EVALUATION OF HEAT AND DROUGHT STRESS TOLERANCE OF DIVERSE TROPICAL SORGHUM GERMPLASM

By

Elton Ndlovu

Submitted in fulfilment of the requirements of the degree of Doctor of Philosophy (Botany)

Research Centre for Plant Growth and Development
School of Life Sciences
University of KwaZulu-Natal
Pietermaritzburg Campus

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Evaluation of heat and drought stress tolerance of diverse tropical sorghum germplasm

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Student's Full Name: ELTON NDLOVU

Student Number: 220112118

Thesis title: Evaluation of heat and drought stress tolerance of diverse tropical sorghum germplasm

Regular consultation took place between the student and ourselves throughout the investigation. We advised the student to the best of our abilities and approved the final document for submission to the College of Agriculture, Engineering and Science (CAES), Higher Degrees Office for examination by the University appointed Examiners.

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PROFESSOR JOHANNES VAN STADEN

CO-SUPERVISOR: __[redacted]___

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Publication 1:

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ABA	Abscisic acid
ABC	ATP binding cassette
AEZ	Agro-ecological zones
AMMI	Additive Main Effects and Multiplicative Interaction
ANOVA	Analysis of Variance
APX	Ascorbate peroxidase
ATP	Adenosine triphosphate
CAT	Catalase
CMS	Cellular membrane stability
CTD	Canopy temperature depression
CVt	Coefficient of Variation of germination time
DNA	Deoxyribonucleic acid
DS	Drought stress
ERD	Early response to dehydration
FG	Final germination
GB	Glycine betadine
GGE	Genotype main effects by genotype × interaction
GEI	Genotype × Environment interaction
GI	Germination index
GLAA	Green leaf area at anthesis
GLAD	Green leaf area duration
HIV	Human immunodeficiency virus

HS	Heat stress
HSP	Heat shock protein
HSR	Heat shock regulation
ICRISAT	International Crop for Research Institute for the Semi-arid tropics
IPCA	Interactive principal component analysis
LEA	Late embryogenesis abundant proteins
LSD	Least significant differences
MGT	Mean germination time
NAR	Net assimilation rate
NPGRC	National Plant Genetic Resources Centre
PCA	Principal Component Analysis
PEG	Polyethylene glycol
QTL	Quantitative Trait Loci
ROS	Reactive oxygen species
RUBISCO	Ribulose bisphosphate carboxylase/oxygenase
RWC	Relative water content
SAT	Semi-arid tropics
SLA	Specific leaf area
SLW	Specific leaf weight
SSA	sub-Saharan Africa
SPS	Sucrose-phosphate-synthase
WUE	Water use efficiency

ABSTRACT

Heat and drought stresses are a global concern affecting crop productivity due to climate change and variability induced weather vagaries at all phases of crop growth. Sorghum is a critically essential, resilient and diverse crop that displays genotypic variations in its ability to withstand such harsh conditions limiting germination, crop stand and ultimately leading to yield losses. However, it is not spared from undesirable effects of heat and drought stresses occurring separately or in combination impacting on different stages of its growth and development. The vast genetic resources in gene banks have not been evaluated for the aforementioned stresses with a view to deploy them against climate change.

Thus, in the current study, 48 uncharacterized sorghum genotypes and two check varieties, bringing the total to 50 genotypes, were assessed under drought, heat and combined stresses at germination, seedling and pre-flowering stages. The previously 48 uncharacterized sorghum genotypes of African origin were selected from a pool of 300 obtained from the ICRISAT and National Gene Bank of Zimbabwe, multiplied and characterized through preliminary studies at Lupane State University experimental plots in Zimbabwe. These genotypes were selected on the basis of yield potential and desirable agro-morphological characteristics which made them ideal candidate varieties for adoption by farmers. A series of laboratory and pot experiments were then carried out to assess the effects of heat and drought stresses on morphological and physiological traits at various stages of growth. Data on seedling germination, morphological, physiological and yield performance were collected and tested and evaluate the performance of the selected genotypes under heat and drought stresses subjected using various multivariate statistical tests.

Germination tests revealed that combined stress suppressed germination percentage and delayed onset of germination. Germination index, a good indicator of both the extent and speed of germination, significantly differed under the stress treatments, genotypes and their interactions. Furthermore, highly significant differences ($p<0.001$) were observed between heat treatments, genotypes and their interactions for both basal and acquired thermotolerance assays, signifying adverse effects of heat stress and existence of genetic diversity in thermotolerance of the assessed genotypes. Popular varieties “Macia” and “SV4” did not feature among the top resilient performers indicating the risk of subsistence farmers relying on them for resistance to heat stress.

At two seedling stages, genotype, stress and their interactions significantly affected root and shoot length at both early (7-day-old) and late (21-day-old) stages. These findings indicated that the combined stresses significantly suppressed shoot length more than root length at both stages. The F-test revealed significant main effects of stress, genotype and their interaction on some of the morpho-physiological traits assessed at pre-flowering and physiological maturity stages in two consecutive seasons of 2020/21 and 2022/23. The combined heat and drought stresses negatively affected all the traits except stem basal diameter and showed no significant effect on relative leaf water content. Noteworthy were yield reductions of 7.2 and 5% observed in the 2021/22 and 2022/23 seasons, respectively, attributed to combined stresses. Significant effect of the interaction between genotype and stress was observed in canopy temperature depression, stem basal diameter, panicle length and yield in the two seasons. Principal component analysis revealed that plant stem height, panicle length, canopy temperature depression and grain weight were contributors of most variation in assessed genotypes under stress. This indicates that breeding efforts can focus on these traits to enhance sorghum stress tolerance. Canopy temperature depression was found to have a weak significant positive correlation ($r^2 = 0.2$) with leaf area, panicle length positively correlated ($r^2 = 0.12$) to grain weight and canopy temperature negatively correlated ($r^2 = -0.12$) to stem basal diameter in Pearson's correlation analysis. The path coefficient analysis showed that panicle length and relative leaf water content had the highest significant direct effects (0.1) on grain weight, while stem height had an indirect effect through panicle length indicating that selection for these traits will lead to directly or indirectly enhanced yield under combined stresses.

