stages. Also, Prajapat et al. (2020) noted a significant reduction in Fv/Fm values under drought stress.

Drought stress can degrade chlorophyll, reduce the plant's photosynthetic capacity and directly affect the chlorophyll a/b ratio, which decreased photosynthesis efficiency (Jaleel et al., 2009). Lower chlorophyll content under drought conditions may indicate a genotype's potential drought

tolerance. Stress often causes stomatal closure to reduce water loss, limiting carbon dioxide uptake and decreasing chlorophyll synthesis. This response is primarily related to the plant's adaptive mechanism to conserve water while maintaining metabolic functions (Vassileva et al., 2011). Under drought conditions, durum wheat plants may enhance their antioxidant mechanisms to prevent oxidative damage, contributing to chlorophyll content maintenance. However, severe drought stress can overwhelm these defenses, leading to significant chlorophyll loss (Hussain et al., 2021). Different varieties of durum wheat show variable degrees of chlorophyll retention under drought conditions. Breeding programs often focus on selecting varieties with higher chlorophyll stability to improve resilience against drought (Balmaceda et al., 2023).

Reduced tillering capacity is generally associated with higher grain yields in water-limited conditions. Our study showed a negative relationship between tiller number and grain yield under both non-stressed and stressed conditions. ETDW/15DZ04, ETDW/15DZ023 in both conditions, MCD3-14 in non-stressed, and Fetan in stressed conditions showed this trend with higher yield and fewer tillers. Studies have shown that wheat genotypes with reduced tillering capacity produce higher grain yields than high-tillering genotypes in drought-stressed conditions due to the formation of fertile spikelets (Houshmandfar et al., 2020, Javed et al., 2022). Additionally, drought can affect spike length by limiting water and nutrient uptake during the growth period. Also Tabassam et al. (2014) reported that drought stress affects spike length and decreases grain yield. Shorter spikes may develop under stressed conditions due to reduced cell elongation and overall plant growth.

Proline plays a crucial role in counteracting drought stress damage by accumulating in the main plant organs. The increase in proline content under stress conditions shows its role in stabilizing proteins and membranes, contributing to stress resilience. The result of this study indicates proline content accumulation differs under stressed and non-stressed conditions, ranging from 0.56 to 1.24 μg g-1 fresh wt. under non-stressed conditions, and from 0.9 to 2.29 μg g-1 fresh wt. under stressed conditions. This result is consistent with Ali (2019), Bowne et al. (2012), and Qayyum et al. (2018) who reported genotypic differences in proline concentration and accumulation in wheat genotypes exposed to drought stress. Genotypes with higher proline

contents, such as 31778, 31831, ETDW/15DZ023, and ETDW/15DZ049, had higher grain yields. Effects of drought stress and increased proline content have also been observed by Ahmad et al. (2018) and Kadam et al. (2017). Proline accumulation as a response to drought stress has also been observed in other crops, including cowpea (*Vigna unguiculata* (L.) Walp.) (Zegaoui et al., 2017), bread wheat (*Triticum aestivum* L.) (Ahmed et al., 2017), peanut plants (Furlan et al., 2020) and maize (*Zea mays* L.) (Köşkeroğlu and Tuna, 2010), making proline accumulation a common response across various plant species, aiding in osmotic adjustment and enhancing their ability to withstand drought stress.

The interaction between RLPH and RBSB is crucial for understanding plant performance under drought conditions. Varieties exhibiting lower RLPH and higher RBSB generally shows better drought tolerance and higher grain yields. These ratios provide intuitions into the balance of growth traits that enable durum wheat to thrive under water-limited conditions. By focusing on these interactions, plant breeders can select and develop wheat varieties with optimized root systems, ensuring improved drought resilience and consistent grain production (Chen et al., 2021). Root characteristics play a crucial role in plant adaptation to water-limited environments. RLPH and RBSB are essential parameters that influence water uptake efficiency and overall plant performance. Lower RLPH indicates a more extensive root system, which enhances the plant's ability to access deeper soil moisture and higher RBSB indicates a greater investment in root biomass, improving water and nutrient absorption capacity. These traits are valuable in drought-prone areas, where water availability is limited in the upper soil layers. The balance between root and shoot growth is vital for maintaining plant health and productivity under stress conditions (Yadav et al., 2022).

In the genetic variability analysis, the GCV values were higher than ECV values for all studied agronomic traits except for LN, while and all physiological traits, except for CT under non-stressed conditions, showed higher ECV than PCV. Under stressed conditions, similar trend was observed for physiological parameters except CCI and SMN, while agronomic traits such as SL and RBSB exhibited higher ECV than PCV. Kumar et al. (2018a) found similar results, with higher ECV values than PCV for physiological traits under both stressed and non-stressed conditions, consistent with our findings. Ahmad et al. (2018) also reported higher GCV than

ECV for most traits, including DTH, GFP, SL, and GY. In this study, traits showed low to high heritability estimates (11.1% to 93.8%), with moderate to high heritability in DTH, LA, SL, RLPH, PC, and GY, under both conditions, aligning with findings from Hailu et al. (2016), Zemede et al. (2019) and Alemu et al. (2020c). The high heritability indicates less environmental influence and greater potential for improvement through direct selection. GAM ranged from low to high for most traits in both conditions, with intermediate to high heritability and high GAM observed for DTH, LA, SL, RLPH, TN, and GY, suggesting that these traits are governed by additive genetic effects, making them suitable for early selection in breeding programs aimed at improving durum wheat grain yield (Bayisa and Amanuel, 2021, Sewore and Abe, 2024).

The PCA results align with multiple studies emphasizing the importance of specific traits under varying conditions. Traits such as chlorophyll content, proline accumulation, Relative leaf water content (RLWC), and root characteristics consistently emerge as significant contributors to plant performance (Flexas et al., 2002, Ashraf et al., 2017). These traits are crucial for plant growth and productivity and serve as indicators of the plant's adaptive mechanisms to environmental stresses. The prominence of chlorophyll-related traits (Chl_a, Chl_b, and Chl_a+b) in multiple components shows their central role in photosynthesis and stress response (Osipova et al., 2024). Traits such as LA and stomatal characteristics (SMW, SMN) are consistently relevant, with larger leaf area potentially enhancing light capture and controlled stomatal behavior regulating water loss (Ashraf and Harris, 2013). The cumulative variance explained by the PCA indicates that a subset of traits accounts for most of the observed variation, guiding researchers to focus on these key components for targeted breeding efforts (Mwadzingeni et al., 2016b).

The cluster mean values of the traits represent the average values for the landraces in each cluster and can be used to compare the performance and characteristics of these clusters. The variation in cluster mean values among the clusters indicates different responses to drought stress. Grouping the genotypes assigns landraces to clusters based on their similarity and dissimilarity for the traits, allowing classification into different categories and selection of promising lines for drought tolerance breeding (Saed-Moucheshi et al., 2015, Dukamo et al., 2023). Our study grouped the 24 landraces into six clusters under non-stressed conditions and eight clusters under stressed conditions. Under non-stressed conditions, cluster III to VI included genotypes with the

best performance, while cluster II contained the least promising lines for breeding for drought tolerance. Under stressed conditions, cluster I included genotypes with higher yields and traits conducive to withstanding drought stress. The intersecting landraces (31831, 34217, and 34493) under both stressed and non-stressed conditions, along with those having higher grain yield and other relevant traits under stressed conditions (ETDW/15DZ04, ETDW/15DZ049, 34522, and ETDW/15DZ023), can be selected as the most promising lines for breeding for drought tolerance.

Significant phenotypic and genotypic correlations among the studied durum wheat landraces under stressed and non-stressed conditions. Under stressed conditions, traits such as DTH and TN exhibited negative correlations with GY, while GFP, CT, and RLWC showed positive correlations. These results align with findings by Flexas et al. (2002) and Ashraf et al. (2017) emphasizing the importance of chlorophyll content, proline accumulation, and root characteristics in plant performance. Although chlorophyll measurements (Chl_a, Chl_b, Chl_a+b) showed weak correlations with GY, CT, GFP, and RLWC consistently exhibited positive correlations, showing their relevance as adaptive traits (Farooq et al., 2014). At the genotypic level, CT showed a strong positive correlation with GY and chlorophyll b (Chl_b), while under non-stressed conditions, DTH and CT had negative correlations with GY, and chlorophyll components and RLWC displayed strong positive correlations which is an indication of the significance of chlorophyll content and water retention in enhancing GY under different environmental conditions (Mecha et al., 2017, Bayisa and Amanuel, 2021).

Path coefficient analysis further elucidated the direct and indirect effects of traits on GY. Under non-stressed conditions, traits like LA, RLPH, RLWC, Chl_a+b, Fv/Fm, SMW and SMN exhibited positive direct effects on GY at both phenotypic and genotypic levels. Chl_b exerted the highest positive direct effect on GY at the genotypic level, showing its critical role in photosynthesis and stress response. Conversely, GFP and PC exhibited negative direct effects on GY, suggesting that their positive correlations with GY were mediated through indirect effects via other traits. Under stressed conditions, GFP, CCI, RLPH, Chl_a, SMW, and SMN had high positive direct effects on GY, while LN, CT, TN, RBSB, RLWC, and Chl_a+b had negative direct effects. These negative direct effects were counterbalanced by positive indirect

effects through other traits showing the complex interaction between physiological and morphological traits in determining GY. This study underlines the significant role of chlorophyll content, water retention, and specific morphological traits in enhancing GY under both stressed and non-stressed conditions, providing valuable understandings for breeding programs aimed at improving drought tolerance and productivity in durum wheat (Aman et al., 2020, Sodagar et al., 2020, Bayisa and Amanuel, 2021).

5.5. CONCLUSION AND RECOMMENDATION

The study utilizes morphological, physiological, and biochemical traits to screen for drought-tolerant genotypes. Significant effects were observed across all studied traits due to the treatments, locations, and their interactions. The importance of CCI reading, PC, and RLWC in coping with drought stress was highlighted. High RLWC and PC are crucial for maintaining cell turgor and metabolic processes, while chlorophyll content has a direct impact on photosynthetic efficiency and overall plant health. Specific genotypes, such as ETDW/15DZ023, ETDW/15DZ04, and 31831, consistently performed well under both stressed and non-stressed conditions, underlining the value of selecting stable genotypes for breeding programs. The research further explores the complex relationships between various traits and grain yield under drought conditions. Traits like CCI readings, TN, stomatal characteristics, and root-to-shoot ratios play vital roles in assessing plant development and yield potential under drought condition. Higher root allocation is advantageous for accessing water from depth and the Fv/Fm ratio serves as an indicator for screening drought tolerance. The study stresses the importance of multi-environment trials to consider for genetic and environmental factors affecting yield. Notably, some genotypes, including ETDW/15DZ23, 31831, ETDW/15DZ04, 34217, and 34522, exhibit enhanced water use efficiency and higher grain yields despite water limitations, showing their potential in reducing yield losses under drought stress.

CHAPTER SIX

GENETIC DIVERSITY ANALYSIS AND POPULATION STRUCTURE OF SELECTED ETHIOPIAN DURUM WHEAT (*T. TURGIDUM SUBSP. DURUM*) LANDRACES USING DARTSEQ MARKERS

Abstract

Genetic variability is crucial for developing high-yield varieties adaptable to diverse climatic conditions. Ethiopia, a center of diversity for durum wheat, holds significant genetic resources in its germplasm. However, these landraces, have limited responses to current and future crop breeding challenges. This study investigates SNP-based genetic diversity, linkage disequilibrium, and population structure of durum wheat using 94 genotypes categorized into high land and mid-land populations based on altitude based agro-ecological patterns. The study identified 14,136 high-quality SNP markers with known physical positions, distributed across the A (6691) and B (7445) genomes. The 2B chromosome had the highest marker density, while the 4A had the lowest. Genome-wide mean values of Nei's gene diversity (0.199) and polymorphism information content (0.184) indicate significant genetic diversity within this collection. The minor allele frequency ranged from 0.005 to 0.5, with a mean of 0.184. Structure analysis classified the landraces into three distinct subpopulations (K = 3), though the grouping pattern did not correlate with agroecological patterns, suggesting high admixture likely due to historical seed exchanges among Ethiopian farming communities. This was further confirmed by the Analysis of Molecular Variance (AMOVA). The findings provide intuitions for wheat genetic improvement, supporting association mapping and genomic prediction. High genetic diversity in Ethiopian durum wheat landraces offers valuable traits for stress resilience, emphasizing the need for policies that conserve diverse landraces to enhance food quality and sustainable, climate-resilient production.

Keywords: Ethiopian durum wheat; Genetic diversity; Landraces; linkage disequilibrium; population structure; SNP markers.

6.1. INTRODUCTION

Durum wheat, scientifically known as *Triticum durum*, is a vital cereal crop highly valued for its use in pasta, couscous, and various other food products (Sissons, 2022). In the global context, modern wheat cultivars primarily consist of two species: hexaploid bread wheat (*Triticum aestivum*) and tetraploid durum wheat (*Triticum durum*) (Kabbaj et al., 2017). While bread wheat contribute to about 90–95% of world wheat production (Singh et al., 2023) durum wheat covers the remaining 5% (Singh et al., 2023). In Ethiopia, wheat holds a significant position as the country's second most widely produced cereal crop after maize and the third most important staple food after maize and sorghum(Dibaba, 2019). Although hard red wheat dominates national production, durum wheat contributes around 10–15% (Usman et al., 2018).

Ethiopia, a center of the primary gene pool for various crops, including durum wheat (Mengistu et al., 2016). The Ethiopian Biodiversity Institute (EBI) maintains an extensive collection of durum wheat genotypes, accounting for 12% of its total holdings (Mengistu et al., 2016, Alemu et al., 2020a). Ethiopian durum wheat has attracted attention due to its uniqueness and potential as a source of novel alleles (Dinsa, 2023). Studies by Vavilov and Zohary have highlighted the high genetic diversity present in Ethiopian durum wheat, suggesting its significance as a possible secondary center of domestication for the crop (Sertse et al., 2023). Durum wheat has a long history in Ethiopia, likely introduced into the northern highlands around 3000 BC (Badebo et al., 2009, Alemu et al., 2020a). The wide range of agroecological conditions coupled with diverse farming practices has contributed to the high genetic variation observed in cultivated durum wheat in Ethiopia (Mengistu et al., 2018, Dagnaw et al., 2022).

Mechanisms for detecting and analyzing genetic diversity have evolved from traditional morphological surveys to molecular examinations of DNA variation (Avise, 2012, Alemu et al., 2020a). Single nucleotide polymorphisms (SNPs) are the most abundant class of DNA markers (Jehan and Lakhanpaul, 2006, LaFramboise, 2009), providing stable and reliable markers for studying complex genetic traits (Ramesh et al., 2020, Hasan et al., 2021). They have been extensively used in genome-wide association studies, genetic resource characterization, and marker-assisted breeding (Kumar et al., 2021). The development of high-throughput sequencing

methods and SNP-based maps has revolutionized genetic research in durum wheat, enabling more comprehensive analyses of genetic diversity and population structure (Maccaferri et al., 2015, Colasuonno et al., 2021, Pandurangan et al., 2021, Taranto et al., 2023).

Various studies have explored the genetic diversity of durum wheat using different molecular marker techniques. For example, RAPD and Microsatellites (also known as SSRs), have been employed in previous investigations, although with limitations in coverage and resolution (Jlassi et al., 2021, Dagnaw et al., 2023). However, recent advancements in SNP genotyping technologies, such as hybridization arrays and high-throughput sequencing, have facilitated more comprehensive assessments of genetic diversity in durum wheat populations (Fayaz et al., 2019, Rani et al., 2024). These techniques have enabled researchers to analyze population structure, genetic divergence, and linkage disequilibrium across diverse germplasm collections (Maccaferri et al., 2015, Kumar et al., 2021).

The diversity arrays technology (Raman et al.) represents a rapid cost-effective method for high-throughput marker analysis across entire genomes without the need for prior sequence knowledge renowned for its versatility and efficiency. DArT has emerged as a cornerstone technology in genetic studies (Nadeem et al., 2021, Chaudhary et al., 2023). Notably Crossa et al. (2007) pioneered the creation of the first wheat genome association map using this groundbreaking approach. Since then, DArT has found extensive application across various species including staple cereals like barley (*Hordeum vulgare* L.), common wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* L.) (Crossa et al., 2007, Pundir et al., 2023, Rani et al., 2024). Beyond its role in genetic mapping and association analyses DArT empowers researchers to investigate deeper into the complex nature of genetic diversity and population genetics correlations (Silva-Junior and Grattapaglia, 2015, Tehseen et al., 2021).

Genetic diversity analyses of within-population and between-population have provided understandings into the evolutionary history and domestication processes of durum wheat (Özbek, 2014, Ben Krima et al., 2020). Ethiopian durum wheat landraces have been subjected to genetic characterization using various genomic tools, revealing high levels of diversity and distinctness in morphological and molecular traits (Haile et al., 2013, Mengistu et al., 2016,

Negisho et al., 2021). Despite significant progress, a considerable proportion of Ethiopian durum wheat germplasm remains underutilized in breeding programs (Kidane et al., 2017, Megerssa et al., 2020). Further research utilizing genome-wide DNA markers is needed to fully explore the genetic potential of durum wheat landraces and identify valuable genes for crop improvement (Mulugeta et al., 2023, Alemu et al., 2020a).

The study of Ethiopian durum wheat landrace diversity and population structure using molecular SNP data represents a critical aspect of crop genetics and breeding (Kabbaj et al., 2017, Mulugeta et al., 2023, Alemu et al., 2020a). Understanding the abundance and distribution of genetic diversity within and between populations is essential for conservation efforts and to develop improved cultivars resilient to changing environmental conditions (Khoury et al., 2022, Alemu et al., 2020a). Advanced molecular marker techniques offer powerful tools for unraveling the genetic architecture of durum wheat populations, paving the way for targeted breeding strategies and sustainable agriculture (Singha et al., 2022, Visioni et al., 2023). Genetic diversity assessment is fundamental to understand the genetic landscape of these landraces, providing awareness into the extent and distribution of genetic variation relevant to drought tolerance traits. Therefore, the objective of the current study was to assess the genetic diversity and population structure of 94 Ethiopian durum wheat landraces using DArT based SNP markers. This study may provide vital information on the genetic composition of the studied durum wheat landraces for drought tolerance breeding.

6.2. MATERIALS AND METHODS

6.2.1. Experimental plant materials

For the present study 94 durum wheat genotypes comprised of 86 landraces and eight improved varieties from Debrezeit agricultural research center (41) and Ethiopian biodiversity institute (53) were considered. The durum wheat genotypes were grouped into two based on altitudinal agroecological pattern of the origin of collection as high land and mid-land populations. The released varieties grouped into mid-land as they are selected for growing in low altitude or low moisture areas. The altitudinal-based agroecological classification was done according to Hurni (1998).