Germination tests under stress grouped genotypes into six distinct clusters based on their performance. Genotypes NPGRC1593, NPGRC1782, NPGRC1476, and IS224426 performed exceptionally well under all the stresses and outperformed check varieties. While at early emergence stage two genotypes were consistently amongst the top ten performers in terms of basal thermotolerance in the two sets, these are genotypes NPGRC1704 and IS24426. Genotypes NPGRC3093 and IS24272 consistently demonstrated superiority in acquired thermotolerance. Genotypes NPGRC1704, IS9567, NPGRC1197, NPGRC1868 and NPGRC1782 exhibited potential in both basal and acquired thermotolerance. Genotypes NPGRC1478 and IS30164 showed resistance to drought stress applied separately and combined stresses for both shoot and root length at both early and late seedling stages. Genotypes NPGRC1478 and IS30164 exhibited dynamic stability and specific adaptability to heat stress and combined stresses at both seedling stages. In yield performance, genotypes IS24426, IS12391 and NPGRC3093, were identified as the most stable and resilient in terms of grain weight. The genotypes were grouped into seven distinct clusters using the hierarchical clustering technique with one cluster with four genotypes identified for dual purposes (grain and forage) characterized by high plant stem height, leaf area and grain weight.

To improve crop establishment and increase agricultural yields, breeding and crop improvement programs should focus on genotypes that can withstand both stresses. The identified sorghum genotypes can be used as potential donors towards resilience to combined stresses at various ontogenetic phases that were investigated and are recommended for utilization in hot and dry agro-ecologies of sub-Saharan Africa due to their potential vigor in germination, early establishment and pre-flowering stages. The study formed the basis for breeding for pre-anthesis combined heat and drought stresses in sorghum using proxies such as panicle length. Further studies on multi-environment trials and stability analysis are recommended using the most consistent genotypes in assessed traits in this study. Amongst these were genotypes IS24426, NPGRC1782, IS24272 and IS12391 for informed evaluation and deployment in target environments.

CHAPTER 1: GENERAL INTRODUCTION

1.1. Background of the study

Cereal crops such as maize and wheat remain the most preferred and widely grown in sub-Saharan African (SSA) regions, although farmers in marginal areas have no capacity to produce enough to satisfy existing food security demands **(STEWART and LAL, 2018)**. Crop production in marginal areas is mostly dependent on rainfed cropping systems **(HADEBE et al., 2017)**. In light of the inevitably increasing impacts of climate change and variability in SSA, increased adoption of hardy small grain crops such as sorghum [*Sorghum bicolor* (L.) Moench] is very crucial **(CHADALAVADA et al., 2021b)**. Sorghum is indigenous to Africa, highly nutritious and widely consumed as grain, porridge, bread and alcoholic beverages in many parts of Africa and Asia **(DERA, 2017)**. Other uses include fodder, fuel and industrial uses in many parts of the world **(TONAPI et al., 2021)**. Though more well adapted than maize and wheat to drier and hot conditions that dominate SSA, productivity of sorghum is still critically constrained by abiotic and biotic stresses **(HADEBE et al., 2017; SHANKER et al., 2011)**.

Zimbabwe, like most countries in southern Africa, is experiencing recurrent droughts and heat waves varying from a few days to weeks. Marginal areas such as Lupane, which is in agro-ecological zone IV (450-650 mm of rainfall annually) in Matabeleland North Province of Zimbabwe, are characterized by several episodes of extreme temperatures and drought **(PHIRI et al., 2020)**. Drought is a period of low rainfall below normal and insufficient availability of soil moisture **(BOYER, 2019)**. Drought results in undesirable morpho-physiological changes in plants known as drought stress (DS) **(FAHAD et al., 2017)**. The two distinct types of DS to which sorghum is sensitive to are pre- and post-flowering **(WHEELER et al., 2015)**. Heat stress (HS) is the rise in soil and air temperatures above normal for a period long enough to cause irreversible damage to plant physiology, growth and development **(LAMAOUI et al., 2018**). Specifically, for sorghum, the optimum air temperature range is 26-34 ℃ during the vegetative stage **(KUMAR et al., 2009)** and 25-28 ℃ at reproductive stages **(PRASAD and STAGGENBORG, 2008)**. Annual rainfall of (450-650 mm) is ideal for most medium- to late-maturing varieties **(ASSEFA et al., 2010)**.

In most hot and dry regions, HS and DS tend to coincide intermittently during the production season for rainfed cereal crops such as sorghum **(SINHA et al., 2021; ZANDALINAS et al., 2023)**. Climate predictions made over a decade ago pointed out that global mean surface temperature increases of 1.4 to 4 ℃ in the 21st century are no longer just projections but a reality **(IPCC, 2014)**. Henceforth, in marginal areas of very erratic rainfall and extremely high temperatures, instability in yield for crops that are even said to be resilient, such as sorghum, is likely to prevail for a long time **(HADEBE et al., 2017; BEGNA et al., 2022)**.