6.2.2. Genomic DNA extraction and SNP genotyping

DNA extraction and SNP genotyping of durum wheat landraces were conducted at SEQART AFRICA, located within the International Livestock Research Institute in Nairobi. DNA was extracted from young, lyophilized leaves using the Nucleomag Plant Extraction Kit, following the CTAB tissue lysis method as per the manufacturer's instructions. The extracted genomic DNA has a concentration ranging from 50 to 100 ng/µl, with its quality and quantity tested on 0.8% agarose gel. Libraries were constructed following the protocol outlined by Egea et al. (2017) which involved employing DArTSeq complexity reduction methodology, which entails genomic Deoxyribose nucleic acid digestion utilizing a combination of PstI and HpaII enzymes, followed by ligation of barcoded adapters and a common adapter using ligase enzyme (Egea et al., 2017). The resulting adapter-ligated fragments underwent PCR amplification. Sequencing of the libraries was performed using Single Read sequencing runs, generating reads of 69 bases each. The sequencing process carried out using the Illumina HiSeq2500 Sequencing System. SNP discovery was accomplished using the Genome Studio Project software package (Illumina, San Diego, CA, USA), with the Svevo durum wheat genome assembly v1.0 (GCA_900231445.1) from the International Wheat Genome Sequencing Consortium serving as the reference genome (Maccaferri et al., 2019). Quality control measures were applied to the genotyping data to mitigate potential errors. Single nucleotide polymorphism with a minor allele frequency (MAF) of less than 1%, a SNP call rate below 90%, and markers with more than 25% missing values were filtered out. Consequently, a set of 17,092 high-quality SNPs, including 14,136 with known chromosomal positions across the 14 chromosomes of durum wheat, were retained for the final analysis.

6.2.3. Genetic diversity analysis

Numbers and percent of polymorphic loci, polymorphism information content (PIC), Nei’s gene diversity, and minor allelic frequency (MAF) were calculated using hierfstat package (Goudet, 2005), analysis of molecular variance (AMOVA) according to (Meirmans and Liu, 2018) using poppr package (Kamvar et al., 2014). Other genetic diversity measures, such as a total number of alleles (NA), Private Alleles (Npa), and Allelic Richness (AR), were computed using the PopGenReport package in R (Adamack and Gruber, 2014). Additionally, the Percentage of

Polymorphic Loci (Davila et al.); Observed Heterozygosity (Ho), Expected Heterozygosity (He), Unbiased Expected Heterozygosity (uHe), and Inbreeding Coefficient (Fis) were calculated using the dartR package (Mijangos et al., 2022) function according to (Nei, 1978). Furthermore, the Index of Association for each population (Ia), the Standardized Index of Association (rbarD), and the p-value for rbarD (P.rD) were computed using the poppr package (Kamvar et al., 2014).

6.2.4. Population Structure and Cluster Analysis

The genetic structure of the population was analyzed using the Bayesian clustering algorithm in STRUCTURE 2.3.4 software (Pritchard et al., 2000). From 1 to 10 subpopulations were suggested to analyze the durum wheat population structure and the admixture model with 50,000 burn-in iterations, and 50,000 Monte Carlo Markov chain replications after burn-in was used. The Evanno test (Evanno et al., 2005) was used to identify the true number of sub-populations (K) implemented in the Structure Harvester R package software (Earl and VonHoldt, 2012). Also, to investigate the genetic structure of the populations, a k-means clustering algorithm using FactoMineR package (Lê et al., 2008), To further confirm cluster analysis, Discriminant Analysis of Principal Components (DAPC) using adegenet package (Jombart et al., 2010) and unweighted pair group method of association (UPGMA) dendrogram using poppr package (Kamvar et al., 2014) was carried out. The visualization of the dendrogram was enhanced by (Ciccarelli et al., 2006).

6.2.5. Linkage disequilibrium (LD) analysis

Understanding linkage disequilibrium (LD) among pairs of multiple SNP markers offers valuable information into the correlation structure of different loci based on their allelic variation (Siol et al., 2017). In this study, pairwise LD, measured as r^2, for SNP pairs was computed following the method outlined by (Weir, 1990) using TASSEL version 5.2.8 (Bradbury et al., 2007), with an LD window size of 50 bp. The decay rate was then estimated for significant SNP marker pairs ($r^2 = 0.20$, $p<0.01$) across the entire genome. To visualize the association between genome-wide LD decay and physical distance, a locally weighted linear regression (loess) line was fitted using the R function ‘loess’ (R Core Team, 2021). The LD

decay rate was determined as the physical distance at which the r^2 value dropped to half its average maximum value, following the approach outlined by (Huang et al., 2010).

6.3. RESULTS

6.3.1 SNP Marker Distribution

The contribution of SNPs scored on the A and B genomes was 47.31% and 52.69%, respectively. The number of SNPs ranged from 727 on chromosome 4B to 1373 on chromosome 2B (Figure 6.01a). In all cases the B genome showed a higher number of SNPs except for chromosome 4, where 813 SNPs were detected on chromosome 4A and 727 SNPs on chromosome 7B. Considering the distribution of SNPs across the homoeologous groups, group 4 scored the lowest number of markers (in total 1540, or 10.89%) of the total number of markers with a known chromosome position, while the top highest was group 2 and group 7 with 2497 (17.66%) and 2304 (16.30%) SNP markers, respectively (Table 6.1).

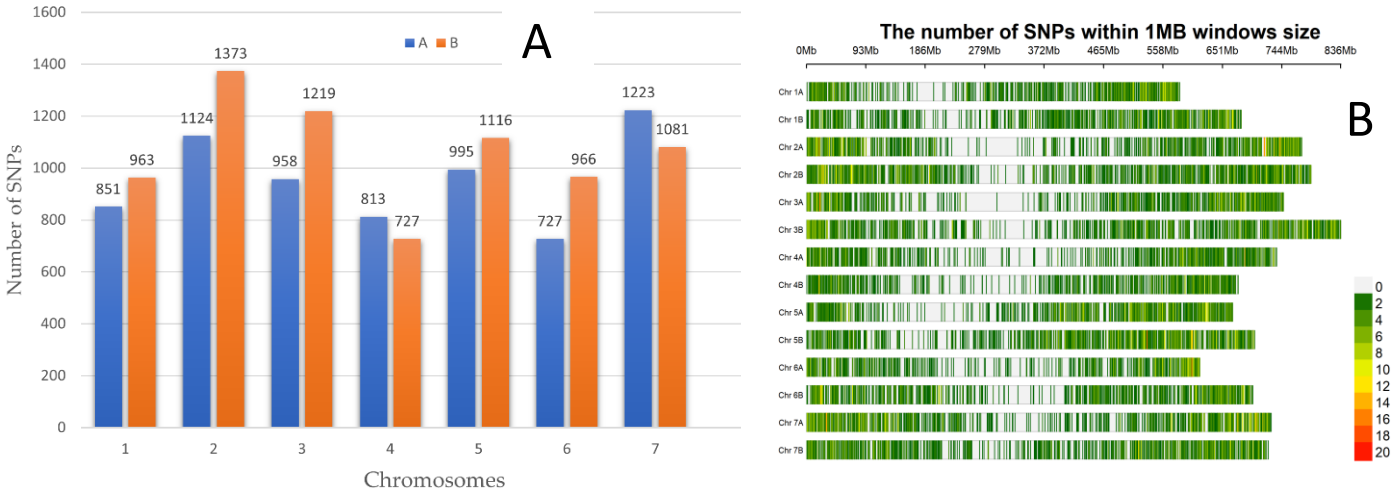

Figure 6.1. Distribution (A) and density (B) of SNP markers along the wheat chromosomes within a population of 94 Ethiopian durum wheat genotypes.

The SNP coverage within one mega base pair (Mbp) in each chromosome confirmed that chromosomes in the A genome had the lowest number and density of SNPs than chromosomes B (Figure 6.1b, Table 6.1). This SNP coverage across the entire genome indicates an average marker density of 1.42 markers per Mbp. Among the individual chromosomes, the B genome

exhibited the highest marker density (1.46 markers/ Mbp), followed by the A genome (1.37 markers/ Mbp). This highlights the slightly denser distribution of markers within the B genome compared to the A genome. When we see the individual chromosomes, chromosome 2B showed the highest marker density (1.74 markers/ Mbp, followed by chromosome 7A (1.68 markers/ Mbp), while chromosome 4B and 4A displayed the lowest (1.07 and 1.10 markers/ Mbp) marker densities respectively.

6.3.2. Genetic diversity analysis

The analysis of SNP markers revealed a diverse landscape of polymorphic information content (PIC) and Nei's gene diversity across chromosomes and genomes. The distribution of SNPs showed varying levels of PIC, gene diversity, and minor allele frequency (MAF) values across the genome, with an overall mean PIC of 0.184, ranging from 0.02 to 0.499. Nei's gene diversity score varied from 0.021 to 0.5 with a mean value of 0.199, while the MAF ranged from 0.009 to 0.5, with a mean of 0.137 (Table 6.1). When compared across chromosomes, chromosome 1B stood out with the highest PIC (0.202) and gene diversity (0.215), contrasting with chromosome 6A, which recorded the lowest PIC (0.167) and gene diversity (0.179). Additionally, several chromosomes, including 2A, 2B, 3B, 5A, and 6B, exhibited slightly lower PIC values compared to the genome-wide average, and certain homoeologous chromosome groups, such as 1, 3, 5, and 7, displayed higher Nei's genetic diversity than the genome-wide average (Table 6.1).

Table 6.1 Mean values of number of SNP and SNP density, minor allelic frequency distribution, Nei's genetic diversity, and polymorphic information content of SNP markers across chromosomes in 94 Ethiopian durum wheat genotypes

Chrom. No.	1A	2A	3A	4A	5A	6A	7A	A	Total
SNP	851	1124	958	813	995	727	1223	6691	14136
SIZE	585.27	775.45	746.67	736.87	669.16	615.67	728.03	4857.12	9964.32
Density	1.45	1.45	1.28	1.1	1.49	1.18	1.68	1.38	1.42
MAF	0.145	0.131	0.142	0.121	0.132	0.12	0.143	0.134	0.137
GD	0.209	0.195	0.204	0.181	0.196	0.179	0.21	0.196	0.199
PIC	0.198	0.185	0.192	0.169	0.182	0.167	0.197	0.195	0.184
Chrom. No.	1B	2B	3B	4B	5B	6B	7B	B	
SNP	963	1373	1219	727	1116	966	1081	7445	
SIZE	681.11	790.34	836.51	676.29	701.37	698.61	722.97	5107.2	
Density	1.41	1.74	1.46	1.07	1.59	1.38	1.5	1.46	

MAF	0.149	0.138	0.136	0.136	0.142	0.135	0.143	0.14	
GD	0.215	0.199	0.2	0.2	0.206	0.197	0.205	0.203	
PIC	0.202	0.188	0.188	0.188	0.193	0.185	0.191	0.19	
Homologues chrom.									
Total SNP	1814	2497	2177	1540	2111	1693	2304		
%age SNP	12.83	17.66	15.4	10.89	14.93	11.98	16.3		

Where MAF: minor allele frequency; GD: Nei's genetic diversity coefficient; PIC: polymorphic information content.

Furthermore, the comparison between homoeologous chromosome groups revealed distinct patterns, with chromosome group one exhibiting the highest gene diversity, PIC, and MAF values, while chromosome group six showed the lowest values. Despite the differences between individual chromosomes, comparable mean values of genetic diversity, PIC, and MAF were observed between the A and B genomes, to indicate a balanced distribution of genetic diversity across the two genomes.

6.3.3 Genetic differentiation of populations

The Analysis of Molecular Variance (AMOVA) was conducted based on both agroecological clusters and identified subpopulations in structure analysis. When examining agroecological clusters, the majority of the genetic variation (51.75%) is observed within populations, while 48.15% is found within samples. A non-significant variation was explained between the agroecological population classification with only 0.1%. On the other hand, when examining structure-based population clusters, a higher percentage (42.65%) of genetic variation is observed between identified subpopulations. Within population, genetic variation is distributed more evenly between samples (19.66%) and within samples (37.69%. The Phi statistic (Φ) of 0.01 between agroecological based populations implied a considerable degree of segregation among them, while a much higher Phi statistic (0.43) between structure analysis based generated populations implies a great differentiation between the populations.

Table 6.2 Analysis of molecular variance (AMOVA). of 94 durum wheat genotypes grouped in two different population cluster

	Df	Sum Sq	Mean Sq	%	Phi(Φ)	Pvalue
Population using agroecological cluster						

Between pop	1	5499.76	5499.76	0.1	0	0.4
Between samples Within pop	92	477535.47	5190.6	51.75	0.52	0.01
Within samples	94	154934	1648.23	48.15	0.52	0.01
population using Structure cluster						
Between pop	2	176592.7	88296.37	42.65	0.43	0.01
Between samples Within pop	91	306442.5	3367.5	19.66	0.34	0.01
Within samples	94	154934	1648.23	37.69	0.62	0.01
Total	187	637969.2	3411.6	100		

6.3.4. Magnitude and pattern of allelic diversity in the populations

The magnitude and pattern of allelic diversity in the populations were shown in Table 6.2. The study on 94 Ethiopian durum wheat genotypes reveals significant genetic diversity across various populations, classified based on agroecological patterns (High land and Mid-land) and structure output clusters (clus1, clus2, and clus3). Mid-land stands out with the highest total number of alleles (34035), and a significant count of private alleles (6839) (Table 6.3).

Table 6.3. Allelic diversity measure in the populations

pop	N	NA	Npa	AR	PPL	Ho	He	uHe	FIS	Ia	rbarD	P.rD
Population using agroecological cluster												
High land	38	27345	149	1.585	60.07	0.068	0.164	0.167	0.518	974	0.108	0.1
Mid-land	56	34035	6839	1.974	99.14	0.116	0.219	0.221	0.357	1804	0.121	0.1
population using Structure cluster												
clus1	62	31109	887	1.409	82.01	0.072	0.133	0.135	0.338	538	0.05	0.1
clus 2	26	26084	202	1.375	52.61	0.077	0.127	0.13	0.419	1018	0.129	0.1
clus 3	6	30407	2652	1.753	77.9	0.434	0.318	0.347	-0.187	221	0.023	0.1

Where N: Sample Size; **NA**: Total Number of Alleles; **Npa**: Private Alleles; **AR**: Allelic Richness; **PP**L: Percentage of Polymorphic Loci; **Ho**: Observed heterozygosity; **He**: Expected heterozygosity; **uHe**: Unbiased expected heterozygosity; **FIS**: Inbreeding Coefficient; **Ia**: Index of association for each population; **rbarD**: Standardized index of association; **P.rD**: The p-value for rbarD.

When comparing based on population structure-based clustering clus3 exhibits a relatively high number of alleles (30407) and private alleles (2652) despite its smaller sample size. The highest allelic richness and percentage of polymorphic loci, 1.974 and 99.14%, respectively, also showed in the Mid-land populations, emphasizing its genetic variability. However, clus3 also shows prominent allelic richness (1.753), and clus1 shows the higher percentage of polymorphic loci (82.01%) among the three-population cluster (Table 6.3).

The result on the heterozygosity measures (Table 6.3) showed clus3 indicates the highest observed heterozygosity (Ho = 0.434), although its expected heterozygosity (He = 0.318) is lower. The other populations in the clusters, clus1 and clus2 show lower observed heterozygosity (0.072 and 0.077) and expected heterozygosity (0.133 and 0.127), than members in the entire population clustering. Midland exhibits the highest expected heterozygosity (He = 0.219) compared to the Highland population. Additionally, clus3 shows the highest unbiased expected heterozygosity (uHe = 0.347), compared to the other two populations in this cluster and also compared with the Highland and Midland populations.

Furthermore, Highland showed the highest inbreeding coefficient (0.518) compared to Midland (0.357). From the three-population structure, clustered groups clus2 showed the highest inbreeding coefficient (0.419), but a negative value (-0.187) was obtained in clus3. Regarding population association, clus2 displays the highest values for Ia (1018) and rbarD (0.129), showing significant genetic association from other populations (Table 6.3).

6.3.5. Population structure and relationship

The population structure of the 94 tetraploid wheat landraces using the STRUCTURE-based clustering method, as shown in Figure 6.2a, indicates that as the number of clusters (K)

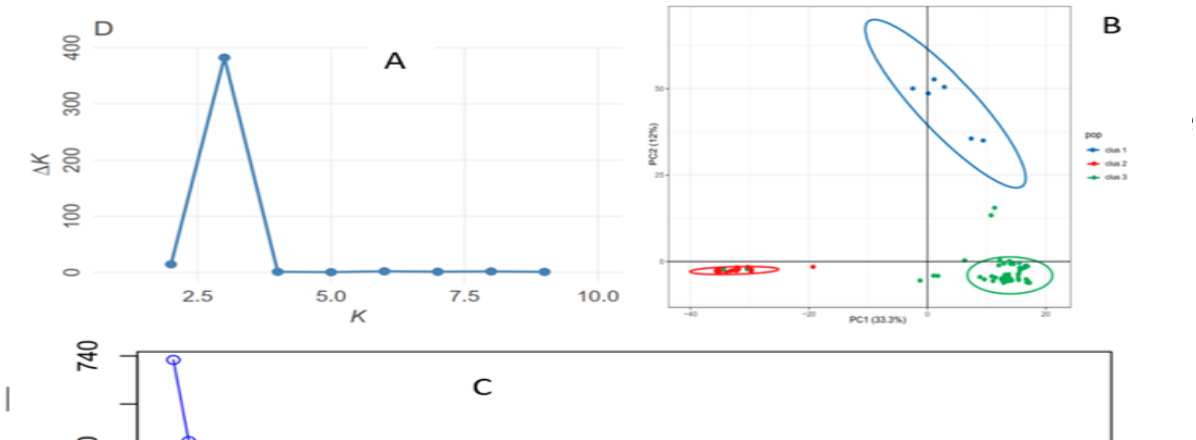

Figure 6.2 Inference of the optimal numbers of sub-populations (clusters) in Ethiopian durum wheat genotypes defined by the Evanno method (A) and by the discriminant analysis of principal components (DAPC) based on the BIC value (C). the PCA of the sub population the first two components are displayed graphically (each sub-population is differentiated by color) (B).