Combined heat and drought stresses disrupt most of the morphological, physiological and biochemical aspects related to plant growth and reproduction starting from germination to maturity **(LIU et al., 2023)**. Excessively high temperatures and water deficits in the soil retard and may even hinder germination and crop establishment **(PATANÈ et al., 2013; AGUILAR-BENÍTEZ et al., 2014)**. Generally, moisture contents below 20-30% field capacity and temperatures above 35 ℃ have been reported to be severe and detrimental to growth and reproduction of crops **(CHADALAVADA et al., 2021b)**. At the cellular level, HS and DS induce oxidative stress that results in protein denaturation, membrane injury and reduced thermostability, which are identified as early symptoms that can be assessed through electrolyte leakage from organs such as the leaves **(GAVUZZI et al., 1997; HOWARTH et al., 1997; GONG et al., 2001)**. Throughout the life cycle of a crop, the two synergistic aforementioned stresses severely affect physiological processes such as cell division, photosynthesis, respiration, water relations and biosynthesis of essential metabolites that eventually affect the growth and yield of sorghum **(NADEEM et al., 2018; NDLOVU et al., 2021)**. Reduced pollen viability, inhibition of root and shoot growth consequently reduced plant biomass and grain yield have been noted in sorghum grown under heat and drought stresses **(OSMOLOVSKAYA et al., 2018; CHADALAVADA et al., 2021a; KHAN et al., 2021)**. The reproductive stages have been widely reported to be very sensitive to combined drought and heat stresses in the lifecycle of sorghum **(TACK et al., 2017; SINHA et al., 2021; BHEEMANAHALLI et al., 2022)**. Sorghum yield losses amounting to 10% were attributed to combined heat and drought stresses by **LOBELL et al. (2015)**.

Drought stress can only be avoided through sufficient irrigation, which is a constraint on the greater population of resource-poor smallholder farmers in marginal areas of SSA. Therefore, under rainfed systems, hardy crops such as sorghum depend on a range of dynamic morphological and physiological adaptation traits to resist, escape or avoid the effects of DS **(GOUFO et al., 2017)**. Mechanisms that enable the plant to sustain physiological and metabolic activities under DS are termed 'drought tolerance'**(PRASAD and STAGGENBORG, 2008; MUTAVA et al., 2012).** Thus, a complex combination of mechanisms enables the crop to survive and produce under DS, rendering it drought resilient. Heat stress cannot be avoided in cropping systems, however, crops have their own inherent and/or acquired tolerance **(SELVARAJ et al., 2011a; AHMAD et al., 2021)**. The inherent ability of a crop to grow and attain potential yield under HS due to evolutionary adaptation is termed 'basal tolerance' **(YEH et al., 2012; BOKSZCZANIN and FRAGKOSTEFANAKIS, 2013)**. The ability to survive potentially lethal extreme temperatures through prior exposure to mild HS is termed 'acquired thermotolerance' **(SELVARAJ et al., 2011b; SONG et al., 2012)**. Prior exposure of the plant to sub-lethal high temperatures or gradually increasing temperatures for a short period of time enables the plant to acquire thermotolerance **(BOKSZCZANIN and FRAGKOSTEFANAKIS, 2013)**. Acquired thermotolerance is associated with the synthesis of HSPs that play a critical role as molecular chaperones assisting in the refolding of proteins under stress **(CHOINSKI, 1999; SETIMELA et al., 2007; DASSOW et al., 2014; KHAN et al., 2021)**. Crops that confer tolerance to combined stresses are likely to be more adapted to semi-arid tropics (SATs).

Many studies acknowledge that any form of tolerance is species- and stage-of-growth-dependent **(MIOT et al., 2011; YADAV et al., 2014; ANDR, 2020)**. At crop emergence, genotypes that can maintain seedling vigour and viability, even if exposed to HS, can be implicated to have a higher thermotolerance ability **(NAHAR et al., 2016; SARI and JUNIARTI, 2023)**. Some sorghum genotypes exhibit such tolerance to harsh environmental conditions because they possess various distinct leaf and root traits that are associated with HS and DS tolerance mechanisms **(PRASAD and STAGGENBORG, 2008; KLUPÁCS et al., 2009; SAHA et al., 2018; AHMAD et al., 2021)**. These are the most sought after attributes by plant breeders for the development of varieties that can withstand HS and DS in marginal areas of SSA **(YAHAYA et al., 2023)**. Such attributes can be ascertained at various critical stages of crop growth using throughput laboratory and field techniques that explore morphological, physiological and biochemical related traits that indicate tolerance.

The first line of screening, especially large numbers of genotypes for thermotolerance, is through rapid laboratory techniques that evaluate the capacity of seedlings to survive *in vitro* induced stresses like heat and drought. Field screening at various stages of crop growth involves setting up trials under normal and stressed conditions for drought and temperature. Various vegetative growth and yield parameters can be used to derive stress tolerance indices employed in screening for tolerant crop types or genotypes **(FISCHER and MAURER, 1978)**. The effectiveness and precision of the techniques and indicators used is dependent on the environment, stage of crop growth, crop type, genotype, intensity and duration of stress. Much research has focused on HS and DS tolerance in selected genotypes of commonly grown cereal crops, such as wheat, barley, rice and maize. However, despite the inherent drought resilience of sorghum, the increasing climate change and variability related abiotic stresses means that the crop will be grown under more challenging conditions. Most agronomic experiments on the afore-mentioned crops have quantified the response to HS and DS independently in only a limited population of genotypes. Sorghum is well adapted and has a very low risk of failure, therefore, research on crops with high energy and nutrition needs to be promoted. The results of research of this nature may increase adoption and resilience in marginal communities, especially for communities prone to malnutrition and HIV prevalence, dominated by the elderly and children **(AMADOU et al., 2013; GEBREMICHAEL et al., 2018)**. In this study, the independent and combined effects of HS and DS were evaluated on diverse sorghum germplasms using various laboratory, field techniques and statistical tools.

1.2. Statement of the problem

Despite the potential demand for sorghum in hot and dry areas due to its high adaptation capacity, the crop has been continuously neglected by mainstream research. Much focus is still on high-value grain crops such as wheat, barley and maize, which poor-resource farmers have no capacity to produce at a large scale due to their high production costs, especially in marginal areas neglecting hardy small grain crops such as sorghum and millets **(DERA, 2017; HADEBE et al., 2017)**. The increasing incidences of heat and drought stresses in the region pose a serious challenge in crop production and productivity.

Understanding the complex interaction of factors that determine crop tolerance to abiotic stress is limited. In most instances, other critical factors, such as the stage of crop growth, functional traits, crop type, genotype and effects of combinatory stresses like HS and DS, have been avoided due to the complex nature of the experimental designs and statistical analysis required. For instance, HS and DS induction requires specialized growth chambers or temperature regulating facilities and rain exclusion shelters, which are costly unless the method of altering planting dates to coincide the HS and DS period of the season with desired stages is used. As such, vast research material in gene banks worldwide has not been evaluated especially for combined stresses. Heat and drought stresses usually coincide but their effects are usually investigated independently under sub-optimal environmental conditions, hence results remain inconclusive.

For a long time, mainstream research and crop improvement has focused on crop yield rather than on tolerance to abiotic stresses such as heat, drought and their combination **(VILE et al., 2012).** The interaction of cellular and functional traits tends to be genotype specific. According to **KUPRA et al. (2017)** root traits at seedling stage have received less attention in screening for drought tolerance in sorghum. Most research studies employ either limited laboratory or field screening techniques or statistical data analytic techniques in the evaluation of HS and DS tolerance. However, each technique has its own limitations in terms of robustness and precision to explain the complex relationships. All aforementioned factors contribute to limited empirical evidence that can promote adoption of research findings and the improvement of sorghum. Consequently, food security remains threatened in sorghum dependent communities dominated by resource-poor farmers with limited capacity to adapt to climate change and variability.

1.3. Justification of the study

The ever-increasing food demand, predicted temperature increases and drought frequency recurrences, due to climate change and variability, necessitates the need for holistic research studies to elucidate HS and DS tolerance in neglected dryland crops such as sorghum. Although, in many studies, the effects of these two abiotic stresses have been studied independently it is most certain that when combined their effects on crop growth and yield are more detrimental, hence the importance of studying them together **(QASEEM et al., 2019; HANDAYANI and WATANABE, 2020)**. After all, in most cases, the two coincide or lead to one another, especially in semi-arid areas. In SATs, drought occurs intermittently from the beginning to the end of the cropping seasons, hence, its effects begin with crop establishment, which necessitates studies from germination and seedling stages. At the seedling stage, studies involving roots are more feasible, as it is easy to uproot, clean and take measurements. Physiological studies at cellular to morphological level provide better insights and a holistic approach for understanding biological systems that affect crop productivity. Such evaluation or characterization studies are also important for germplasm recommendation when breeding for stresses. Although precise laboratory, field screening techniques, yield and tolerance indices have been identified, their applicability is dependent on genotype and stage of crop growth and hence cannot be generalized. Since sorghum has a wide diversity and is grown in a range of environments, for different uses, such variation in responses needs to be explored.

The use of various laboratory and field screening techniques and multivariate statistical tools will allow the simultaneous identification of similarities between genotypes according to their morpho-physiological traits and response to abiotic stress. In this way, it may be possible to recognize different sets of variables associated with biological systems such as thermotolerance. This study used path coefficient analysis, which enables partitioning and quantification of interrelated independent variables of different components and their direct and indirect effects on grain yield as a response to stress tolerance. Such knowledge will play a pivotal role in enhancing crop breeding initiatives to improve heat and moisture stress tolerance and in the management of cropping systems. Furthermore, the study made use of low-cost methods of simultaneously inducing HS and DS in the field by altering planting dates to coincide HS and DS periods of the season with the stages of interest. Although the method is dependent on weather patterns that are not static, it mimics natural environmental conditions, hence, the results will be more relevant and applicable to farming conditions. Thus, this research is anticipating to identify sorghum genotypes that are potentially tolerant to heat, drought and combined stresses at different stages of its ontogenesis.

1.4. Aims, objectives and hypotheses

This study aimed at investigating the effects of heat, drought and combined stress on various morpho-physiological traits at different growth and developmental stages of diverse African sorghum genotypes with a view to inform screening for HS and DS tolerance for future crop improvement studies and deployment in marginal areas.

The specific objectives of the study were to:

- To assess the response of 50 diverse sorghum germplasms to *in vitro* induced drought, heat and combined stresses at germination;
- To evaluate *in vitro* basal and acquired thermotolerance in seedlings of 50 selected high potential diverse sorghum germplasms;
- To assess shoot and root length growth of 50 selected high potential tropical sorghum genotypes under drought, heat and combined heat and drought stresses at early and late seedling stages; and
- To determine the morpho-physiological effects of combined heat and drought stresses induced on 50 diverse tropical sorghum germplasms 10 days before booting at pre-flowering stage.

The following hypotheses were based on the objectives:

- Synergistic effects of HS and DS effects are more detrimental than when each acts independently at germination stage regardless of the sorghum genotype;
- Basal and acquired thermotolerance is heritable and varies with sorghum genetic background and germplasm source;
- Apical meristems of sorghum seedlings are differentially affected by drought, heat stress and their combination regardless of the genotype; and
- Interaction of HS and DS has greater detrimental effects on morpho-physiological traits of sorghum at pre-flowering stage regardless of the genotype.