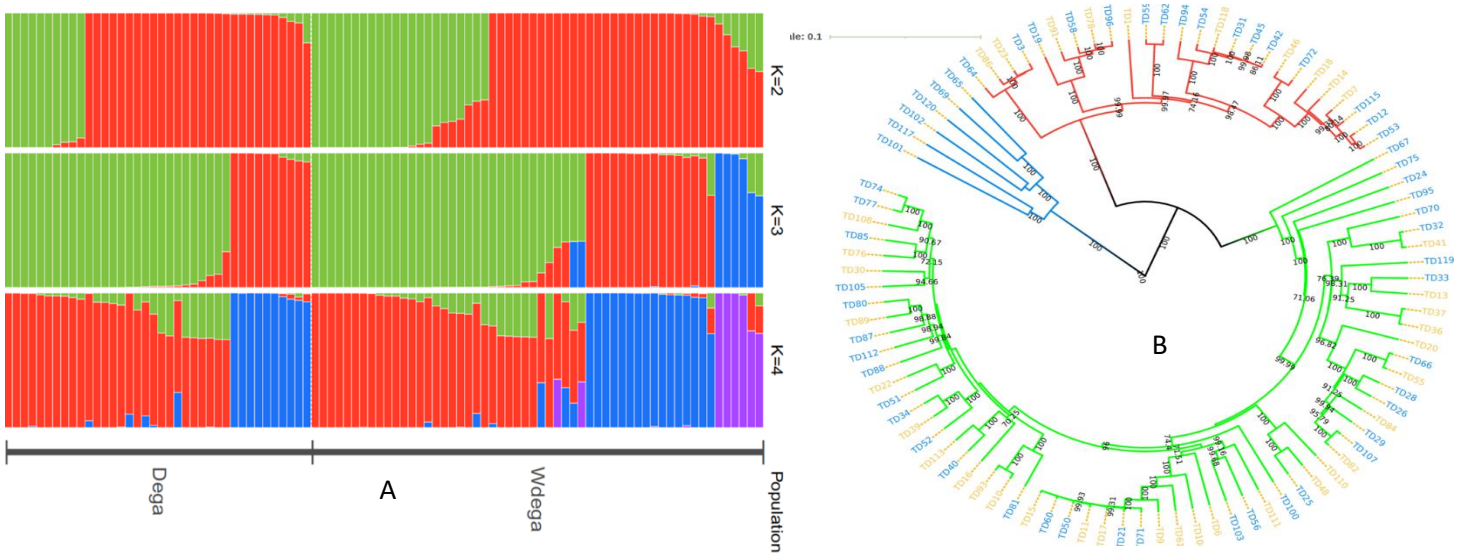

Figure 6.3 Genetic structure of Ethiopian durum wheat genotypes with the Bayesian clustering model in STRUCTURE (A) and UPGMA clustering dendrogram generated using 17,092 SNP markers and 94 tetraploid wheat landraces(B). Colors of genotypes are clusters inferred from STRUCTURE-based analysis

The discriminant analysis of principal components (DAPC). This approach did not yield a clear minimum Bayesian information criterion (Ning et al.) at a specific K value, where BIC values would decrease simultaneously with an increase, forming an elbow at the optimal K value (Figure 6.2b). However, it did suggest that fewer than five clusters could be optimal. Consequently, the genotypes were divided into three clusters as per the results from the STRUCTURE-inferred clustering, with 62, 26, and 6 genotypes grouped into sub-populations 1, 2, and 3, respectively (Supplementary Table 7).

Principal component analysis (PCA) was conducted with all Polymorphic SNPs generated from the panel. The first, and second principal components explained 33.1 %, and 12% of the total variance, respectively (Figure 6.2c). The smaller numbers of variance explained by the third and consecutive PCs indicated that only a few PCs couldn't capture the existing genetic variance in Ethiopian durum wheat. A cluster analysis was also performed using UPGMA to construct a dendrogram from a pairwise similarity matrix (Figure 6.3b). The UPGMA clustering method also divided the panel into three classes, consistent with the results from the structure analysis.

6.3.6. Pattern and extent of linkage disequilibrium (LD)

A scatter plot showing the LD decay over the whole genome is presented in Figure 4. The LD Decay Curve, depicted by the red curve, illustrates the decreasing LD as the physical distance between SNP pairs increases. Concurrently, the Half Decay Threshold, indicated by the horizontal blue line at r^2 =0.2, serves as a pivotal reference point for assessing LD persistence. At the Intersection Point, marked by the vertical light green line at 4.58 Mbp, the LD decay curve intersects with the half decay threshold, providing an estimate of the average distance over which LD remains significant. This intersection point not only signifies the extent of LD but also offers breeders crucial information on genomic regions likely to be co-inherited, thereby aiding in the design of targeted breeding strategies.

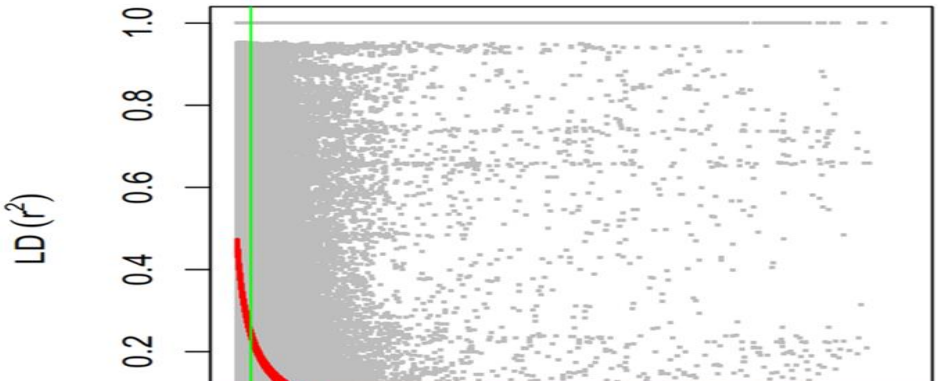

Figure 6.4 Scatter plot of genome-wide LD decay against total physical distance (Mbp) based on the r^2 values of the marker pairs.

The horizontal blue line represents the half decay r^2 value of the genome ($r^2 = 0.2$). The red curve line is the smoothing spline regression model fitted to LD decay. The vertical light green line in Mbp (4,58Mbp) indicates the intersection between the half decay and the LD decay curve.

Table 6.4. linkage disequilibrium measurement of the studied durum wheat accession for each chromosome, A, and B genomes, and in total.

	SNP/Chr	TSNP	SSNP	%SSNP	R.2	DPrime	pDiseq
1A	829	10458	3509	33.553	0.229	0.817	0.289
1B	965	12359	3586	29.015	0.186	0.816	0.312
2A	1126	14824	4509	30.417	0.222	0.832	0.337
2B	1400	17872	5655	31.642	0.226	0.844	0.315
3A	946	12014	4205	35.001	0.235	0.839	0.278
3B	1224	16269	4583	28.17	0.199	0.816	0.362
4A	817	9538	2590	27.155	0.188	0.821	0.367
4B	727	8401	3053	36.341	0.25	0.846	0.282
5A	1005	12288	3272	26.628	0.188	0.816	0.372
5B	1113	13491	4239	31.421	0.205	0.818	0.303
6A	727	7187	1837	25.56	0.172	0.794	0.383
6B	964	11488	3264	28.412	0.204	0.832	0.346
7A	1200	17191	5257	30.58	0.2	0.828	0.342
7B	1085	13513	3722	27.544	0.19	0.808	0.32

A Genome	6650	83500	25179	30.154	0.205	0.821	0.338
B Genome	7478	93393	28102	30.09	0.208	0.826	0.32
Whole	14128	176893	53281	30.12	0.207	0.823	0.329

Where: SNP/Chr Number of SNP per chromosome; TSNP: total number of SNP pair; SSNP: significant number of SNP pair; %SSNP: percentage of significant number of SNP pair; R.2: square of the correlation coefficient between SNP pairs; DPrime: also called D' is a standardized measure of linkage disequilibrium; pDiseq: P-value for Disequilibrium

Table 6.4 represents the average SNP/chromosome, the total number of SNP pairs, the number of significant SNP pairs percentage of significant SNP pars and r^2 located on the same chromosome. At the genome level, with an average of 0.205, the A genome had moderate LD, while the B genome had an LD of 0.2054 The LD within each genome ranged from 0.172 (6 A) to 0. 236 (3 A) and 0.186 (1B) to 0.25 (4B). Across chromosomes, variations in LD metrics indicate distinct patterns of genetic linkage and recombination rates. Chromosome 4B displays high LD values, while chromosome 6A exhibits weaker LD. Some chromosomes, such as 1A, 3A, and 4B, reveal relatively high percentages of SNPs in strong LD, implying genomic regions with reduced recombination and higher conservation. The B and A genomes possessed the highest number of significant marker pairs (28102), and the least number (25179), respectively (Table 6.4).

6.4. DISCUSSION

The investigation of screened SNPs in this study shows a notable difference in their distribution, with a greater abundance observed in the B genome compared to the A genome. This observation aligns with previous research that consistently identifies a higher SNP count on the B genome, underscoring the significance of this genome in shaping the genetic diversity of durum wheat (Kabbaj et al., 2017, Alemu et al., 2020a, Negisho et al., 2021). It also offers valuable understanding into the underlying genomic structure and diversity of durum wheat chromosomes, shedding light on the mechanisms driving genetic variation (Zhou et al., 2020, Marzario et al., 2023). Furthermore, the uneven distribution of markers implies the existence of potential genomic hotspots characterized by heightened diversity and adaptation (Soriano et al., 2021).

Polymorphism Information Content (PIC) is used to evaluate the informativeness of SNP markers, providing insight into the genetic diversity of a DNA segment within a population. PIC acts as an indicator of the evolutionary pressures on alleles, reflecting historical mutations and selection over time (Kabbaj et al., 2017, Taranto et al., 2023). PIC values are influenced by both the type of marker and the germplasm under study; for instance, multi-locus markers like SSRs generally have PIC values ranging from 0 to 1.0, while bi-allelic markers like SNPs have a maximum PIC value of 0.5, a 50% occurrence of each allele (Botstein et al., 1980). In this study, the average PIC value calculated was 0.184, which aligns closely with the findings of Ren et al. (2013), who reported a PIC value of 0.188 in a global durum wheat collection of 150 genotypes. Similarly, Alemu et al. (2020a) observed that landraces exhibited lower PIC, gene diversity (GD), and minor allele frequency (MAF) values than improved varieties. Lower values in durum wheat landraces from diverse clusters were also noted by Kabbaj et al. (2017), suggesting that broader geographical sampling could enhance genetic diversity. Rabieyan et al. (2023) confirmed this, finding increased PIC, GD, and MAF across a diverse, multi-country sample of durum wheat. Moreover, chromosomes 1, 3, 5, and 7 showed higher Nei's genetic diversity than the genome-wide average, indicating regions with enriched genetic diversity (Tehseen et al., 2021).

The PIC value of 0.184 in this study indicates a moderate level of genetic diversity among the 94 durum wheat landraces, reflecting the DArTSeq markers' ability to detect valuable genetic variation. While not particularly high, this diversity level provides a foundation for identifying advantageous alleles associated with key traits, such as drought tolerance. By leveraging these genetic resources, breeding programs can select parental lines with complementary traits, aiming to improve the adaptability and resilience of durum wheat varieties under different environmental pressures. For agricultural practices and food production, this moderate genetic diversity contributes to yield stability and resilience, especially in water-limited or stress-prone regions. Applying this diversity in breeding can facilitate the development of durum wheat varieties suited to challenging conditions, ultimately supporting sustainable agriculture and food security. However, to better capture the complexity of traits like drought tolerance, increasing

marker density or integrating additional genomic tools could further enhance the effectiveness of selection.

The results of the AMOVA also revealed different genetic variation patterns among populations classified based on agroecological and structure-based clusters. Considering the agroecological zones-based population, the majority of the genetic variation (51.75%) is observed between samples within populations, while 48.15% is found within samples. This observed genetic variation between samples within populations may have arisen during domestication processes or as a result of seed exchange among farmers and local traders from adjacent and non-adjacent regions. Alemu et al. (2020a) also reported higher genetic variation between groups (61.02%) than within individuals within the group (38.98%) using durum wheat landraces and cultivars from Ethiopia. Similarly, Kabbaj et al. (2017) and Roncallo et al. (2021) reported higher genetic variation between individuals within populations. However, the percentage of genetic variation between populations is relatively low (0.1%), showing limited differentiation among agroecological clusters. This result is supported by Rabieyan et al. (2023) who obtained genetic variation of 9.40% between populations and 90.60% within populations classified based on region. Conversely, a higher percentage (42.65%) of genetic variation was observed between structure clusters, suggesting greater genetic differentiation within the structure-identified population cluster, as noted by Rabieyan et al. (2023) and Mulugeta et al. (2023), showing significant genetic variation within durum wheat landraces and emphasizing the importance of considering both geographical and genetic factors in conservation and breeding programs aimed at preserving and enhancing the genetic diversity of durum wheat.

A fundamental component of harnessing genetic diversity is understanding the genetic population structure, which provides crucial information regarding available genetic resources (Eltaher et al., 2018), thereby contributing to the development of future conservation strategies and broadening the genetic base of crops. Population stratification was assessed using DAPC, STRUCTURE analysis, and the neighbor-joining method, all of which consistently classified the population into three distinct groups comprising 62, 26, and 6 genotypes (Table 6.3). Although DAPC did not yield a clear minimum Bayesian information criterion (Ning et al.) at

a specific K value, wherein BIC values typically exhibit a spontaneous decrease with a simultaneous increase, forming an elbow at the optimal K value, as depicted in Figure 2b.

The absence of discernible associations based on geographical coordinates, such as altitudes and latitudes, contrasts with findings from Ren et al. (2013), who observed significant relationships between ecological factors, geography, and SNP allele frequency variation in wild emmer across diverse environmental conditions in Israel and Turkey. In contrast, traditional farming practices in Ethiopia revealed a reliance on seeds from the previous year's harvest, with frequent exchanges among farmers, as documented by Marzang et al. (2020) and Salsman et al. (2021) hinting at the potential clustering of durum wheat landraces based on geographical patterns.

However, analyses including structure analysis, PCoA, and UPGMA clustering failed to segregate landraces based on their regional origins and altitudinal classification used in this paper. This lack of distinct grouping patterns challenges the assumption of clear relationships between genetic diversity and geographical derivation. Historical seed exchange practices, documented in studies by Kabbaj et al. (2017) and Shaygan et al. (2021), likely contribute to this observed genetic mixing. In Ethiopia, the informal farmer-to-farmer seed exchange system dominates, underlining the significant role of genetic distance in parent selection for breeding programs. Despite this genetic blending, the wide latitudinal variation and geographic isolation of collection sites, spanning from 1470 to 3120 meters above sea level, underline the rich genetic diversity and adaptive potential of Ethiopian durum wheat landraces. This diversity presents valuable opportunities for breeding and improvement programs aimed at addressing both biotic and abiotic stresses across diverse altitudinal ranges (Negisho et al., 2021).

Understanding the landscape of linkage disequilibrium (LD) across the durum wheat genome is paramount for outlining inherited genomic regions (Roncallo et al., 2021, Broccanello et al., 2023). which is not only informs the mapping resolution of targeted genomic regions but also aids in decision-making regarding mapping strategies. (Ibrokhim and Abdukarimov, 2008). LD estimation in durum wheat has been conducted using various DNA markers (Maccaferri et al., 2005, Taranto et al., 2023). This study revealed a significant proportion of significant SNP pairs (30.12%, $r^2 \geq 0.2$, $p < 0.01$) across the durum wheat genome, surpassing previous findings of

13.4% by Roncallo et al. (2021), 27.6% by Mekonnen et al. (2021), and 19.8% by Mulugeta et al. (2023). Moreover, we observed a high genomic mean r^2 of 0.20 (all linked SNP pairs in LD, $p<0.01$) across the entire durum wheat set in contrast to previous studies (Alemu et al., 2020b, Mekonnen et al., 2021).

These results indicate the significant influence of LD elements due to genetic linkage and residual LD arising from selection pressures, genetic recombination rates, and evolutionary history, contributing to the observed high genetic diversity (Fayaz et al., 2019, Roncallo et al., 2021). Consistent with prior research, this study also identified distinct variations in LD patterns and decay distances across individual chromosomes and genomic regions of durum wheat (Maccaferri et al., 2019, Alemu et al., 2020a, Roncallo et al., 2021, Taranto et al., 2023). The presence of significant variation in LD measures, particularly exemplified by the contrasting LD patterns of chromosomes 4B and 6A, suggests evolutionary dynamics underlying allele associations and recombination rates (Cui et al., 2017). The pronounced LD observed in chromosome 4B, characterized by high R^2 values and lower pDiseq, suggests the presence of conserved genomic regions under strong selective constraints (Mahboubi et al., 2020). Conversely, the weaker LD observed in chromosome 6A, accompanied by higher pDiseq values, may indicate regions experiencing higher rates of recombination and genetic diversity (Hao et al., 2020). This differential LD pattern across chromosomes shows the complex interaction between genetic drift, selection, and recombination in shaping genomic diversity and adaptation in wheat populations (Cui et al., 2017).

The LD decay patterns observed across wheat chromosomes provide targeted awareness for marker-assisted breeding, especially in selecting genomic regions associated with agronomic traits relevant to wheat improvement. For instance, chromosomes like 4B, which display slower LD decay rates, likely contain conserved genomic regions under selective pressure, possibly related to traits such as drought tolerance, pest resistance, or yield stability. These regions are valuable for marker-assisted selection as they can facilitate the development of resilient durum wheat varieties adapted to stress-prone environments, directly supporting improved agricultural practices and food production (Kole et al., 2015). On the other hand, chromosomes with faster LD decay, like 6A, may contain genomic regions with higher recombination rates, making them

suitable for fine mapping and the identification of specific alleles contributing to complex traits. This enables precise trait mapping for drought resistance or nutrient efficiency, enhancing the use of landraces in breeding programs to develop high-performance cultivars. Understanding these LD decay dynamics and the distribution of SNPs across chromosomes is thus critical for optimizing crop improvement, accelerating the selection of beneficial traits, and supporting sustainable food production in challenging agricultural environments (Budhlakoti et al., 2022, Chao et al., 2010, Thomson, 2014).

The findings from this study on Ethiopian durum wheat landraces have significant implications for agricultural policy and food quality, as well as for improving landraces for traits like drought tolerance. The high genetic diversity and unique allelic richness, particularly in the Mid-land population, indicate the value of these landraces as resources for breeding programs. Policies supporting the conservation and utilization of these diverse genetic resources are essential, as they can stabilize yields in climate-prone regions. Additionally, the SNP marker distribution and linkage disequilibrium (LD) patterns observed across chromosomes provide actionable comprehension for marker-assisted breeding. Chromosomes with high marker density and LD persistence, such as 2B and 7A, may harbor genomic regions linked to drought tolerance, making them ideal candidates for targeted trait improvement in low-input agricultural practices. By integrating these findings into breeding programs, it is possible to develop resilient, high-quality wheat varieties adapted to specific agroecological needs, directly supporting sustainable food production and food security initiatives.

6. 5. CONCLUSION AND RECOMMENDATION

In conclusion, our study on Ethiopian durum wheat landraces' diversity and population structure revealed significant insights into the genomic architecture, genetic diversity, and evolutionary dynamics of this important crop species. The observed differences in SNP marker distribution across chromosomes and homologous genomes suggest the presence of potential biases, likely arising from inequalities in the availability of genetic resources or the complex nature of specific chromosomal segments. This variability in marker density among chromosomes provides valuable information about the underlying genomic structure and diversity of durum wheat chromosomes, emphasizing the importance of targeted investigations to elucidate the functional significance of these regions in wheat evolution.