1.5. Thesis outline

This thesis consists of seven Chapters, the background of the study, research problem, significance of the study and objectives (Chapter 1), and a detailed literature review (Chapter 2). Chapters 3, 4, 5 and 6 represent each of the four specific objectives of the study, and the summary of the findings of this study, and general conclusions are contained in Chapter 7. The citation and reference style of this study is in line with the South African Journal of Botany guidelines.

CHAPTER 2: LITERATURE REVIEW

2.1. Effects of heat and drought stresses on phenology and physiology of sorghum

Sorghum passes through three stages of development which are seedling development, panicle initiation and reproduction **(ANANDA et al., 2011; HARIPRASANNA and PATIL, 2015)**. Depending on the genotype, climatic and edaphic factors during the growing season, the length of time needed for the plant to go through each stage tends to vary **(TIRFESSA et al., 2020)**. Compared to grain sorghum planted later in the season, the crop planted early in the season when temperatures are still cool will go through the stages more slowly **(ROOZEBOOM and PRASAD, 2019)**. Sorghum tolerates alkaline salts as compared to other grain crops, therefore it can be grown on soils with a pH range of 5.5 and 8.5 successfully in soils with a clay content of 10-30% **(DAI et al., 2014)**. Abiotic stresses particularly heat and drought stresses, affect the various morphological and physiological processes of sorghum during growth and reproduction which will be discussed in subsequent sections.

2.1.1. Germination and seedling emergence

Under *in vitro* conditions protrusion of 2 mm of the radicle from the seed is considered germination **(KAUR et al., 2015)**. In field conditions hypocotyl emergence occurs in sorghum, which means a shoot pops out of the seed and pushes through the soil's surface within three to 10 days after planting **(CRAUFURD et al., 1996)**. Sorghum and other tropical crops need high temperatures for good germination and growth. Eighty percent of germination occurs within 10-12 days at a minimum temperature of 15 °C or higher at a depth of 10 cm. Germination requires a temperature range of 16-18 °C in soil moisture that is close to field capacity, sowing depth of less than 5 cm and a seedling vigour percentage of at least 90% **(ROOZEBOOM and PRASAD, 2019)**. The seed supplies the seedlings with food stores and nutrients and from this the hypocotyl, or immature shoot, extends and absorbs water **(AMZALLAG et al., 1997)**.

Germination begins with imbibition followed by the biochemical stage, active cell division, elongation and differentiation. During the imbibition and biochemical stages, water plays a critical role in the activation of hydrolytic enzymes (α-amylase, cellulase, endoxylanases, lipase and arabinofuranosidases) that unlock the food reserves in the endosperm for a constant supply of energy for cell division during germination **(WANG et al., 2012; CHENG et al., 2016)**. The enzymes function best within restricted temperature margins. Thus, water deficiency and excessive soil temperatures tend to slow down or inhibit the process of initiation of germination and seedling establishment which is also referred to as enforced dormancy **(KUMAR et al., 2022)**.

Imbibition at high temperatures was observed to accentuate the negative effects of HS on germinating seeds, a clear indication that if thermal and DS coincide during germination, their combined negative effects are far reaching **(YADAV et al., 2016)**. Active cell division and elongation necessary for seedling emergence cannot be initiated under such stressed conditions. According to **CRAUFURD and PEACOCK (1993)** soil temperatures above 45 ℃ hinder germination and epicotyl emergence in sorghum. Reduced plant stem height was observed in seedlings of related cereals, maize and pearl millet (*Pennisetum glaucum*) grown under drought- and heat-stressed conditions **(ÁLVAREZ-IGLESIAS et al., 2017; TANDZI et al., 2018; QUEIROZ et al., 2019)**. This explains the importance of seed vigor tests as a measure for thermotolerance in sorghum **(NYONI et al., 2020)**.

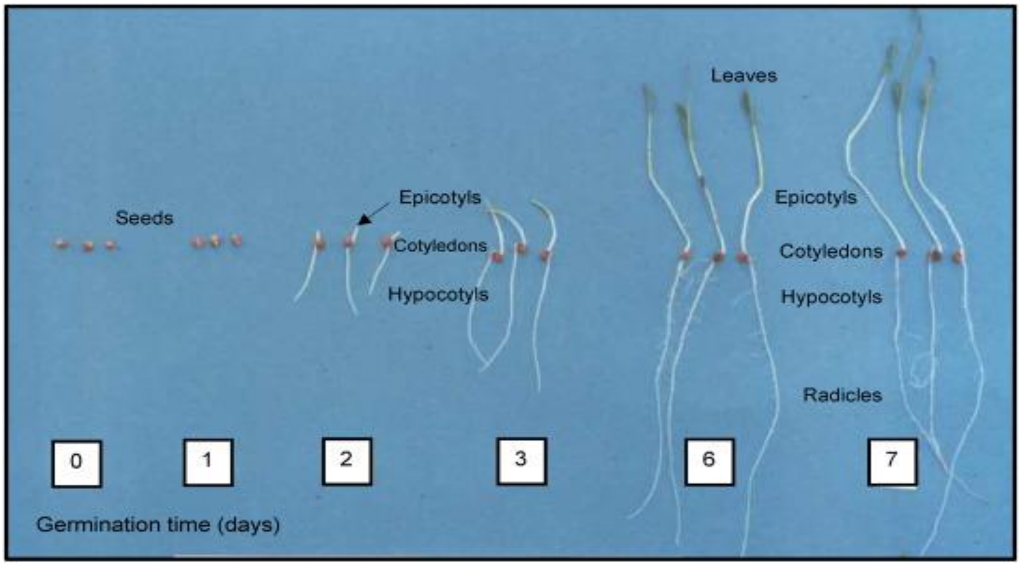

Figure 1. Germination and emergence stages of Sorghum (Sorghum bicolor L.) (Source: **PAIVA et al., 2022**)

2.1.2. Vegetative growth and development

Vegetative growth is the primary stage of plant development. This is the period when maximum leaf expansion, tillering, stem elongation and increase in girth occurs, which later aids in the development and growth of grains **(KONG et al., 2014)**. The phase continues up to the booting stage which is the initiation of the panicle **(FLORES, 2011)**. During this phase of growth, the shaping of the reproductive organs i.e. the panicle occurs and the maximum number of seeds per panicle is established **(FAROOQ, 2016)**. The most important climatic factors that affect crop growth and reproduction at this stage are temperature, day length and precipitation **(HATFIELD and PRUEGER, 2015; KRUPA et al., 2017)**. The length of the first growth stage mostly depends on air temperature and the number of leaves and is very sensitive to day length **(LOHANI et al., 2020)**. In general, early maturing genotypes bear 15 leaves per plant, but medium and late maturity genotypes have 17-19 leaves, respectively **(GERIK et al., 2003)**. Temperature significantly affects growth and development after germination. The temperature range between 27-30 ℃ is good for growth and development of sorghum **(MUNDIA et al., 2019)**. It is well adapted to semi-arid regions with a minimum annual precipitation of 350-400 mm **(KLUPÁCS et al., 2009; ASSEFA et al., 2010)**. At early vegetative stage termed ‘growth stage I’, the plant can withstand drought, hail, and cold temperatures with little impact on grain output **(BIBI et al., 2012)**. Warm weather of 20-30 °C, and a frost-free period of approximately 120-140 days is generally ideal for sorghum **(MASHAO and PRINSLOO, 1994)**.

During vegetative growth, stem elongation, an increase in stem girth, leaf number, leaf area and biomass all depend on active cell division, cell enlargement and differentiation **(FAHAD et al., 2017)**. Water plays a pivotal role in the maintenance of cell turgidity and translocation of assimilates to the actively growing meristematic parts of the plant **(TAKELE and FARRANT, 2013)**. Optimum temperatures allow production, partitioning of assimilates, respiration and transpiration, which all support growth and development in plants **(CRAWFORD et al., 2012; ZHAO et al., 2013)**. Thus, heat and drought stresses result in loss of turgidity in cells and a reduced supply of assimilates due to a slow net photosynthetic rate, which hinders mitosis and cell enlargement, consequently retarding plant growth **(EMENDACK et al., 2018; KAPOOR et al., 2020; ZUFFO et al., 2022)**. Reduced plant height, stem girth, leaf area, leaf number and biomass have been linked to heat and drought stresses in most cereals, including sorghum **(GRAY and**

BRADY, 2016; AHMADI et al., 2018a; RAD et al., 2023). PRASAD and STAGGENBORG (2008) noted that cell enlargement is more sensitive to combined HS and DS than cell division, hence stimulating leaf appearance and suppressing leaf expansion.

Thus, heat and drought stresses that occur at the pre-flowering stage subject sorghum to stunted growth, susceptibility to lodging, pests, diseases such as stalk rot and premature death **(ASSEFA et al., 2010; ZANDALINAS et al., 2021; OGUZ et al., 2022)**. Optimum temperature for panicle initiation ranges from 25-28 ℃ and sorghum is most sensitive even to slight temperature changes at this stage especially at 10 days before flowering **(PRASAD and STAGGENBORG, 2014)**. Adequate water supply is needed at this stage **(MONGI et al., 2010; NAIM et al., 2017)**. During this time, plants are vulnerable to DS including temperature extremes, nutritional deficiencies, or excesses, all of which can lead to fewer seeds being produced **(TARI et al., 2013; CAMARGO and AUGHAN, 2021; ZANDALINAS et al., 2021)**. Since grain yield is mostly dependent on the number of seeds per plant, it is regarded as the most crucial time for grain development **(JABERELDAR et al., 2017; NAIM et al., 2017)**. During this time, the rate of water absorption by the plant significantly increases since the leaf area is at its peak, intercepting the most light at this stage **(PRASAD and STAGGENBORG, 2014; OSMOLOVSKAYA et al., 2018)**. The head is almost formed but is still protected by the flag leaf sheath **(ROOZEBOOM and PRASAD, 2019) (Figure 1)**. Elongation of the peduncle has started, which will fully cause the head to protrude from the flag leaf sheath **(ALAM et al., 2014)**. The size of the head is determined hence stressful conditions, such as DS or herbicide damage, may prevent the head from fully emerging from the flag sheath at this point **(CHADALAVADA et al., 2021a)**. Under conditions of extremely high temperatures and acute moisture stress, death of the head can happen before booting **(COHEN et al., 2021)**.

Figure 2. Sorghum at booting stage, which is a pre-flowering stage, marking the end of the vegetative stage phase (**Source: Ndlovu Elton, 2023**).

2.1.3. Effects of drought and heat stress on Photosynthesis

Crops that are more adapted to SAT conditions, such as sorghum **(HADEBE et al., 2017; JABERELDAR et al., 2017; ABREHA et al., 2022; BEGNA, 2022)**, are known for maintaining photosynthetic capacity for an extended period under such conditions. However, depending on the stress type and severity, they eventually succumb to the effects **(COSTA et al., 2021)**. Under moderate HS, the decline in photosynthetic capacity is attributed to the inactivation of enzyme activity in the chloroplasts **(PRASAD et al., 2021; DOS SANTOS et al., 2022)**. While under severe HS, the electron harvesting capacity aided by the cyclic electron chain of Photosystem II is reduced or halted by structural and physiological changes of the thylakoid membranes **(HASANUZZAMAN et al., 2013; WANG et al., 2016; NADEEM et al., 2018)**. Severe HS also reduces subunits of the RUBISCO binding proteins due to denaturing effects **(GURURANI et al., 2015; FELLER, 2016; OGDEN et al., 2020)**. Day/night temperatures of over 40/30 ℃ result in lipid peroxidation and breakdown of chloroplast and thylakoid membranes in sorghum **(NADEEM et al., 2018; SARSHAD et al., 2021; COTRINA CABELLO et al., 2023)**. Available evidence is, however, inconclusive on which is the most sensitive photosystem to HS.