Additionally, our analysis of genetic variation within and between populations highlighted significant genetic diversity within durum wheat landraces, with limited differentiation observed among agroecological clusters but greater genetic differentiation within structure-identified population clusters. The investigation into population structure and LD patterns across the durum wheat genome provides valuable intuitions into the genetic architecture and diversity of this important crop. Despite the absence of clear associations with geographical coordinates, our study underlines the rich genetic diversity present in durum wheat landraces. This diversity, particularly evident in cluster 3, holds promise for enhancing wheat breeding programs and developing cultivars resilient to changing environmental conditions. By understanding the complex interaction between population structure, allelic diversity, and geographical factors, we can better harness the genetic potential of durum wheat to address future challenges in agriculture and food security.

CHAPTER SEVEN
7. SUMMARY, CONCLUSION AND RECOMMENDATIONS

7.1 General Summary

Drought poses a global challenge to durum wheat production and productivity. Durum wheat, a tetraploid species, ranks as the second most cultivated wheat species after common wheat. Developing drought-adapted wheat varieties is crucial for boosting grain yield in water-limited environments. Broad genetic diversity is essential to provide the gene pool needed for the long-term development of drought-tolerant varieties. Ethiopia's diverse agro-ecological landscape presents a unique opportunity to explore and leverage a wide array of genetic traits found in local wheat varieties. These landraces, evolved under various climates and soil conditions, show significant adaptability to various stress factors, including drought. The genetic variation among Ethiopian durum wheat landraces makes them a promising resource for creating new cultivars with enhanced drought tolerance and other desirable agronomic traits.

The main goal of this study was to characterize selected Ethiopian durum wheat germplasm and identify the best genotypes for drought stress tolerance. The research aimed to evaluate the potential of these genotypes for efficient selection as breeding materials. A total of 104 durum wheat genotypes from the Debrezeit Agricultural Research Center (DZARC) and the Ethiopian Biodiversity Institute (EBI) underwent evaluation for thirteen agronomic traits under both stressed and non-stress conditions at two locations: Dera (altitude 1500 masl) and Debrezeit (altitude 1920 masl). These locations provided varied environmental conditions to assess the adaptability and performance of the wheat genotypes. The study, conducted on 100 landraces and 4 local checks, utilized an augmented design. Data was collected on 13 traits encompassing yield and yield components, phenology, and canopy condition to understand the performance of these genotypes under different environmental stresses.

There were significant differences between landraces for different traits with different sources of variation. The mean grain yield of the genotypes under non-stressed conditions was 4.03 tons per hectare, while under drought-stressed conditions, it was reduced to 1.94 tons per hectare. Several landraces out yielded the checks under both environmental conditions. Intermediate to

high estimates of the phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in a broad sense (h^2b), and genetic advance in percent of the mean (Jeyabalasingh et al.) were observed for all the studied traits except for days to flowering, thousand seed weight under stress, days to maturity, leaf chlorophyll concentration measurement, and canopy temperature measurement under both conditions. The estimation of variability parameters showed that genotypic variation was higher than environmental variation for most traits. Traits such as the number of tillers, spike length, kernel per spike, and grain yield exhibited higher values for h^2b and GAM under both non-stressed and stressed conditions, showing considerable genetic control over these traits. The significant genetic variation observed for these traits suggests that selection for drought tolerance can be effectively implemented in breeding programs.

High heritability and genetic advance indicate that additive genes likely control these traits, making them more amenable to selection. Spikelets per spike, kernels per spike, and thousand-seed weight were positively correlated with grain yield, with correlation coefficients of 0.68, 0.72, and 0.65, respectively, under non-stressed conditions, and 0.59, 0.61, and 0.57, respectively, under stressed conditions. In contrast, grain yield exhibited a weak negative correlation with days to heading and days to maturity, with correlation coefficients of -0.32 and -0.35, respectively, under non-stressed conditions, and -0.29 and -0.31, respectively, under stressed conditions. This negative correlation indicates that genotypes with shorter heading or maturity days can be selected for drought tolerance breeding. These traits enable the crop to complete its life cycle before the onset of late-season drought, minimizing water stress during critical stages like grain filling. Early-maturing plants also use available soil moisture more efficiently, reducing losses from evaporation and competition with weeds while avoiding the combined stresses of heat and drought during flowering and grain development. Short-duration genotypes enhance adaptation to variable climates and contribute to yield stability in arid and semi-arid regions.

Principal components analysis revealed that six traits were the major loadings on the first two principal components, which described 41.0% and 37.9% of the total morphological variance under non-stressed and stressed conditions, respectively. Cluster analysis grouped the landraces

into six clusters, each showing variation in performance for different traits under non-stressed and stressed conditions. Cluster I, consisting of 17 landraces, showed high mean values for grain yield, number of tillers, and spike length under non-stressed conditions, while cluster IV, with 12 landraces, exhibited high mean values for grain yield and kernel per spike under stressed conditions. Significant genetic variability among the evaluated durum wheat landraces suggests an opportunity for improvement of grain yield through the hybridization of genotypes from different clusters and subsequent selection.

Furthermore, data on grain yield from drought-stressed and non-stressed environments were recorded for two seasons. Nine drought tolerance indices, Tolerance (TOL), Mean Productivity (MP) Geometric Mean (GMP), Harmonic Mean (HM), Stress Susceptibility Index (STI), Stress Tolerance Index, (SSI), Yield Index (YI), Yield Stability Index (YSI) and Relative Stress Index (RSI) were calculated for each genotype based on grain yields under both conditions.

This study identified genotypes with superior agronomic traits that outperform the best checks. These genotypes can be used as potential parents for yield improvement programs under drought stress. Genotype ETDW/15DZ23 yielded 5.32 tons per hectare under non-stressed conditions and 2.45 tons per hectare under stressed conditions, while the check variety yielded 4.70 and 1.80 tons per hectare, respectively. The evaluation of the studied Ethiopian durum wheat landraces, based on stress indices, differentiated them according to their yield under both stressed and non-stressed conditions. Notably, genotypes ETDW/15DZ23, 34493, and ETDW/15DZ4 had high grain yields under non-stressed and stressed conditions.

They are robust and have potential utility in breeding programs. These genotypes also possessed high values for indices such as STI, MP, GMP, YSI, and YI, which are useful indices for selecting genotypes for breeding under stress conditions. Genotype 34493 recorded higher values for STI (1.27), MP (4.29 tons per hectare), GMP (3.88 tons per hectare), YSI (0.88), and YI (1.26). A significant positive correlation ($r = 0.88$) was observed between grain yield under non-stressed (Yp) and stressed (Ys) conditions. Except for SSI and TOL, which had a negative correlation, Yp showed a significant positive correlation with all other indices. This indicates a high correlation between the indices (MP, GMP, and STI) with the average performance of

genotypes under drought stress conditions. Based on the result of stress indices and yield under stressed and non-stressed conditions, 20 landraces and 4 checks were selected for further analysis under greenhouse conditions to assess their stress-adaptive traits using agromorphological, physiological, and proline content parameters.

These 20 selected landraces and 4 checks were grown in pots under non-stressed (70% pot capacity) and stressed (35% pot capacity) conditions, arranged in a completely randomized design (CRD) with three replicates. Data on yield, yield-related traits, and physio-biochemical attributes were recorded and analyzed to determine their stress-adaptive and drought tolerance ability for future breeding efforts. In addition to phenological and yield related parameters, traits such as Relative leaf water content, stomatal characteristics, chlorophyll content, and proline accumulation were measured to evaluate the physiological responses of the genotypes to drought stress.

The results indicated significant effects of genotype and stress conditions observed across all studied parameters, with drought stress having a notable impact on the morpho-physiological and biochemical attributes of all tested durum wheat genotypes. Relative leaf water content decreased by 34%, stomatal characteristics by 41%, and chlorophyll content by 29% under drought stress compared to non-stressed conditions. Important traits for drought tolerance included earlier heading, optimal tiller production, and a short grain-filling period. The study also highlighted the role of high proline content and relative leaf water content in maintaining metabolic processes under drought conditions. Proline content increased by 52%, and relative leaf water content rose by 34% under drought stress compared to non-stressed conditions. A comprehensive analysis of phenotypic and genotypic correlations, combined with path coefficient analysis, provided valuable insights into the direct and indirect effects of these traits on grain yield. Cluster analysis categorized the studied landraces based on their potential to endure drought stress. Landraces in specific clusters, under both stress and non-stress conditions, demonstrated an ability to withstand drought while yielding reasonably well. Consequently, landraces ETDW/15DZ023, ETDW/15DZ04, 34217, and 31831 maintained high yields under both non-stressed and stressed conditions. These landraces exhibited greater

drought tolerance compared to the other tested genotypes, underscoring their potential for use in breeding programs aimed at enhancing drought resilience in durum wheat.

Furthermore, the study examined SNP-based genetic diversity, linkage disequilibrium, and population structure of durum wheat using 94 genotypes (90 landraces and 4 improved varieties). These genotypes were categorized into highland and midland populations based on altitude-related agro-ecological patterns. This analysis aimed to assess the genetic diversity and population structure among the Ethiopian durum wheat landraces, providing vital information on the genetic composition of the studied durum wheat landraces for drought tolerance breeding. Using SNP markers allowed for a high-resolution analysis of genetic diversity and population structure, providing insight into the genetic relationships and evolutionary history of the durum wheat landraces. The identification of genetic loci associated with drought tolerance can facilitate marker-assisted selection in breeding programs, accelerating the development of new cultivars with improved stress resilience.

Genetic diversity analysis and population structure using DArTSeq markers identified 14,136 high-quality SNP markers with known physical positions distributed across the A and B genomes. The 2B chromosome had the highest marker density, with 2,541 SNPs, while the 4A had the lowest, with 812 SNPs. Genome-wide mean values of Nei's gene diversity (0.199) and polymorphism information content (0.184) indicate significant genetic diversity within this collection. The minor allele frequency ranged from 0.005 to 0.5, with a mean of 0.13 4. Analysis of molecular variance (AMOVA) showed that 10% of the total genetic variation was attributed to differences between populations, while 90% was within populations. These findings offer valuable information for wheat genetic improvement, crucial for association mapping and genomic prediction studies. Sustainable utilization and conservation of this genetic resource are indispensable for coping with climate change and biotic stresses, thereby ensuring durum wheat production stability in the face of global environmental challenges. The genetic diversity present in Ethiopian durum wheat landraces represents a valuable asset for breeding programs worldwide, providing opportunities to develop improved varieties with enhanced resilience to various stresses.

7.2 Conclusions Remarks

The present study examined the genetic diversity, population structure, and phenotypic performance of Ethiopian durum wheat landraces under both non-stressful and drought-stressful conditions, providing a significant understanding of their adaptation and potential for breeding. There were considerable differences among the landraces for various agronomic traits across different environmental conditions, with several landraces outperforming the checks in terms of grain yield. Specifically, landraces such as ETDW/15DZ23, 34522, and 34493 showed consistently high performance, signifying their strong adaptability and potential for inclusion in breeding programs. The clustering of landraces into distinct groups based on their genetic and phenotypic characteristics highlighted the presence of considerable genetic diversity, which exhibited the greatest inter-cluster distances under both conditions. This genetic diversity is crucial for breeding programs aimed at enhancing the resilience of durum wheat to environmental stresses.

Furthermore, the study's use of stress tolerance indices, including STI, MP, GMP, and HM, provided valuable tools for identifying genotypes that maintain high yields under drought conditions. The positive correlations between these indices and grain yield under stress conditions reinforce their utility in selecting drought-tolerant cultivars. Genotypes such as ETDW/15DZ23, 34493, and 34217 were identified as the most drought-tolerant, making them suitable candidates for further research and development. Additionally, the study's detailed examination of physiological traits such as chlorophyll content, Relative leaf water content, and proline analysis provided deeper realizations into the mechanisms of drought tolerance. These traits are critical for maintaining plant health and productivity under water-limited conditions, and their strong performance in drought-prone environments indicates their importance in breeding for climate resilience.

The genetic diversity and population structure analysis provided a detailed understanding of the genomic architecture of Ethiopian durum wheat landraces. The study identified significant genetic diversity within these landraces, with variability in SNP marker distribution across chromosomes. This diversity, particularly evident in cluster 3, holds promise for enhancing wheat breeding programs aimed at developing cultivars resilient to changing environmental

conditions. The findings underline the importance of considering both genetic and environmental factors in breeding strategies, paving the way for the development of durum wheat varieties that are not only high-yielding but also resilient to environmental stresses such as drought.

The identification of key traits and superior genotypes offers a solid foundation for developing durum wheat cultivars that can withstand the challenges posed by climate change, particularly in regions prone to drought. The findings also emphasize the need for ongoing research into the genetic and physiological mechanisms underlying drought tolerance, as well as the importance of multi-environment trials to ensure the stability and reliability of selected genotypes.

7.3 Recommendations

Considering the high genetic diversity within Ethiopian Durum wheat landraces, it would be better to develop drought-tolerant varieties by emphasizing the following suggestions/recommendations.

- ✓ It is recommended that breeding programs incorporate the identified high-performing drought-tolerant genotypes, such as ETDW/15DZ23, 34493, ETDW/15DZ4, 34522, and 34217.
- ✓ These genotypes should be genetic resources to develop new cultivars with enhanced drought tolerance and yield potential.
- ✓ Extensive field trials should be conducted in different drought-prone regions to validate the performance of the identified genotypes under varying environmental conditions, assess their stability and adaptability, and ensure their suitability for large-scale cultivation.

Further physiological and biochemical studies should be conducted to understand the mechanisms underlying drought tolerance in the identified genotypes.

- ✓ Investigating traits such as root depth, water-use efficiency, stomatal conductance and osmotic adjustment can provide valuable insights into the adaptive strategies of drought-tolerant genotypes.

Continue using stress tolerance indices like STI, MP, GMP, and HM in breeding programs to identify and select drought-tolerant genotypes effectively.

- ✓ These indices have reliable performance predictors under stress and non-stress conditions.
- ✓ The clustering of genotypes based on stress tolerance indices should be further explored to identify groups with similar performance characteristics.

Conduct targeted investigations on chromosomes with significant LD variations, such as 4B and 6A, to identify genomic regions associated with important agronomic traits.

- ✓ This will enhance marker-assisted selection and genetic improvement efforts.

Conducting genome-wide association studies (GWAS) to identify genes linked to specific traits associated with drought tolerance.

- ✓ It is recommended to pursue further marker-trait association studies on durum wheat landraces, specifically to identify marker-trait associations related to drought tolerance to facilitate the development of drought-tolerant cultivars.

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APPENDIX

Supplementary Table 1. Passport data and their field plot number for the studied durum wheat landraces and checks.

field plot	Accession Number	Region	Zone	District (Wereda)	Latitude N/S	Longitude E/W	Altitude
1	31696	Amhara	Debub Gondar	Tach Gayint	11°38'00"N	38°32'00"E	2750
2	DW-NVT LM 12						
3	Kuami						
4	31698	Amhara	Debub Gondar	Lay Gayint	01°44'00"N	38°29'00"E	3120
5	31703	Amhara	Semen Wello	Dawuntna Delant	11°34'00"N	39°10'00"E	2920
6	ETDW/15DZ 003	Oromia	North Shewa	Aleltu	09°19'39"N	39°15'17"E	2633
7	31755	Oromia	Mirab Harerge	Kuni	09°00'00"N	41°53'00"E	2367
8	Fetan		CIMMYT				
9	ETDW/15DZ 010	Oromia	North Shewa	Aleltu	09°19'39"N	39°15'17"E	2633
10	31759	Oromia	Semen Shewa	Berehna Aleltu	09°17'00"N	39°15'00"E	2514
11	ETDW/15DZ 057	Oromia	North Shewa	D.Libanos	09°71'20"N	38°88'00"E	2618
12	Alem Tena		ICARDA				
13	31761	Oromia	Arsi	Tiyo	07°58'00"N	39°10'00"E	2490
14	31778	Amhara	Misrak Gojjam	Enarj Enawga	10°53'52"N	38°04'19"E	2550
15	ETDW/15DZ 006	Oromia	North Shewa	Aleltu	09°19'39"N	39°15'17"E	2633
16	31789	Amhara	Misrak Gojjam	Enarj Enawga	10°53'52"N	38°04'19"E	2550
17	ETDW/15DZ 038	Amhara	North Shewa	D.Birhan	09°41'47"N	39°30'36"E	2621
18	31794	Amhara	Misrak Gojjam	Enarj Enawga	10°53'52"N	38°04'19"E	2550
19	Ude		CIMMYT				
20	31797	Amhara	Misrak Gojjam	Enarj Enawga	10°48'25"N	38°07'08"E	2550
21	MCD15-49	Oromia	East Shewa	Gimbichu	07°27'04"N	37°36'36"E	2100
22	31806	Amhara	Misrak Gojjam	Enarj Enawga	10°51'09"N	38°08'08"E	2550
23	31831	Oromia	Misrak Shewa	Gimbichu	09°13'00"N	39°24'16"E	2540
24	MCD3-15	Oromia	East Shewa	Gimbichu	07°27'04"N	37°36'36"E	2101
25	33205	Amhara	Mirab Gojjam	Bahir Dar Zuria	11°30'98"N	36°54'04"E	2056
26	MCD12-30	Oromia	East Shewa	Gimbichu	07°27'04"N	37°36'36"E	2102

28	33230	Oromia	Misrak Shewa	Lume	08°50'00"N	39°19'00"E	2260
29	ETDW/15DZ 4	Oromia	West Shewa	Ambo	08°57'13"N	37°55'43"E	2101
30	33235	Oromia	Misrak Shewa	Lume	09°47'00"N	03°46'00"E	2300
31	33239	Oromia	Misrak Shewa	Akaki	08°52'00"N	38°48'00"E	1800
32	MCD4-15	Oromia	East Shewa	Gimbichu	07°27'04"N	37°36'36"E	2102
33	33241	Oromia	Misrak Shewa	Lume	08°51'00"N	39°15'00"E	1577
34	DW-NVT LM 5						
36	33244	Oromia	Misrak Shewa	Akaki	08°54'00"N	03°54'00"E	2300
37	33254	Oromia	Jimma	Mana	07°36'00"N	36°43'00"E	2533
39	DZDW17002	Amhara	S.Shewa	Lemi	94°68'03"N	38°59'73"E	2600
40	33293						1470
41	33300	Oromia	Bale	Agarfa	07°20'00"N	39°45'00"E	2580
42	ETDW/15DZ 39	Oromia	West Shewa	Ambo	08°57'13"N	37°55'43"E	2101
43	33306	Amhara	Misrak Gojjam	Awabel	10°15'00"N	37°50'00"E	2450
45	MCD4-41	Oromia	East Shewa	Gimbichu	07°27'04"N	37°36'36"E	2102
46	33403	Oromia	Semen Shewa	Degem	09°40'03"N	38°39'59"E	2890
47	DZDW17004	Amhara	D.Wello	D.Wello	10°20'57"N	39°14'74"E	2621
48	33405	Oromia	Semen Shewa	Gerar Jarso	38°73'15"N	09°78'52"E	2840
50	MCD13-42	Oromia	East Shewa	Gimbichu	07°27'04"N	37°36'36"E	2102
51	34217	Amhara	Mirab Gojjam	Dembecha	10°34'00"N	37°29'00"E	2145
52	34219	Oromia	Misrak Shewa	Ada'a Chukala	08°45'00"N	38°59'00"E	2083
53	DW-NVT LM 4						
54	34295	Tigray	Central Zone	Axum	14°08'00"N	38°42'00"E	1800
55	MCD2-17	Oromia	East Shewa	Gimbichu	07°27'04"N	37°36'36"E	2102
56	34310	Tigray	Central Zone	Axum	14°07'00"N	38°43'00"E	2000
58	34415	Oromia	Mirab Harerge	Tulo	09°13'00"N	41°07'00"E	2030
59	DW-NVT LM 11						
60	34418	Oromia	Mirab Harerge	Tulo	09°15'00"N	41°07'00"E	2200
61	MCD1-32	Oromia	East Shewa	Gimbichu	07°27'04"N	37°36'36"E	2102
62	34423	Amhara	Debub Wello	Tehuledere	11°31'00"N	39°37'00"E	2090
64	ETDW/15DZ 23	Oromia	West Shewa	Ambo	08°57'13"N	37°55'43"E	2101

65	34451	Oromia	Misrak Shewa	Ada'a Chukala	08°49'00"N	38°54'00"E	2000
66	34452	Oromia	Misrak Harerge	Haro Maya	09°26'00"N	41°47'00"E	2100
67	DW-PVT LM 8						
69	34481	Oromia	Mirab Shewa	Walisona Goro	08°27'00"N	37°58'00"E	2000
70	34484	Oromia	Mirab Shewa	Walisona Goro	08°27'00"N	37°58'00"E	2000
71	MCD10-11	Oromia	East Shewa	Gimbichu	07°27'04"N	37°36'36"E	2102
72	34493	Oromia	Semen Shewa	Wara Jarso	09°49'50"N	38°15'13"E	2080
74	MCD15-7	Oromia	East Shewa	Gimbichu	07°27'04"N	37°36'36"E	2102
75	34496	Oromia	Mirab Shewa	Becho	08°50'00"N	38°26'00"E	2060
76	ETDW/15DZ 073						
77	34500	Oromia	Misrak Shewa	Ada'a Chukala	08°40'00"N	39°06'00"E	2000
78	ETDW/15DZ 049	Oromia	North Shewa	D.Libanos	09°71'20"N	38°88'00"E	2618
80	34510	Amhara	Misrak Gojjam	Enemay	10°29'00"N	38°20'00"E	2120
81	MCD12-2	Oromia	East Shewa	Gimbichu	07°27'04"N	37°36'36"E	2102
82	34516	Amhara	Misrak Gojjam	Enemay	10°20'53"N	38°09'02"E	2150
84	34520	Amhara	Misrak Gojjam	Enemay	10°19'37"N	38°08'52"E	2150
85	ETDW/15DZ 35	Oromia	West Shewa	Ambo	08°57'13"N	37°55'43"E	2101
86	34522	Amhara	Misrak Gojjam	Enemay	10°29'46"N	38°09'17"E	2170
87	MCD4-4	Oromia	East Shewa	Gimbichu	07°27'04"N	37°36'36"E	2102
88	34545	Amhara	Misrak Gojjam	Dejen	10°10'20"N	38°07'38"E	2200
89	34560	Amhara	Misrak Gojjam	Enemay	10°24'09"N	38°10'57"E	2170
91	ETDW/15DZ 012	Oromia	Semien Shewa	Sheno	09°19'27"N	39°17'57"E	2850
92	34566	Amhara	Misrak Gojjam	Enemay	10°22'52"N	38°10'01"E	2180
93	34571	Amhara	Misrak Gojjam	Enemay	10°22'08"N	38°06'46"E	2190
94	MCD2-29	Oromia	East Shewa	Gimbichu	07°27'04"N	37°36'36"E	2102
95	34573	Amhara	Misrak Gojjam	Enemay	00-00-00-N	00-00-00-E	2190
96	MCD3-14	Oromia	East Shewa	Gimbichu	07°27'04"N	37°36'36"E	2102
97	34580	Amhara	Misrak Gojjam	Dejen	10°13'46"N	38°07'15"E	2200
98	DW-NVT LM 6						
100	34594	Amhara	Oromia	Chefe Golana	10°44'57"N	39°48'30"E	2150
101	MCD3-27	Oromia	East Shewa	Gimbichu	07°27'04"N	37°36'36"E	2102

102	34596				14°81'39"N	38°80'67"N	1950
103	34600	Oromia	Misrak Shewa	Lume	09°47'00"N	39°16'00"E	2200
104	ETDW/15DZ 014	Oromia	Semien Shewa	Sheno	09°19'27"N	39°17'57"E	2850
105	34602	Amhara	Semen Gondar	Gondar Zuria	12°38'00"N	37°28'00"E	2100
107	MCD14-29	Oromia	East Shewa	Gimbichu	07°27'04"N	37°36'36"E	2102
108	34607	Amhara	Semen Gondar	Gondar Zuria	12°38'00"N	37°28'00"E	2100
110	34611	Amhara	Semen Gondar	Gondar Zuria	12°38'00"N	37°28'00"E	2100
111	ETDW/15DZ 043	Oromia	North Shewa	D.Libanos	09°71'20"N	38°88'00"E	2618
112	34613	Oromia	Mirab Shewa	Alem Gena	08°50'00"N	38°22'00"E	1773
113	DZDW1702,04	Amhara	S.Shewa	Lemi	94°68'03"N	38°59'73"E	2600
114	34620	Amhara	Mirab Gojjam	Dembecha	10°34'00"N	37°29'00"E	2145
116	34622	Oromia	Misrak Shewa	Ada'a Chukala	08°49'00"N	39°00'00"E	1915
117	34625	Oromia	Misrak Shewa	Ada'a Chukala	08°49'00"N	39°00'00"E	1915
118	DZDW17010,8	Amhara	M.Gojjam	Debey Tila Tigil	10°30'41"N	37°58'76"E	2626
119	34632						1910
120	34641						2160

Supplementary Table 2. Standard errors of mean and *Least Significant Increase* for comparison of adjusted means for 13 studied traits at Debrezeit and Dera location

Standard Errors Difference	Loc.	BY	CT	DTF	GY	DTH	PH	KPS
Difference between means of two check varieties (Sc)	Debrezeit	52.39	1.48	2.59	0.22	1.88	4.88	1.71
Difference between adjusted yields of two test varieties in the same block (Sd)		117.14	3.3	5.78	0.49	4.21	10.91	3.83
Difference between adjusted yields of two test varieties in different blocks (Sv)		130.97	3.69	6.47	0.54	4.71	12.2	4.28
Difference between the adjusted yield of a test variety and a check mean (Svc)		101.45	2.86	5.01	0.42	3.65	9.45	3.32
A least significant increase (LSI)		180.78	5.10	8.93	0.75	6.50	16.84	5.92
Difference between means of two check varieties (Sc)	Dera	75.74	1.46	2.3	0.19	1.71	3.03	1.98
Difference between adjusted yields of two test varieties in the same block (Sd)		169.35	3.26	5.14	0.43	3.82	6.77	4.43
Difference between adjusted yields of two test varieties in different blocks (Sv)		189.34	3.64	5.75	0.49	4.27	7.57	4.95
Difference between the adjusted yield of a test variety and a check mean (Svc)		146.66	2.82	4.45	0.38	3.31	5.86	3.83
A least significant increase (LSI)		261.35	5.03	7.93	0.68	5.90	10.44	6.83

Supplementary Table 2. Contd …

Standard Errors Difference	Loc.	DTM	LCC	SL	SPS	NT	TSW
Difference between means of two check varieties (Sc)	Debrezeit	1.57	1.66	0.37	1	0.84	3.16
Difference between adjusted yields of two test varieties in the same block (Sd)		3.5	3.72	0.82	2.24	1.87	7.06
Difference between adjusted yields of two test varieties in different blocks (Sv)		3.92	4.15	0.92	2.5	2.09	7.9
Difference between the adjusted yield of a test variety and a check mean (Svc)		3.04	3.22	0.71	1.94	1.62	6.12
A least significant increase (LSI)		5.42	5.74	1.27	3.46	2.89	10.91
Difference between means of two check varieties (Sc)	Dera	1.8	2.49	0.42	1.05	0.73	1.24
Difference between adjusted yields of two test varieties in the same block (Sd)		4.03	5.57	0.94	2.34	1.64	2.77
Difference between adjusted yields of two test varieties in different blocks (Sv)		4.51	6.22	1.05	2.61	1.83	3.1
Difference between the adjusted yield of a test variety and a check mean (Svc)		3.49	4.82	0.82	2.03	1.42	2.4
A least significant increase (LSI)		6.22	8.59	1.46	3.62	2.53	4.28

Loc: location, DF: Degree of freedom, BY: Biomass yield, CT: canopy temperature measurement, DTF: days to 50% flowering, GY: grain yield per plot, DTH: days to heading, PH: plant height, KPS: kennels per spike, DTM: Days to maturity, LCC: Leaf chlorophyll content measurement; SL: spike length, SPS: the number of spikelets per spike, NT: number of tillers, and TSW: thousand seed weight.

Supplementary Table 3. Mean performance of checks and adjusted performance of durum wheat genotypes at Debrezeit for the thirteen yield and yield-related traits

Landraces	BY	CT	DTF	GY	DTH	PH	KPS	DTM	LCC	SL	SPS	NT	TSW	D^2	D^2 P
31696	940.78	31.14	70.35	2.17	57.6	82.12	24.99	104.25	48.78	7.53	20.19	17.59	31.39	4.52	0.972
31698	892.81	33.78	68.35	0.91	49.6	63.71	17.29	67.68	42.54	6.83	16.81	21.16	32.69	10.26	0.593
31755	789.36	40.81	72.35	1.89	56.6	98.57	25.48	111.25	50.28	7.23	10.31	21.24	48.7	28.75	04
31759	987.91	28.66	72.35	1.87	51.6	91.9	23.49	111.25	52.48	9.38	18.69	21.44	29.29	22.27	0.035
31761	927.36	28.06	70.35	0.93	55.6	80.92	28.12	103.25	48.31	8.44	13.93	17.88	41.99	14.05	0.298
31778	590.16	28.48	75.35	3.7	56.6	100.03	33.79	109.25	49.14	11.13	24.43	16.42	49.27	6.85	0.867
31789	814.81	29.72	79.35	2.03	57.6	69.69	21.11	105.25	47.36	9.73	16.31	21.96	51.4	12.75	0.388
31794	790.25	31.46	74.35	3.38	52.6	92.5	30.33	112.25	39.68	7.28	16.93	22.58	45.44	11.31	0.503
31797	984.88	38.37	74.35	3.11	51.6	90.11	27.73	99.25	44.74	8.98	22.93	20.19	48.88	13.72	0.319
31806	1032.73	31.05	79.35	3.37	50.6	68.59	20.56	104.25	46.56	8.93	14.19	20.92	53.87	19.81	0.071
31831	801.48	32.01	72.35	4.54	51.6	67.25	48.19	109.25	46.39	11.88	23.43	19.81	59.62	30.91	02
33205	802.29	34.18	78.62	1.42	64.35	75.83	20.79	115.5	42.11	8.87	18.47	17.36	28.36	13.40	0.341
33230	1015.17	34.59	88.62	2.17	72.35	88.6	24.29	105.5	51.91	8.77	21.97	22.86	21.4	15.05	0.239
33235	869.43	27.92	74.62	3.28	51.35	71.38	29.27	108.5	43.75	7.61	19.71	18.78	41.78	6.03	0.914

33239	648.07	30.74	64.66	2.56	55.35	84.87	21.41	104.5	44.53	9.17	19.09	20.31	38.42	7.49	0.824
33241	1092.94	32.21	82.66	2.14	54.35	102.55	26.79	111.5	43.16	9.37	24.47	23.79	33.25	20.62	0.056
33244	848.07	29.17	82.66	0.75	71.35	79.95	30.6	102.5	47.55	7.46	20.09	23.22	25.19	13.45	0.337
33254	578.01	32.33	68.66	1.92	45.35	91.99	21.41	109.5	34.01	6.71	19.09	20.18	31.41	21.68	0.041
33300	975.96	33.68	80.66	1.64	70.35	64.95	22.91	104.5	45.65	8.52	20.59	19.65	27.55	9.31	0.677
33306	749.55	26.23	70.66	2.18	61.35	87.66	29.42	113.5	43.66	9.62	20.97	22	49.86	10.44	0.577
33403	825.25	32.96	78.66	2.45	63.35	103.7	30.45	103.5	49.93	8.37	23.83	17.05	38.33	7.83	0.798
33405	858.82	37.13	81.66	2.16	63.35	88.6	29.39	112.5	33.6	7.42	21.09	16.86	42.91	20.38	0.060
34217	636.46	26.97	82.85	4.34	66.6	104.32	24.57	103.25	45.7	11.84	24.7	23.23	48	16.10	0.187
34219	640.14	30.32	74.85	2.19	56.6	101.58	21.37	112.25	46.95	7.89	22.2	24.1	53.78	10.71	0.554
34295	803.03	28.72	74.85	2.92	61.6	93.13	19.99	106.25	50.53	10.2	20.82	21.48	42.78	5.17	0.952
34415	549.98	31.8	74.85	3.02	56.6	105.79	25.35	109.25	49.33	10.24	24.2	19.25	47.62	10.42	0.579
34418	718.2	30.57	63.85	1.99	52.6	106.56	20.45	99.25	50.41	8.89	20.94	21.48	36.48	8.41	0.752
34423	640.48	31.18	57.85	2.27	44.6	80.07	19.87	92.25	46.35	8.94	20.7	21.66	47.63	10.41	0.580
34451	790.79	36.42	66.85	0.7	54.6	68.86	19.61	104.25	48.46	9.44	20.44	20.41	28.75	8.90	0.711

34452	669.28	26.62	79.85	1.32	64.6	85.59	20.87	106.25	43.96	8.04	21.7	18.99	49.15	9.57	0.654
34481	896.99	36.41	77.85	1.65	65.6	72.18	35.2	94.25	45.13	7.51	19.2	16.65	34.92	11.39	0.496
34484	645.73	34.64	74.85	1.18	67.6	95.14	20.37	103.25	45.43	7.59	21.2	21.98	29.93	9.20	0.685
34493	726.29	34.72	72.85	5.22	64.6	85.67	26.84	99.25	55.01	12.79	23.32	23.06	47.48	27.32	07
34496	739.08	28.25	59.6	2.79	43.1	90.94	24.56	97	42.14	7.17	21.82	19.92	40.82	10.37	0.583
34500	737.8	29.04	73.6	2.51	63.1	73.48	32.19	112	39.33	7.01	19.68	13.87	46.48	12.43	0.412
34510	646.01	31.28	69.6	3.66	62.1	72.92	26.23	106	44.91	7.27	23.82	19.36	43.47	8.63	0.734
34516	735.4	27.05	74.6	3.6	65.1	80.04	26.85	115	46.81	8.07	24.44	17.91	40.44	8.36	0.756
34520	924.88	28.21	73.6	3.3	52.1	85.65	42.57	111	46.59	7.72	19.44	17.31	36.01	8.29	0.762
34522	830.16	26.43	74.6	6.08	61.1	103.49	31.09	114	38.43	10.47	28.68	15.17	41.15	21.86	0.039
34545	739.53	33.84	83.6	3.86	62.1	70.67	28.23	106	47.74	9.27	25.82	19.02	38.9	10.16	0.602
34560	922.83	35.21	75.6	4.15	65.1	104.44	25.81	108	51.19	7.37	23.4	20.95	50.1	11.56	0.482
34571	836.9	33.76	77.6	3.35	68.1	78.18	30.92	113	43.09	8.32	24.18	21.72	34.54	11.07	0.523
34573	741.53	33.77	88.6	3.73	62.1	79.98	27.75	112	49.99	7.62	21.18	16.99	28.46	15.29	0.226
34594	659.57	33.21	78.6	0.89	65.35	83.1	25	114	48.85	7.35	23.69	20.79	26.8	11.61	0.477

34596	761.09	34.32	71.6	1.89	57.35	70.89	27.44	103	50.92	8.15	20.28	17.4	39.79	4.52	0.972
34600	769.21	35.24	75.61		65.35	74.71	19.09	114	47.76	6.9	17.78	16.01	37.1	7.64	0.813
34602	854.7	33.88	73.61	1.57	62.35	100.13	22.21	96	48.32	8.45	20.9	17.75	29.16	8.70	0.728
34607	702.71	40.12	75.61	2.67	54.35	95.15	27.91	113	53.43	6.1	22.54	23.12	38.62	20.16	0.064
34611	849.4	35.28	66.61	1.13	57.35	87.07	24.09	98	47.57	7.55	22.78	19.22	43.59	8.53	0.742
34613	673.21	35.81	77.61	1.52	65.35	70.46	29.52	117	42.84	8.35	19.9	11.67	31.04	22.69	0.031
34620	855.13	35.53	67.61	2.16	63.35	87.29	23	106	45.87	7.3	21.69	22.3	27.05	12.39	0.415
34622	874.68	37.33	73.61	2.32	65.35	93.72	25.71	103	59.92	9.4	24.4	20.73	48.96	15.02	0.240
34625	857.81	36.13	73.61	2.87	60.35	85.1	21.63	101	58.27	6.7	19.16	16.19	45.46	18.20	0.110
34632	758.47	35.29	68.61	0.89	56.35	98.2	24.45	105	51.47	7.35	21.04	22.64	41.25	9.70	0.642
34641	851.53	41.38	86.61	2.93	66.35	73.17	37.05	115	56.9	8.79	21.54	15.2	47.55	23.39	0.025
Alemtena	830.92	28.14	66.61	3.36	54.2	90.45	31.09	103.8	43.94	6.38	24.37	17.33	48.05	4.32	0.977
DW-NVT LM 11	676.77	28.12	81.85	1.15	68.6	70.75	20.37	109.25	46.68	8.44	21.2	16.5	37.44	8.83	0.718
DW-NVT LM 4	788.24	33.51	82.85	1.74	70.6	89.66	20.11	111.25	43.96	8.49	20.94	19.16	49.13	8.75	0.724
DW-NVT LM 5	744.88	31.83	78.61	3.04	51.35	75.36	23.62	109.5	43.11	8.26	19.71	18.32	52.2	8.50	0.745