Moderate DS conditions reduce photosynthetic capacity through reduced intercellular carbon dioxide levels due to stomatal closure that inhibits carboxylation activities **(FAROOQ et al., 2009)**. Deactivation of RUBISCO and an enzyme active in source-sink loading, sucrose-phosphate-synthase (SPS), has also been reported in severely drought-stressed crops **(JAIN et al., 2010; COTRINA CABELLO et al., 2023)**. Increased solute concentrations in the stroma due to water limitations result in deactivation of enzyme activity **(FAHAD et al., 2017)**. Following arguments by various researchers on the most detrimental type of stress to photosynthesis, their assertion is sound that moderate water stress takes precedence in reducing the rate of photosynthesis over HS **(FLEXAS et al., 2004)**. Although this position may still be contested, one emerging fact is that the combined effects of heat and drought stresses impact photosynthesis more severely than their independent effects **(LAWAS et al., 2018; COHEN et al., 2021)**. A combination of limited soil moisture and excessive temperatures leads to high evapotranspiration, resulting in disturbed photochemistry and eventually in a reduction photosynthetic capacity **(KUMAR et al., 2017; HUSSAIN et al., 2019)**.

The reduced turgidity of cells and their organelles exposes the photosynthetic apparatus to physical damage. Functional and structural changes in the photosynthetic apparatus and enzyme deactivation under HS and DS arrest photosynthetic capacity **(GETNET et al., 2015)**. Thus, photosynthesis is undoubtedly one of the most important physiological factors severely affected by the combined occurrence of HS and DS, consequently leading to reduced growth, productive capacity and yield in sorghum **(QASEEM et al., 2019)**. The combined negative effects of HS and DS are manifested in all the components of photosynthesis, including the light harvesting photosystems and photosynthetic pigments, the cyclic and non-cyclic electron transport systems, and carboxylation pathways, resulting in reduced photosynthetic capacity.

2.1.4. Plant nutrient and water relations

Plant water relations are crucial physiological aspects that cannot be ignored when reviewing the effects of heat and drought stresses on cropping. They integrate the relative water content (RWC) of various plant organs, leaf water potential, stomatal conductance, transpiration rate, root water uptake, canopy temperature and metabolite status of plants **(ZHANG et al., 2019a)**. Cellular water content, especially in leaves and roots, helps in the maintenance of the anatomic structure and metabolic activities of plants **(TAKELE and FARRANT, 2013**). Some water is lost from leaves through stomatal openings during transpiration. The rate of which depends on leaf function and atmospheric conditions, chiefly atmospheric temperatures. Thus, the water status of plants is of major concern under heat and drought stresses. They usually coincide in areas that dominate sorghum production in SSA **(CHATURVEDI et al., 2021; ZANDALINAS et al., 2023)**.

Unavailability of soil water lowers soil water potential (ψ_w - ability of water to leave a system) **(MACHADO and PAULSEN, 2001; BIBI et al., 2012)**, below that of plant roots, which limits their water uptake and decreases water content or water potential in plant tissues. Similarly, excessive atmospheric temperatures result in increased evapotranspiration due to a steep vapour pressure deficit **(ZHAO et al., 2013)** between plant leaves and ambient air, which would normally increase root water uptake and the transpiration stream for replenishment if soil water is not limiting, otherwise, the water content or water potential of plant tissues is lowered. The combined effects of HS and DS likely plunge plant tissues to their lowest water potential or water content and turgor pressure or potential (ψ_p).

Drought stress leads to variations in efficiency in the uptake of various mineral elements by different crop species. Mineral uptake is generally compromised under water-limiting conditions **(FAHAD et al., 2017)**. The effect of HS on mineral uptake has not been widely studied. It is speculated that root conductance tends to be reduced under HS **(BIBI et al., 2012)**, as to date its effects on root growth inhibition have not been established **(HASANUZZAMAN et al., 2013)**. Leaf water content has also been reported to be related to increased canopy temperature because warm canopies are a result of the absence of the transpiration cooling after stomatal closure that is triggered by low root and leaf water potential **(POUR-ABOUGHADAREH et al., 2019; ZHANG et al., 2019a)**. Canopy temperature as a tool for determining leaf water status shall be further discussed as a drought tolerance mechanism in subsequent sections.

2.1.5. Oxidative stress and membrane stability

Oxidative stress is a widely reported physiological response of various crops, including sorghum, to both heat and drought stresses **(SCARPECI et al., 2008; HASANUZZAMAN et al., 2013; AZZOUZ-OLDEN et al., 2020)**. It is characterized by excess generation of reducing agents and accumulation of dangerous ROS **(LOHANI et al., 2020; TRIPATHI et al., 2020)**. Excess electrons from light-harvesting photosystems I and II are a result of stomatal closure under heat and drought stresses. This leads to reduced intercellular carbon dioxide and consequently uncoupling of enzymes and various metabolic processes associated with carboxylation **(OSMOLOVSKAYA et al., 2018; AHMAD et al., 2021)**.