DW-NVT LM 6	947.71	34.14	74.6	2.34	62.35	88.87	25.93	103	47.24	8.8	24.62	18.7	42.8	5.31	0.947
DW-PVT LM 8	620.69	32.43	62.85	0.68	44.6	76.12	18.99	103.25	49.08	8.14	19.82	21.68	38.28	11.64	0.475
DZDW17002	883.94	28.08	76.6	3.04	55.35	74.88	23.15	108.5	40.53	6.97	20.83	18.14	35.71	5.57	0.936
DZDW17004	1045.41	33.75	86.6	1.04	77.35	77.62	21.67	81.7	43.98	8.11	23.03	20.36	27.61	36.36	00
DZDW17010,8	949.87	32.9	79.6	2.5	58.35	76.34	26.28	110	48.96	8.3	20.78	20.82	46.97	4.59	0.970
DZDW1702,04	487.44	37.44	73.6	3.15	58.35	58.93	31.06	106	54.44	11.85	23.9	18.27	44.13	24.05	0.020
ETDW/15DZ 23	915.21	29.62	76.85	6.13	64.6	88.95	26.49	110.25	45	13.04	27.32	17.51	46.5	16.19	0.183
ETDW/15DZ 35	753.17	28.03	83.6	3.46	69.1	90.39	24.97	111	42.11	7.82	22.56	20.65	41.91	6.77	0.873
ETDW/15DZ 39	1040.29	31.63	84.6	2	70.35	76.82	25.02	119.5	43.71	7.47	21.83	19.66	33.92	10.92	0.536
ETDW/15DZ 4	1048.02	26.92	59.6	5.73	45.35	97.75	28.84	103.5	41.25	13.02	24.59	17.1	44.42	27.75	06
ETDW/15DZ 003	791.32	33.1	69.35	2.1	55.6	63.83	23.12	110.25	43.63	7.63	18.32	18.14	42.12	6.29	0.901
ETDW/15DZ 006	1002.31	30.22	79.35	2.54	53.6	71.54	42.33	106.25	49.27	8.63	17.31	16.81	49.23	10.81	0.546
ETDW/15DZ 010	592.41	29.12	73.35	3.9	58.6	87.45	32.37	109.25	46.38	11.68	23.69	17.57	54.42	5.80	0.926
ETDW/15DZ 012	520.41	30.02	76.6	3.41	60.1	97.71	26.47	102	48.23	8.17	24.06	21.89	28.31	16.77	0.159
ETDW/15DZ 014	953.39	30.93	92.6	2.5	68.35	79.29	24.21	116	48.05	9.8	22.9	20.01	45.81	12.92	0.375

ETDW/15DZ038	822.99	31.45	85.35	3.23	61.6	102.63	28.11	116.25	53.51	9.23	23.31	18.08	51.91	11.38	0.497
ETDW/15DZ043	490.66	33.47	81.6	2.61	67.35	82.84	20.85	111	51.54	7.35	19.54	19.55	43.96	12.84	0.381
ETDW/15DZ049	971.65	35.94	71.6	3.63	59.1	78.48	25.67	111	45.74	9.92	22.94	13.95	49.98	13.51	0.333
ETDW/15DZ057	1087.92	30.64	67.35	2.81	43.6	102.23	23.87	97.25	46.68	9.08	19.07	19.64	33.28	14.89	0.248
ETDW/15DZ073	648.21	28.05	74.6	2.71	63.1	71.4	25.59	112	46.29	8.67	23.18	13.35	37.22	11.85	0.457
Fetan	842.11	28.88	67.84	52.8	86.03	30.64	101.2	44.48	10.65	24.41	17.36	46.3	5.74	0.929	
Kuami	803.7	30.44	68	2.84	54	94.88	27.73	105.2	45.33	10.5	22.87	18	41.85	2.64	0.998
MCD1-32	645.04	29.12	69.85	1.54	54.6	88.84	27.74	103.25	42.98	8.49	24.68	24.53	53.81	15.88	0.197
MCD10-11	748.28	30.7	65.85	1.87	55.6	70.79	22.39	106.25	53.15	8	21.93	23.02	42.3	10.70	0.555
MCD12-2	850.4	38.87	62.6	3.39	44.1	86.35	26.35	95	50.19	8.32	23.94	16	35.72	19.12	0.086
MCD12-30	1050.43	30.39	80.6	4.05	52.35	89.01	23.66	113.5	38.48	7.87	19.71	22.2	48.69	14.28	0.283
MCD13-42	666.54	27.7	76.85	1.45	60.6	95.43	22.49	107.25	44.31	7.79	23.32	15.57	47.26	13.35	0.344
MCD14-29	994.19	35.33	57.6	0.88	49.35	94.21	21.23	90	46.84	7	15.66	21.85	36.55	16.60	0.165
MCD15-49	784.66	31.47	83.35	2.78	67.6	96.96	23.23	116.25	47.04	8.83	18.43	23.92	40	6.89	0.865
MCD15-7	743.39	29.19	76.6	2.92	59.1	101.73	20.85	100	40.61	8.17	18.44	13.65	37.57	16.46	0.171

MCD2-17	774.01	28.5	72.85	2.69	62.6	103.1	23.77	103.25	43.78	10.14	17.08	17.82	48.63	12.75	0.388
MCD2-29	736.65	27.85	79.6	2.95	69.1	76.11	36.85	116	43.13	7.32	22.82	18.38	41.14	7.60	0.815
MCD3-14	849	27.54	72.6	4.88	63.1	86.75	35.17	112	44.79	10.67	27.82	19.74	49.22	9.99	0.617
MCD3-15	590.96	30.16	68.35	2.55	52.6	75.99	25.08	103.25	51.51	8.73	15.81	16.54	50.38	11.19	0.513
MCD3-27	989.53	34.48	79.6	2.28	63.35	59.24	23.59	112	52.67	8.9	22.28	22.08	25.78	16.59	0.166
MCD4-15	1003.9	29.91	51.6	1.69	45.35	61.81	23.41	88.5	42.78	7.67	21.09	16.87	40.23	20.78	0.054
MCD4-4	750.84	30.62	75.6	2.79	60.1	80.41	25.73	111	45.12	6.62	23.32	18.29	30.11	5.39	0.944
MCD4-41	1023.74	28.45	85.6	2.31	72.35	95.81	26.54	111.5	42.13	8.52	21.97	20.83	47.17	12.13	0.435
Ude	768.72	31.95	66	4.28	56.4	90.46	27.6	103.8	46.64	8.43	24.74	17.31	43.92	17.20	0.142

Supplementary Table 4. Mean performance of checks and adjusted performance of durum wheat genotypes at Dera for the thirteen yield and yield-related traits

Treatment	BY	CT	DTF	GY	DTH	PH	KPS	DTM	LCC	SL	SPS	NT	TSW	D^2	D^2 P
31696	705.2	31.44	58.55	1.12	52.25	90.29	20.86	94.8	46.26	5.33	18.64	16.04	38.96	5.27	0.948
31703	409.5	34.08	69.55	0.59	50.25	90.04	8.96	104.8	32.36	4.63	16.58	19.61	31.25	23.20	0.026
31755	406.1	31.11	68.55	0.63	58.25	90.54	15.36	103.8	45.39	6.03	14.1	15.69	28.14	13.37	0.343
31759	928.5	28.96	63.55	1.26	47.25	81.79	14.03	101.8	42.42	7.18	12.1	16.89	36.41	12.22	0.428
31761	279.2	28.36	62.55	0.36	53.25	88.79	16.15	98.8	38.32	6.24	14.42	16.33	33.55	9.88	0.626
31778	809.2	28.78	59.55	3.04	47.25	88.79	25.2	95.8	43.39	8.93	19.56	14.87	36.23	7.59	0.816
31789	598.1	30.02	60.55	1.31	52.25	80.54	15.02	86.8	47.02	7.53	16.1	17.41	37.92	11.16	0.515

31794	588.56	31.76	58.55	1.48	43.25	85.54	19.2	92.8	37.09	5.08	18.98	18.03	34.74	10.28	0.591
31797	395.2	28.67	60.55	2.47	49.25	96.52	27.63	94.8	37.06	6.78	20.55	18.64	35.13	11	0.529
31806	502.1	31.35	71.55	1.76	44.25	80.54	15.58	98.8	39.99	6.73	14.1	16.07	40.08	12.73	0.389
31831	283	32.31	57.55	3.62	50.25	73.79	36.86	86.8	47.53	8.68	19.02	18.26	41.17	21.06	0.050
33205	918.64	36.98	77.8	1.36	59.75	78.48	20.72	107.3	47.3	6.41	17.54	12.06	42.31	11.77	0.465
33230	916.54	30.39	78.8	1.72	58.75	69.61	29.13	100.3	41.53	6.31	20.87	17.56	38.77	14.82	0.251
33235	396.64	30.72	71.8	2.29	46.75	71.98	23.9	107.3	38.07	5.17	20.54	16.48	43.41	17.81	0.122
33239	821.84	33.54	56.8	1.61	45.75	81.73	23.13	96.3	38.73	6.71	18.97	14.01	42.22	7.94	0.789
33241	415.64	35.01	78.8	1.7	49.75	84.18	19.8	100.3	36.2	6.91	15.45	17.49	37.9	13.46	0.336
33244	194.74	28.97	68.8	0.61	54.75	78.23	18.46	92.3	41.2	5.01	14.15	16.92	36.18	14.14	0.292
33254	525.64	35.13	65.8	1.17	45.75	84.98	21.66	102.3	36.67	6.26	17.54	13.88	39.09	6.61	0.882
33300	896.29	36.48	70.8	1.21	60.75	68.48	18.46	96.3	40.67	6.06	13.87	17.35	44.51	10.61	0.563
33306	397.74	29.03	74.8	2.04	57.75	85.98	24.13	102.3	37.33	7.16	20.65	15.7	37.4	7.90	0.793
33403	915.24	35.76	64.8	2.42	52.75	83.86	28.13	96.3	48.03	6.91	20.37	14.75	35.65	9.20	0.686
33405	616.54	44.93	81.8	1.68	59.75	79.73	32.46	102.3	32.13	4.96	21.54	11.56	47.85	27.14	07
34217	360.71	29.27	75.55	3.6	59.75	93.01	17.63	99.05	39.29	9.39	18.55	20.18	36.95	20.10	0.065
34219	365.41	32.62	61.55	1.03	53.75	90.88	15.47	95.05	36.92	5.44	14.92	21.05	33.55	10.43	0.578
34295	681.61	31.02	69.55	0.35	59.75	82.63	28.63	94.05	41.99	7.75	18.93	14.43	36.25	16.20	0.182
34415	278.61	34.1	53.55	2.6	43.75	96.38	29.96	93.05	52.19	7.79	21.85	13.2	36.78	13.60	0.327
34418	581.71	34.87	55.55	1.12	48.75	97.38	16.35	86.05	43.66	6.44	14.92	18.43	35.67	7.95	0.789
34423	366.21	33.48	51.55	1.73	46.75	79.21	12.04	90.05	41.29	6.49	10.25	18.61	40.71	11.45	0.491
34451	661.61	34.72	62.55	0.21	52.75	74.54	14.45	98.05	43.69	6.99	13.59	17.36	36.07	9.39	0.669
34452	558.52	28.92	56.55	0.68	56.75	74.38	14.27	95.05	40.06	5.59	14.92	15.94	34.74	11.59	0.479
34481	877.21	38.71	64.55	0.54	55.75	74.2	24.39	85.05	35.22	6.06	15.93	13.6	39.7	16.31	0.177
34484	377.71	36.94	66.55	0.58	60.75	85.55	16.3	91.05	43.69	5.14	16.92	18.93	38.73	10.46	0.576
34493	588.34	33.02	63.55	4.54	53.75	88.63	31.39	91.05	48.76	10.34	15	20.01	37.53	27.90	06
34496	568.61	31.8	49.05	1.74	36.75	88.56	21.11	88.55	37.43	4.96	15.73	18.37	36.78	10.22	0.597
34500	555.71	32.59	57.05	1.24	44.75	72.06	26.05	95.55	32.23	4.81	19.14	12.32	34.08	10.12	0.605
34510	441.81	34.83	54.05	1.38	42.75	74.06	23.34	98.55	38.93	5.07	15.73	17.81	44.15	12.55	0.402
34516	546.81	30.6	52.05	2.07	46.75	78.39	18.48	98.55	35.93	5.87	11.73	16.36	35.66	14.77	0.254
34520	867.71	31.76	67.05	2.12	52.75	88.06	19.02	100.55	43.47	5.52	12.08	15.76	41.62	13.37	0.343

34522	746.31	29.98	52.05	4.66	49.75	86.89	35.05	92.55	43.1	8.27	22.02	9.62	38.16	16.83	0.156
34545	573.01	33.39	61.05	2.6	47.75	76.06	26.91	97.55	48.77	7.07	20.06	14.47	43	6.16	0.908
34560	854.71	33.76	58.05	2.37	49.75	87.93	23.07	96.55	44.37	5.17	18.06	17.4	45.5	9.21	0.685
34566	88.36	36.14	85.05	2.58	58.75	14.96	37.81	116.55	37.1	8.12	25.6	87.48	37.05	23.10	0.027
34571	767.11	39.31	69.05	2.58	56.75	80.56	33.05	100.55	46.6	6.12	21.4	20.17	51.58	18.23	0.109
34573	588.51	37.32	66.05	2.17	52.75	77.73	20.48	93.55	45.97	5.42	17.56	15.44	49.62	12.94	0.374
34580	100	37.32	70.05	0.52	50.5	77.78	39.79	100.3	43.36	5.35	26.36	6.8	38.25	25.04	0.015
34594	445.76	34.26	79.05	0.52	56.5	88.95	14.56	101.3	43.89	5.15	11.02	19.24	41.44	16.58	0.166
34596	570.36	35.37	52.05	1.17	42.5	83.11	24.61	90.3	46.23	5.95	19.69	11.85	38.62	7.19	0.844
34600	652.96	36.29	57.05	0.48	49.5	87.11	17.56	97.3	44.23	5.7	13.36	14.46	32.72	10.62	0.561
34602	759.46	34.93	53.05	1.21	47.5	102.45	19.13	83.3	42.73	6.25	16.69	16.2	44.1	18.36	0.105
34607	474.46	41.17	70.05	2.06	57.5	88.21	22.01	102.3	52.33	5.9	20.02	14.57	45.58	15.78	0.202
34611	755.46	36.33	64.05	1.23	52.5	81.11	17.79	90.3	48.16	5.35	17.36	17.67	39.77	8.49	0.745
34613	468.36	36.86	65.05	1.48	52.5	80.11	21.89	107.3	34.56	6.15	17.36	10.12	40.41	15.54	0.213
34620	761.16	36.58	57.05	1.42	51.5	83.11	16.1	101.3	46.36	5.1	16.02	18.75	40.67	11.71	0.469
34622	795.16	38.38	63.05	1.97	53.5	88.94	21.79	97.3	51.39	7.2	18.92	19.18	46.95	11.95	0.450
34625	775.36	37.18	56.05	1	47.5	86.28	18.29	95.3	48.96	4.5	15.69	14.64	41.62	7.26	0.840
34632	562.46	33.34	57.05	0.84	48.5	93.45	23.13	96.3	42.99	5.15	21.02	15.09	41.11	9.76	0.637
34641	756.76	32.43	72.05	2	59.5	86.34	31.46	99.3	47.29	6.59	20.36	13.65	36.06	8.51	0.744
Alemtena	776.63	30.14	54.4	2.85	46.8	87.38	30.29	90.2	38.02	4.48	19.84	15.93	35.81	16.84	0.156
DW-NVT LM 11	565.51	30.42	64.55	0.5	63.75	81.88	15.01	89.05	41.19	5.99	17.25	13.45	35.48	15.70	0.205
DW-NVT LM 4	659.2	36.81	60.55	0.63	59.75	85.13	17.63	91.05	49.26	6.04	18.53	13.11	37.4	10.88	0.539
DW-NVT LM 5	805.84	34.63	68.8	2.08	48.75	68.48	20.46	103.3	37.33	5.82	16.54	16.02	42.09	8.74	0.725
DW-NVT LM 6	872.46	35.19	56.05	1.59	46.5	82.28	33.46	94.3	53.89	6.6	22.58	17.15	38.4	13.64	0.325
DW-PVT LM 8	357.61	34.73	46.55	0.11	44.75	83.55	14.2	90.05	42.53	5.69	12.59	18.63	36.08	12.16	0.433
DZDW17002	795.95	30.88	60.8	1.42	44.75	73.98	21.5	88.3	34.43	4.51	16.51	12.84	39.21	12.06	0.441
DZDW17010,8	922.91	33.95	60.05	2.09	47.5	78.28	29.79	93.3	45.66	6.1	18.76	14.27	37.56	6.89	0.865
DZDW1702,04	347.36	31.49	61.05	1.13	48.5	68.35	22.56	101.3	50.79	8.65	17.83	16.72	37.26	21.83	0.039
ETDW/15DZ 23	257.01	27.92	65.55	4.96	57.75	79.48	29.63	94.05	35.49	9.59	21.47	14.46	35.78	20.38	0.060
ETDW/15DZ 35	707.73	31.58	63.05	2.15	57.75	74.9	24.78	95.55	42.83	6.62	16.06	19.1	42.92	8.57	0.739
ETDW/15DZ 39	418.64	34.43	76.8	1.66	65.75	67.73	18.92	99.3	39.27	5.02	14.54	13.36	38.25	9.57	0.654

ETDW/15DZ 4	698.64	29.72	56.8	5.47	45.75	78.73	20.56	99.3	43.03	9.57	16.67	10.8	39.18	25.41	0.013
ETDW/15DZ 003	407	38.4	55.55	1.05	48.25	71.29	26.86	87.8	48.79	5.43	20.14	12.59	32.33	22.56	0.032
ETDW/15DZ 006	588.06	30.52	62.55	1.65	56.25	83.32	30.2	98.8	47.26	6.43	19.35	15.26	38.9	7.20	0.844
ETDW/15DZ 010	795.1	29.42	69.55	3.19	53.25	91.79	22.53	98.8	50.09	9.48	18.94	16.02	32.15	14	0.301
ETDW/15DZ 012	346.81	33.57	55.05	2.51	51.75	82.8	21.17	93.55	51.8	5.97	18.73	13.34	40.26	13.21	0.354
ETDW/15DZ 014	936.85	31.98	75.05	1.21	54.5	93.78	15.56	100.3	42.53	7.6	16.52	14.46	35.39	14.12	0.293
ETDW/15DZ 038	409.2	31.75	70.55	2.42	54.25	92.04	30.53	106.8	50.06	7.03	18.59	14.53	34.71	14.30	0.282
ETDW/15DZ 043	352.46	34.52	64.05	0.43	59.5	87.78	19.23	95.3	47.56	5.15	15.68	14	37.6	8.76	0.723
ETDW/15DZ 049	970.61	39.49	61.05	2.71	54.75	68.79	20.23	89.55	39.7	7.72	16.06	12.4	40.38	18.60	0.099
ETDW/15DZ 057	602.9	30.94	58.55	1.2	44.25	90.79	13.84	91.8	51.46	6.88	12.1	18.09	33.7	11.65	0.474
ETDW/15DZ 073	454.61	31.6	56.05	1.75	45.75	75.4	18.46	93.55	35.83	6.47	16.06	11.8	36.54	7.05	0.854
Fetan	720.27	30.88	54.6	3.25	46	86.7	31.42	88.6	38.92	8.35	21.15	15.36	37.07	8.33	0.758
Kuami	660.61	32.04	55.6	2.4	48.2	86.55	30.57	96	38.2	8.2	21.31	17.2	37.45	11.67	0.473
MCD1-32	377.71	34.42	57.55	1	50.75	80.51	21.63	93.05	40.72	6.04	19.89	14.48	34.49	6.39	0.895
MCD10-11	609.01	33	59.55	0.65	54.75	81.21	16.32	91.05	42.89	6.55	15.92	19.97	38.18	6.14	0.909
MCD12-2	772.81	32.42	52.05	1.88	37.75	91.06	20.69	81.55	43.47	6.11	13.73	14.45	41.58	15.52	0.214
MCD12-30	399.74	33.19	75.8	2.05	49.75	71.73	22.1	101.3	32.6	5.41	16.54	11.9	34.83	13.72	0.319
MCD13-42	543.71	30	66.55	0.85	57.75	96.88	15.47	98.05	43.66	5.34	14.25	12.52	34.31	13.06	0.365
MCD14-29	975.76	36.38	52.05	0.38	45.5	91.11	18.19	85.3	47.19	5.8	14.69	16.73	39.76	9.62	0.649
MCD15-49	895.5	31.77	64.55	1.45	60.25	83.04	18.41	96.8	40.42	6.63	16.77	19.37	33.74	11.40	0.495
MCD15-7	612.38	32.74	54.05	2.21	46.75	84.81	20.23	83.55	39.6	5.97	13.4	10.1	37.9	13.54	0.331
MCD2-17	653.28	30.8	57.55	2.13	49.75	93.38	8.39	94.05	43.92	7.69	9.89	14.77	34.7	11.21	0.511
MCD2-29	552.71	31.4	59.05	2.19	56.75	69.8	34.38	88.55	41.73	5.12	21.58	16.83	42.07	14.99	0.242
MCD3-14	772.71	31.09	68.05	2.88	54.75	71.56	37.76	101.55	46.33	8.47	24.1	13.19	41.29	10.77	0.549
MCD3-15	379.1	30.46	52.55	1.88	42.25	87.04	20.88	90.8	42.72	7.53	15.77	14.99	35.09	6.07	0.913
MCD3-27	971.46	28.53	62.05	1.43	55.5	73.45	29.79	94.3	47.09	6.7	21.36	16.53	37.86	13.53	0.332
MCD4-15	1008.44	37.71	56.8	1.13	44.75	65.23	14.67	83.3	31.8	5.21	10.87	14.57	39.16	20.58	0.057
MCD4-4	652.71	34.17	62.05	1.22	43.75	82.56	24.15	93.55	36.57	5.41	15.73	16.74	42.03	7.30	0.837
MCD4-41	497.74	31.25	72.8	1.81	62.75	76.23	23.28	92.3	37.63	6.06	17.87	18.53	37.13	10.22	0.596
Ude	740.26	30.55	54.6	3.22	45	85.43	28.22	92.4	40.47	6.13	19.96	15.31	37.46	6.93	0.862

Supplementary Table 5. Analysis of stress tolerance indices for the studied durum wheat landraces for 2020/21 cropping season.

Genotype Code	Yp	Ys	TOL	MP	GMP	HM	SSI	STI	YI	YSI	RSI
31696	2	1.18	0.82	1.59	1.54	1.48	1.21	0.33	0.67	0.59	0.89
Kuami	2.84	2.4	0.44	2.62	2.61	2.6	0.46	0.96	1.36	0.85	1.28
ETDW/15DZ 003	1.93	1.11	0.82	1.52	1.46	1.41	1.26	0.3	0.63	0.58	0.87
31755	1.72	0.69	1.03	1.21	1.09	0.98	1.77	0.17	0.39	0.4	0.61
Fetan	4	3.25	0.75	3.63	3.61	3.59	0.56	1.83	1.84	0.81	1.23
ETDW/15DZ 010	3.73	3.25	0.48	3.49	3.48	3.47	0.38	1.71	1.84	0.87	1.32
31759	1.7	1.32	0.38	1.51	1.5	1.49	0.66	0.32	0.75	0.78	1.17
ETDW/15DZ 057	2.64	1.26	1.38	1.95	1.82	1.71	1.55	0.47	0.71	0.48	0.72
Alemtena	3.36	2.85	0.51	3.11	3.09	3.08	0.45	1.35	1.62	0.85	1.28
31761	0.76	0.42	0.34	0.59	0.56	0.54	1.32	0.05	0.24	0.55	0.83
31778	3.53	3.1	0.43	3.32	3.31	3.3	0.36	1.54	1.76	0.88	1.33
ETDW/15DZ 006	2.37	1.71	0.66	2.04	2.01	1.99	0.82	0.57	0.97	0.72	1.09
31789	1.86	1.37	0.49	1.62	1.6	1.58	0.78	0.36	0.78	0.74	1.11
ETDW/15DZ 038	3.06	2.48	0.58	2.77	2.75	2.74	0.56	1.07	1.41	0.81	1.22
31794	3.21	1.54	1.67	2.38	2.22	2.08	1.54	0.7	0.87	0.48	0.72
Ude	4.28	3.22	1.06	3.75	3.71	3.68	0.73	1.94	1.83	0.75	1.14
31797	2.94	2.53	0.41	2.74	2.73	2.72	0.41	1.05	1.43	0.86	1.3
MCD15-49	2.61	1.51	1.1	2.06	1.99	1.91	1.25	0.56	0.86	0.58	0.87
31806	3.2	1.82	1.38	2.51	2.41	2.32	1.28	0.82	1.03	0.57	0.86
31831	4.37	3.68	0.69	4.03	4.01	4	0.47	2.27	2.09	0.84	1.27
MCD3-15	2.38	1.94	0.44	2.16	2.15	2.14	0.55	0.65	1.1	0.82	1.23
33205	1.33	1.06	0.27	1.2	1.19	1.18	0.6	0.2	0.6	0.8	1.2
MCD12-30	3.96	1.75	2.21	2.86	2.63	2.43	1.65	0.98	0.99	0.44	0.67
33230	2.01	1.42	0.59	1.72	1.69	1.66	0.87	0.4	0.81	0.71	1.07
ETDW/15DZ #4	5.64	5.17	0.47	5.41	5.4	5.39	0.25	4.11	2.93	0.92	1.38
33235	3.19	1.99	1.2	2.59	2.52	2.45	1.11	0.9	1.13	0.62	0.94
33239	2.47	1.31	1.16	1.89	1.8	1.71	1.39	0.46	0.74	0.53	0.8
MCD4-15	1.6	0.83	0.77	1.22	1.15	1.09	1.43	0.19	0.47	0.52	0.78
33241	2.05	1.4	0.65	1.73	1.69	1.66	0.94	0.4	0.79	0.68	1.03

DW-NVT LM 5	2.95	1.78	1.17	2.37	2.29	2.22	1.17	0.74	1.01	0.6	0.91
33244	0.66	0.31	0.35	0.49	0.45	0.42	1.57	0.03	0.18	0.47	0.71
33254	1.83	0.87	0.96	1.35	1.26	1.18	1.55	0.22	0.49	0.48	0.72
DZDW17002	2.95	1.12	1.83	2.04	1.82	1.62	1.84	0.47	0.64	0.38	0.57
33300	1.55	0.91	0.64	1.23	1.19	1.15	1.22	0.2	0.52	0.59	0.89
ETDW/15DZ #39	1.91	1.36	0.55	1.64	1.61	1.59	0.85	0.37	0.77	0.71	1.08
33306	2.09	1.74	0.35	1.92	1.91	1.9	0.5	0.51	0.99	0.83	1.26
MCD4-41	2.22	1.51	0.71	1.87	1.83	1.8	0.95	0.47	0.86	0.68	1.03
33403	2.36	2.12	0.24	2.24	2.24	2.23	0.3	0.71	1.2	0.9	1.36
33405	2.07	1.38	0.69	1.73	1.69	1.66	0.99	0.4	0.78	0.67	1.01
MCD13-42	2.02	1.4	0.62	1.71	1.68	1.65	0.91	0.4	0.79	0.69	1.05
34217	4.91	4.15	0.76	4.53	4.51	4.5	0.46	2.87	2.35	0.85	1.28
34219	2.76	1.58	1.18	2.17	2.09	2.01	1.27	0.62	0.9	0.57	0.86
DW-NVT LM 4	2.31	1.18	1.13	1.75	1.65	1.56	1.45	0.38	0.67	0.51	0.77
34295	3.49	0.9	2.59	2.2	1.77	1.43	2.2	0.44	0.51	0.26	0.39
MCD2-17	3.26	2.68	0.58	2.97	2.96	2.94	0.53	1.23	1.52	0.82	1.24
34415	3.59	3.15	0.44	3.37	3.36	3.36	0.36	1.6	1.79	0.88	1.32
DW-NVT LM 11	1.72	1.05	0.67	1.39	1.34	1.3	1.15	0.25	0.6	0.61	0.92
34418	2.56	1.67	0.89	2.12	2.07	2.02	1.03	0.6	0.95	0.65	0.98
MCD1-32	2.11	1.55	0.56	1.83	1.81	1.79	0.79	0.46	0.88	0.73	1.11
34423	2.84	2.28	0.56	2.56	2.54	2.53	0.58	0.91	1.29	0.8	1.21
ETDW/15DZ #23	6.7	5.51	1.19	6.11	6.08	6.05	0.53	5.21	3.12	0.82	1.24
34451	1.27	0.76	0.51	1.02	0.98	0.95	1.19	0.14	0.43	0.6	0.9
34452	1.89	1.23	0.66	1.56	1.52	1.49	1.03	0.33	0.7	0.65	0.98
DW-PVT LM 8	1.25	0.66	0.59	0.96	0.91	0.86	1.4	0.12	0.37	0.53	0.8
34481	2.22	1.09	1.13	1.66	1.56	1.46	1.51	0.34	0.62	0.49	0.74
34484	1.75	1.13	0.62	1.44	1.41	1.37	1.05	0.28	0.64	0.65	0.97
MCD10-11	2.44	1.2	1.24	1.82	1.71	1.61	1.51	0.41	0.68	0.49	0.74
34493	5.79	5.09	0.7	5.44	5.43	5.42	0.36	4.16	2.89	0.88	1.33
MCD15-7	1.95	1.59	0.36	1.77	1.76	1.75	0.55	0.44	0.9	0.82	1.23
34496	1.82	1.12	0.7	1.47	1.43	1.39	1.14	0.29	0.64	0.62	0.93

ETDW/15DZ 073	1.74	1.13	0.61	1.44	1.4	1.37	1.04	0.28	0.64	0.65	0.98
34500	1.54	0.62	0.92	1.08	0.98	0.88	1.77	0.13	0.35	0.4	0.61
ETDW/15DZ 049	2.66	2.09	0.57	2.38	2.36	2.34	0.63	0.78	1.19	0.79	1.19
34510	2.69	0.76	1.93	1.73	1.43	1.19	2.12	0.29	0.43	0.28	0.43
MCD12-2	2.42	1.26	1.16	1.84	1.75	1.66	1.42	0.43	0.71	0.52	0.79
34516	2.63	1.45	1.18	2.04	1.95	1.87	1.33	0.54	0.82	0.55	0.83
34520	2.33	1.5	0.83	1.92	1.87	1.83	1.06	0.49	0.85	0.64	0.97
ETDW/15DZ #35	2.49	1.53	0.96	2.01	1.95	1.9	1.14	0.54	0.87	0.61	0.93
34522	5.11	4.04	1.07	4.58	4.54	4.51	0.62	2.91	2.29	0.79	1.19
MCD4-4	1.82	0.6	1.22	1.21	1.04	0.9	1.99	0.15	0.34	0.33	0.5
34545	2.89	1.98	0.91	2.44	2.39	2.35	0.93	0.81	1.12	0.69	1.03
34560	3.18	1.75	1.43	2.47	2.36	2.26	1.33	0.79	0.99	0.55	0.83
ETDW/15DZ 012	2.44	1.89	0.55	2.17	2.15	2.13	0.67	0.65	1.07	0.77	1.17
34571	2.38	1.96	0.42	2.17	2.16	2.15	0.52	0.66	1.11	0.82	1.24
MCD2-29	1.98	1.57	0.41	1.78	1.76	1.75	0.61	0.44	0.89	0.79	1.2
34573	2.76	1.55	1.21	2.16	2.07	1.99	1.3	0.6	0.88	0.56	0.85
MCD3-14	3.91	2.26	1.65	3.09	2.97	2.86	1.25	1.25	1.28	0.58	0.87
DW-NVT LM 6	3	1.91	1.09	2.46	2.39	2.33	1.08	0.81	1.08	0.64	0.96
34594	1.55	0.84	0.71	1.2	1.14	1.09	1.36	0.18	0.48	0.54	0.82
MCD3-27	2.94	1.75	1.19	2.35	2.27	2.19	1.2	0.73	0.99	0.6	0.9
34596	2.55	1.49	1.06	2.02	1.95	1.88	1.23	0.54	0.84	0.58	0.88
34600	1.66	0.8	0.86	1.23	1.15	1.08	1.53	0.19	0.45	0.48	0.73
ETDW/15DZ 014	3.16	1.53	1.63	2.35	2.2	2.06	1.53	0.68	0.87	0.48	0.73
34602	2.23	1.53	0.7	1.88	1.85	1.81	0.93	0.48	0.87	0.69	1.04
MCD14-29	1.54	0.7	0.84	1.12	1.04	0.96	1.62	0.15	0.4	0.45	0.69
34607	3.33	2.38	0.95	2.86	2.82	2.78	0.84	1.12	1.35	0.71	1.08
34611	1.79	1.55	0.24	1.67	1.67	1.66	0.4	0.39	0.88	0.87	1.31
ETDW/15DZ 043	3.27	0.75	2.52	2.01	1.57	1.22	2.28	0.35	0.43	0.23	0.35
34613	2.18	1.8	0.38	1.99	1.98	1.97	0.52	0.55	1.02	0.83	1.25
DZDW1702,04	3.81	1.45	2.36	2.63	2.35	2.1	1.83	0.78	0.82	0.38	0.57
34620	2.82	1.74	1.08	2.28	2.22	2.15	1.13	0.69	0.99	0.62	0.93

34622	2.98	2.29	0.69	2.64	2.61	2.59	0.69	0.96	1.3	0.77	1.16
34625	3.53	1.32	2.21	2.43	2.16	1.92	1.85	0.66	0.75	0.37	0.56
DZDW17010,8	3.16	2.41	0.75	2.79	2.76	2.73	0.7	1.07	1.37	0.76	1.15
34632	1.55	1.16	0.39	1.36	1.34	1.33	0.75	0.25	0.66	0.75	1.13
34641	3.59	2.32	1.27	2.96	2.89	2.82	1.05	1.17	1.32	0.65	0.98

Where Yp: yield in non-stress condition: Ys: yield in stress condition; RC: relative change; TOL: Tolerance Index; MP: Mean Productivity; GMP: Geometric Mean Productivity; HM: Harmonic Mean; SSI: Stress Susceptibility Index; STI: Stress Tolerance Index; YI: Yield Index; YSI: Yield Stability Index; RSI: Relative Drought Index.

Supplementary Table 6. Analysis of stress tolerance indices for the studied durum wheat landraces for 2022/23 cropping season.

Genotype Code	Yp	Ys	RC	TOL	MP	GMP	HM	SSI	STI	YI	YSI	RSI
31696	1.39	1.06	23.81	0.33	1.22	1.21	1.2	0.77	0.22	0.59	0.76	1.1
DW-NVT LM 12	1.13	1.03	8.26	0.09	1.08	1.08	1.08	0.27	0.17	0.58	0.92	1.33
Kuami	2.95	2.6	11.78	0.35	2.78	2.77	2.77	0.38	1.15	1.46	0.88	1.28
31698	1.23	1.14	7.72	0.1	1.18	1.18	1.18	0.25	0.21	0.63	0.92	1.34
31703	0.91	0.82	9.26	0.08	0.87	0.86	0.86	0.3	0.11	0.46	0.91	1.31
ETDW/15DZ 003	1.79	1.44	19.72	0.35	1.61	1.6	1.59	0.64	0.38	0.8	0.8	1.16
31755	1.91	1.83	4.35	0.08	1.87	1.87	1.87	0.14	0.52	1.02	0.96	1.38
Fetan	3.95	2.84	28.17	1.11	3.39	3.35	3.3	0.91	1.67	1.59	0.72	1.04
ETDW/15DZ 010	3.99	3.74	6.32	0.25	3.86	3.86	3.86	0.2	2.22	2.09	0.94	1.36
31759	1.7	1.24	26.88	0.46	1.47	1.45	1.44	0.87	0.32	0.69	0.73	1.06
ETDW/15DZ 057	3.33	2.5	24.92	0.83	2.92	2.89	2.86	0.81	1.24	1.4	0.75	1.09
Alemtena	3.58	3.26	9	0.32	3.42	3.42	3.41	0.29	1.74	1.82	0.91	1.32
31761	1.1	1.08	2.17	0.02	1.09	1.09	1.09	0.07	0.18	0.6	0.98	1.42
31778	3.12	2.76	11.7	0.37	2.94	2.93	2.93	0.38	1.28	1.54	0.88	1.28
ETDW/15DZ 006	2.52	1.4	44.33	1.12	1.96	1.88	1.8	1.43	0.53	0.78	0.56	0.81
31789	1.41	1.26	10.99	0.16	1.33	1.33	1.33	0.36	0.26	0.7	0.89	1.29
ETDW/15DZ 038	3.73	2.93	21.58	0.81	3.33	3.3	3.28	0.7	1.63	1.64	0.78	1.14
31794	3.27	1.64	49.94	1.63	2.45	2.31	2.18	1.62	0.8	0.92	0.5	0.72
Ude	4.37	3.18	27.24	1.19	3.77	3.73	3.68	0.88	2.07	1.78	0.73	1.05
31797	2.38	2.22	6.85	0.16	2.3	2.3	2.3	0.22	0.79	1.24	0.93	1.35

MCD15-49	2.52	1.46	41.98	1.06	1.99	1.92	1.85	1.36	0.55	0.82	0.58	0.84
31806	3.99	0.95	76.19	3.04	2.47	1.95	1.53	2.46	0.57	0.53	0.24	0.34
31831	3.88	2.78	28.4	1.1	3.33	3.28	3.24	0.92	1.61	1.55	0.72	1.04
MCD3-15	1.98	1.61	18.79	0.37	1.79	1.78	1.77	0.61	0.47	0.9	0.81	1.18
33205	1.5	1.34	10.47	0.16	1.42	1.42	1.42	0.34	0.3	0.75	0.9	1.3
MCD12-30	3.41	0.99	71.03	2.42	2.2	1.84	1.53	2.3	0.5	0.55	0.29	0.42
33230	2.29	1.54	32.84	0.75	1.91	1.88	1.84	1.06	0.53	0.86	0.67	0.97
ETDW/15DZ #4	5.03	4.77	5.15	0.26	4.9	4.9	4.9	0.17	3.58	2.67	0.95	1.37
33235	3.55	1.07	69.92	2.48	2.31	1.95	1.64	2.26	0.57	0.6	0.3	0.44
33239	2.02	1.67	17.43	0.35	1.84	1.84	1.83	0.56	0.5	0.93	0.83	1.2
MCD4-15	1.55	1.31	15.48	0.24	1.43	1.42	1.42	0.5	0.3	0.73	0.85	1.22
33241	1.3	1.09	16.38	0.21	1.19	1.19	1.18	0.53	0.21	0.61	0.84	1.21
DW-NVT LM 5	2.41	1	58.34	1.41	1.71	1.56	1.42	1.89	0.36	0.56	0.42	0.6
33244	1.57	0.76	51.66	0.81	1.16	1.09	1.02	1.67	0.18	0.42	0.48	0.7
33254	2.04	1.58	22.65	0.46	1.81	1.79	1.78	0.73	0.48	0.88	0.77	1.12
DZDW17002	3.2	1.12	65.13	2.08	2.16	1.89	1.65	2.11	0.53	0.62	0.35	0.5
33300	1.28	1.27	1.17	0.02	1.27	1.27	1.27	0.04	0.24	0.71	0.99	1.43
ETDW/15DZ #39	2.59	1.41	45.56	1.18	2	1.91	1.83	1.47	0.54	0.79	0.54	0.79
33306	1.62	1.32	18.7	0.3	1.47	1.46	1.45	0.61	0.32	0.74	0.81	1.18
MCD4-41	2.89	1.66	42.63	1.23	2.27	2.19	2.11	1.38	0.71	0.93	0.57	0.83
33403	2.95	2.52	14.47	0.43	2.74	2.73	2.72	0.47	1.11	1.41	0.86	1.24
DZDW17004	1.34	1.3	2.99	0.04	1.32	1.32	1.32	0.1	0.26	0.73	0.97	1.4
33405	1.93	1.65	14.51	0.28	1.79	1.78	1.78	0.47	0.48	0.92	0.85	1.24
MCD13-42	2.73	1.43	47.8	1.31	2.08	1.97	1.87	1.55	0.58	0.8	0.52	0.76
34217	4.44	4.04	9.01	0.4	4.24	4.24	4.23	0.29	2.68	2.26	0.91	1.32
34219	3.35	1.3	61.19	2.05	2.33	2.09	1.87	1.98	0.65	0.73	0.39	0.56
DW-NVT LM 4	2.68	2.13	20.71	0.56	2.4	2.39	2.37	0.67	0.85	1.19	0.79	1.15
34295	3.66	1.13	69.04	2.53	2.4	2.04	1.73	2.23	0.62	0.63	0.31	0.45
MCD2-17	3.56	2.05	42.39	1.51	2.81	2.7	2.6	1.37	1.09	1.15	0.58	0.83
34310	1.03	0.88	14.4	0.15	0.95	0.95	0.95	0.47	0.13	0.49	0.86	1.24
34415	3.31	2.84	14.15	0.47	3.07	3.07	3.06	0.46	1.4	1.59	0.86	1.24

DW-NVT LM 11	2.05	2.01	1.95	0.04	2.03	2.03	2.03	0.06	0.61	1.12	0.98	1.42
34418	2.37	1.25	47.43	1.12	1.81	1.72	1.63	1.53	0.44	0.7	0.53	0.76
MCD1-32	2.18	1.81	16.97	0.37	2	1.99	1.98	0.55	0.59	1.01	0.83	1.2
34423	3.09	2.4	22.36	0.69	2.74	2.72	2.7	0.72	1.11	1.34	0.78	1.12
ETDW/15DZ #23	6.81	5.35	21.4	1.46	6.08	6.04	5.99	0.69	5.44	2.99	0.79	1.14
34451	1.37	1.02	25.47	0.35	1.2	1.18	1.17	0.82	0.21	0.57	0.75	1.08
34452	1.37	1.09	20.73	0.28	1.23	1.22	1.21	0.67	0.22	0.61	0.79	1.15
DW-PVT LM 8	1.13	1.05	7.17	0.08	1.09	1.09	1.09	0.23	0.18	0.59	0.93	1.34
34481	2.66	0.89	66.43	1.77	1.78	1.54	1.34	2.15	0.35	0.5	0.34	0.49
34484	1.25	1.08	13.68	0.17	1.16	1.16	1.16	0.44	0.2	0.6	0.86	1.25
MCD10-11	1.96	1.45	25.97	0.51	1.71	1.69	1.67	0.84	0.42	0.81	0.74	1.07
34493	5.32	4.7	11.71	0.62	5.01	5	4.99	0.38	3.73	2.63	0.88	1.28
MCD15-7	2.73	2.55	6.45	0.18	2.64	2.64	2.64	0.21	1.04	1.43	0.94	1.35
34496	1.56	1.39	10.83	0.17	1.48	1.47	1.47	0.35	0.32	0.78	0.89	1.29
ETDW/15DZ 073	1.25	1.17	6.64	0.08	1.21	1.21	1.21	0.21	0.22	0.65	0.93	1.35
34500	1.79	1.69	5.53	0.1	1.74	1.74	1.74	0.18	0.45	0.95	0.94	1.37
ETDW/15DZ 049	2.46	1.6	35	0.86	2.03	1.98	1.94	1.13	0.59	0.89	0.65	0.94
34510	2.16	1.94	10.23	0.22	2.05	2.05	2.04	0.33	0.62	1.08	0.9	1.3
MCD12-2	2.75	1.3	52.91	1.46	2.02	1.89	1.76	1.71	0.53	0.72	0.47	0.68
34516	2.67	2.27	15.17	0.41	2.47	2.46	2.45	0.49	0.9	1.27	0.85	1.23
34520	2.71	1.6	40.96	1.11	2.16	2.08	2.01	1.32	0.65	0.89	0.59	0.85
ETDW/15DZ #35	2.28	2.05	10.09	0.23	2.17	2.16	2.16	0.33	0.7	1.15	0.9	1.3
34522	4.41	3.94	10.7	0.47	4.17	4.17	4.16	0.35	2.59	2.2	0.89	1.29
MCD4-4	1.17	1.03	12.14	0.14	1.1	1.1	1.09	0.39	0.18	0.57	0.88	1.27
34545	3.58	1.41	60.67	2.17	2.49	2.25	2.02	1.96	0.75	0.79	0.39	0.57
34560	3.93	1.65	58.12	2.28	2.79	2.54	2.32	1.88	0.96	0.92	0.42	0.61
ETDW/15DZ 012	2.44	2.09	14.22	0.35	2.27	2.26	2.25	0.46	0.76	1.17	0.86	1.24
34566	1.41	0.7	50.46	0.71	1.06	0.99	0.94	1.63	0.15	0.39	0.5	0.72
34571	2.12	1.43	32.78	0.7	1.77	1.74	1.7	1.06	0.45	0.8	0.67	0.97
MCD2-29	2.15	1.71	20.33	0.44	1.93	1.92	1.91	0.66	0.55	0.96	0.8	1.15
34573	2.26	1.88	17.04	0.39	2.07	2.06	2.05	0.55	0.63	1.05	0.83	1.2

MCD3-14	4.26	2.8	34.39	1.47	3.53	3.45	3.38	1.11	1.78	1.56	0.66	0.95
34580	1.33	0.74	44.53	0.59	1.04	0.99	0.95	1.44	0.15	0.41	0.55	0.8
DW-NVT LM 6	2.7	2.41	10.74	0.29	2.56	2.55	2.55	0.35	0.97	1.35	0.89	1.29
34594	1.54	1.43	6.88	0.11	1.49	1.49	1.49	0.22	0.33	0.8	0.93	1.35
MCD3-27	2.63	1.46	44.49	1.17	2.05	1.96	1.88	1.44	0.57	0.82	0.56	0.8
34596	2.63	1.2	54.45	1.43	1.91	1.78	1.65	1.76	0.47	0.67	0.46	0.66
34600	1.96	1.51	23.01	0.45	1.73	1.72	1.71	0.74	0.44	0.84	0.77	1.11
ETDW/15DZ 014	3.32	1.23	63.04	2.09	2.27	2.02	1.79	2.04	0.61	0.69	0.37	0.53
34602	1.79	1.71	4.64	0.08	1.75	1.75	1.75	0.15	0.46	0.95	0.95	1.38
MCD14-29	1.85	1.79	3.41	0.06	1.82	1.82	1.82	0.11	0.49	1	0.97	1.4
34607	3.86	1.8	53.5	2.07	2.83	2.63	2.45	1.73	1.03	1	0.47	0.67
34611	1.89	1.19	37.14	0.7	1.54	1.5	1.46	1.2	0.33	0.66	0.63	0.91
ETDW/15DZ 043	3.61	1.22	66.23	2.39	2.41	2.1	1.82	2.14	0.66	0.68	0.34	0.49
34613	2.17	1.3	40.32	0.88	1.73	1.68	1.62	1.3	0.42	0.72	0.6	0.86
DZDW1702,04	4.3	1.27	70.49	3.03	2.78	2.34	1.96	2.28	0.81	0.71	0.3	0.43
34620	1.84	1.33	27.88	0.51	1.58	1.56	1.54	0.9	0.36	0.74	0.72	1.04
34622	2.92	2.26	22.47	0.66	2.59	2.57	2.55	0.73	0.99	1.27	0.78	1.12
34625	4.08	1.08	73.46	3	2.58	2.1	1.71	2.38	0.66	0.61	0.27	0.38
DZDW17010,8	3.84	3.61	6.07	0.23	3.72	3.72	3.72	0.2	2.07	2.02	0.94	1.36
34632	2.1	1.4	33.48	0.7	1.75	1.71	1.68	1.08	0.44	0.78	0.67	0.96
34641	3.31	2.17	34.59	1.15	2.74	2.68	2.62	1.12	1.07	1.21	0.65	0.95

Where Yp: yield in non-stress condition: Ys: yield in stress condition; RC: relative change; TOL: Tolerance Index; MP: Mean Productivity; GMP: Geometric Mean Productivity; HM: Harmonic Mean; SSI: Stress Susceptibility Index; STI: Stress Tolerance Index; YI: Yield Index; YSI: Yield Stability Index; RSI: Relative Drought Index.

Supplementary Table 7. Passport data of the landraces used for molecular characterization and their cluster grouping according to the population structure output.

Id	Name of genotype	Pop	Region	Type	Clus	Latitude	Longitude
TD1	31696	High land	Amhara	landrace	clus 2	11.63333	38.53333
TD115	Fetan	Unkn	Unkn	improved	clus 2		

TD118	DZDW17010,8	High land	Amhara	landrace	clus 2	10.51138	37.98777
TD12	Alem Tena	Unkn	Unkn	improved	clus 2		
TD14	31778	High land	Amhara	landrace	clus 2	10.25	37.83333
TD15	ETDW/15DZ 006	High land	Oromia	landrace	clus 2	9.41086	38.4936
TD19	Ude	Unkn	Unkn	improved	clus 2		
TD22	31806	High land	Amhara	landrace	clus 2	10.1424	38.0349
TD3	Kuami	Unkn	Unkn	improved	clus 2		
TD31	33239	Mid-land	Oromia	landrace	clus 2	8.86666	38.8
TD42	ETDW/15DZ_39	Mid-land	Oromia	landrace	clus 2	8.95361	37.92861
TD45	MCD4-41	Mid-land	Oromia	landrace	clus 2	7.45111	37.61
TD46	33403	High land	Oromia	landrace	clus 2	9.6675	38.66638
TD53	DW-NVT LM 4	Unkn	Unkn	improved	clus 2		
TD54	34295	Mid-land	Tigray	landrace	clus 2	14.13333	38.7
TD58	34415	Mid-land	Oromia	landrace	clus 2	9.21666	41.11666
TD59	DW-NVT LM 11	Unkn	Unkn	improved	clus 2		
TD62	34423	Mid-land	Amhara	landrace	clus 2	11.51666	39.61666
TD64	ETDW/15DZ_23	Mid-land	Oromia	landrace	clus 2	8.95361	37.92861
TD7	31755	High land	Oromia	landrace	clus 2	9	41.88333
TD72	34493	Mid-land	Oromia	landrace	clus 2	9.83055	38.25361
TD78	ETDW/15DZ 049	High land	Oromia	landrace	clus 2	9.46803	38.5973
TD86	34522	High land	Amhara	landrace	clus 2	10.28458	38.1052
TD91	ETDW/15DZ 012	High land	Oromia	landrace	clus 2	9.32416	39.29916
TD94	MCD2-29	Mid-land	Oromia	landrace	clus 2	8.89583	38.78916
TD96	MCD3-14	Mid-land	Oromia	landrace	clus 2	8.89583	38.78916
TD101	MCD3-27	Mid-land	Oromia	landrace	clus 3	7.45111	37.61
TD102	34596	Mid-land	Tigray	landrace	clus 3	14.13694	38.135
TD117	34625	Mid-land	Oromia	landrace	clus 3	8.81666	39
TD120	34641	Mid-land	Amhara	landrace	clus 3	10.56666	37.48333

TD65	34451	Mid-land	Oromia	landrace	clus 3	8.81666	38.9
TD69	34481	Mid-land	Oromia	landrace	clus 3	8.45	37.96666
TD10	31759	High land	Oromia	landrace	clus1	9.28333	39.25
TD100	34594	Mid-land	Amhara	landrace	clus1	10.74916	39.80833
TD103	34600	Mid-land	Oromia	landrace	clus1	9.78333	39.26666
TD104	ETDW/15DZ 014	High land	Oromia	landrace	clus1	9.32416	39.29916
TD105	34602	Mid-land	Amhara	landrace	clus1	12.63333	37.46666
TD107	MCD14-29	Mid-land	Oromia	landrace	clus1	7.45111	37.61
TD108	34607	High land	Amhara	landrace	clus1	1317	37.4648
TD11	ETDW/15DZ 057	High land	Oromia	landrace	clus1	10.18888	39.46666
TD110	34611	High land	Amhara	landrace	clus1	1317	37.4648
TD111	ETDW/15DZ 043	High land	Oromia	landrace	clus1	10.13859	39.5406
TD112	34613	Mid-land	Oromia	landrace	clus1	8.83333	38.36666
TD113	DZDW1702,04	High land	Amhara	landrace	clus1	9.98972	39361
TD119	34632	Mid-land	Tigray	landrace	clus1	14.13333	38.7
TD13	31761	High land	Oromia	landrace	clus1	7.96666	39.16666
TD16	31789	High land	Amhara	landrace	clus1	10.89777	38.07194
TD17	ETDW/15DZ 038	High land	Amhara	landrace	clus1	9.69638	39.51
TD18	31794	High land	Amhara	landrace	clus1	10.2515	38.1731
TD20	31797	High land	Amhara	landrace	clus1	10.5026	38.2112
TD21	MCD15-49	Mid-land	Oromia	landrace	clus1	7.45111	37.61
TD23	31831	High land	Oromia	landrace	clus1	9.21666	39.40444
TD24	MCD3-15	Mid-land	Oromia	landrace	clus1	7.45111	37.61
TD25	33205	Mid-land	Amhara	landrace	clus1	11.52722	36.90111
TD26	MCD12-30	Mid-land	Oromia	landrace	clus1	7.45111	37.61
TD28	33230	Mid-land	Oromia	landrace	clus1	8.83333	39.31666
TD29	ETDW/15DZ_4	Mid-land	Oromia	landrace	clus1	8.95361	37.92861
TD30	33235	High land	Oromia	landrace	clus1	9.78333	38.76666

TD32	MCD4-15	Mid-land	Oromia	landrace	clus1	7.45111	37.61
TD33	33241	Mid-land	Oromia	landrace	clus1	8.85	39.25
TD34	DW-NVT LM 5	Unkn	Unkn	improved	clus1		
TD36	33244	High land	Oromia	landrace	clus1	8.9	3.9
TD37	33254	High land	Oromia	landrace	clus1	7.6	36.71666
TD39	DZDW17002	High land	Amhara	landrace	clus1	9.98972	39361
TD40	33293	Mid-land	Oromia	landrace	clus1	8.85	39.25
TD41	33300	High land	Oromia	landrace	clus1	7.33333	39.75
TD48	33405	High land	Oromia	landrace	clus1	10.31444	39.22083
TD50	MCD13-42	Mid-land	Oromia	landrace	clus1	7.45111	37.61
TD51	34217	Mid-land	Amhara	landrace	clus1	10.56666	37.48333
TD52	34219	Mid-land	Oromia	landrace	clus1	8.75	38.98333
TD55	MCD2-17	High land	Oromia	landrace	clus1	9.16583	38.37
TD56	34310	Mid-land	Tigray	landrace	clus1	14.11666	38.71666
TD6	ETDW/15DZ 003	High land	Oromia	landrace	clus1	9.3275	39.25472
TD60	34418	Mid-land	Oromia	landrace	clus1	9.25	41.11666
TD61	MCD1-32	High land	Oromia	landrace	clus1	9.16583	38.37
TD66	34452	Mid-land	Oromia	landrace	clus1	9.43333	41.78333
TD67	DW-PVT LM 8	Unkn	Unkn	improved	clus1		
TD70	34484	Mid-land	Oromia	landrace	clus1	8.45	37.96666
TD71	MCD10-11	Mid-land	Oromia	landrace	clus1	9.16583	38.37
TD74	MCD15-7	Mid-land	Oromia	landrace	clus1	9.16583	38.37
TD75	34496	Mid-land	Oromia	landrace	clus1	8.83333	38.43333
TD76	ETDW/15DZ 073	High land	Amhara	landrace	clus1	10.34916	39.25388
TD77	34500	Mid-land	Oromia	landrace	clus1	8.66666	39.1
TD80	34510	Mid-land	Amhara	landrace	clus1	10.48333	38.33333
TD81	MCD12-2	Mid-land	Oromia	landrace	clus1	8.89583	38.78916
TD82	34516	High land	Amhara	landrace	clus1	7.73333	38.48333

TD84	34520	High land	Amhara	landrace	clus1	10.5048	38.1722
TD85	ETDW/15DZ_35	Mid-land	Oromia	landrace	clus1	8.95361	37.92861
TD87	MCD4-4	Mid-land	Oromia	landrace	clus1	8.89583	38.78916
TD88	34545	Mid-land	Amhara	landrace	clus1	10.17222	38.12722
TD89	34560	High land	Amhara	landrace	clus1	10.13253	38.0801
TD9	ETDW/15DZ 010	High land	Oromia	landrace	clus1	9.55465	38.1931
TD93	34571	High land	Amhara	landrace	clus1	11.56666	39.16666
TD95	34573	Mid-land	Amhara	landrace	clus1	10.48333	38.33333