Photo reduction of oxygen produces a common family member of ROS, superoxide radical ($O_2^{\bullet-}$) through the Mehler reactions in Photosystem I of the chloroplast, electron transfer chains of the mitochondria through photorespiration processes, photorespiration of the glyoxizomal and oxidation processes in the plasma membrane **(FELLER, 2016; GUO et al., 2018; NADEEM et al., 2018)**. Superoxide is a precursor of a more stable ROS called hydrogen peroxide (H_2O_2) formed through reduction processes of the electron transport chains of the chloroplast and mitochondria, plasma membrane, apoplastic and peroxisomal with the aid of the superoxide dismutase enzyme **(NXELE et al., 2017; TRIPATHI et al., 2020)**. Reactions of hydrogen peroxide with superoxide in the Haber-Weiss reactions and with Fe^{2+} in the Fenton reactions form hydroxyl radicals ($OH^{\bullet}$), while photo inhibition and electron transfer chains of photosystem II in the chloroplast generate singlet oxygen **(FAROOQ et al., 2009; HASANUZZAMAN et al., 2013)**. Reportedly, hydrogen peroxide flux detected in leaf tissues of sorghum exposed to 40 ℃ day and 30 ℃ night temperatures is an indication of oxidative stress during HS **(MOHAMMED and TARPLEY, 2009)**.

ROS react with biomolecules such as lipids, proteins, DNA and pigments. Membrane instability is a severe indication of oxidative stress that results in disruptions of the protein and lipid components of cell membranes through peroxidation under heat and drought stresses **(AMALERO et al., 2003; KESKIN et al., 2010; SINHA et al., 2021)**. Quantification of electrolyte leakage in plant tissues exposed to HS, such as leaf discs, is a common method of detecting membrane thermostability when screening for thermotolerance **(IRFAN and ALI, 2018; UPADHYAY, 2019; AHMAD et al., 2021)**.

2.1.6. Reproduction and yield formation

The reproductive stage in crops involves the development of reproductive organs through cell differentiation, growth through cell division and enlargement followed by fertilization. The last growth stage of sorghum begins with flowering through grain filling and dough stages and proceeds until physiological maturity. After the head protrudes through the sheath of the flag leaf, as the peduncle lengthens quickly, the plant begins flowering **(GERIK et al., 2003)**. Half-bloom is defined as the time when 50% of the heads in the field have flowered **(PRASAD et al., 2021b)**. The genotype and environment especially temperatures, photoperiod, availability of water and nutrients influence the time taken to 50% flowering **(GALICIA-JU et al., 2021; BHEEMANAHALLI et al., 2022)**. The average period for modern hybrids is 55-80 days, or 50-70% of the time between planting and physiological maturity **(HARIPRASANNA and PATIL, 2015)**. Most sorghum genotypes are self-pollinated **(MORRIS et al., 2013)**. It takes 4-5 days for the entire head to flower and flowering starts at the top and moves downward **(GERIK et al., 2003)**. Temperature has a significant impact on the duration of flowering **(LOHANI et al., 2020)**. Pollen-blasting and inadequate head-fill can occur during flowering as a result of extreme moisture stress and/or extremely high temperatures **(PRASAD et al., 2015; COHEN et al., 2021; SMITH et al., 2023)**.

About 15-25 days after flowering, when 50% of the seed weight has accumulated and little to no fluid is visible when the seed is crushed is termed the ‘soft dough stage’ **(ASSEFA et al., 2010; HARIPRASANNA and PATIL, 2015)**. Seed is formed over a period of 25-45 days through development phases of milk, soft dough, hard dough, and physiological maturity **(FLORES, 2011)**. The length of this period will significantly affect grain yield because dry matter accumulation is identical among genotypes, with the longer maturing genotypes out-producing the quicker maturing lines **(CARCEDO and GAMBIN, 2019; DE SOUZA et al., 2021)**. At hard dough stage an approximation of 75% of the grain dry weight is accumulated. The majority of nutrients have been absorbed and lower leaves may be lost during this stage **(PRASAD and STAGGENBORG, 2014)**.

The effects of heat, moisture and combined stresses on reproduction and yield formation in most crops are dependent on genotype, time of occurrence and intensity of stress, among other factors **(ABDEL-GHANY et al., 2020)**. Booting and flowering stages in sorghum are reportedly very sensitive to heat and drought stresses **(FLORES, 2011; YADAV et al., 2016; CHADALAVADA et al., 2021b)**. The onset of these stages is delayed by these stresses **(CAMARGO and AUGHAN, 2021; DE SOUZA et al., 2021)**, while the combination of the two during the pre-flowering stage results in damage, abortion of the florets, reduced panicle size, and a significant reduction in seed size, all of which result in decreased yield potential **(JABERELDAR et al., 2017; DE SOUZA et al., 2021; HAVRLENTOVÁ et al., 2021)**. Specifically, temperatures above 32 ℃ adversely affect the reproductive stages in sorghum **(PRASAD et al., 2008)**, while temperatures exceeding 44 ℃ halt the processes. Pollination and fertilization are adversely affected as well through reduced pollen viability and receptive ability of the stigma at high temperatures **(BARNABÁS et al., 2008; PAUPIÈRE et al., 2014; LOHANI et al., 2020; LI and HOWELL, 2021)**. However, there is great genetic variability in the genotypic responses to temperature **(DE SOUZA et al., 2021)**.

During the reproductive stages, temperatures exceeding the optimum by 1-2 ℃ result in grain yield reduction in cereals **(EMENDACK, 2014; BIJU et al., 2018a; RIVERO et al., 2022)**. Temperatures above 32 ℃ during the day and 22 ℃ at night cause significant yield losses **(PRASAD et al., 2008)**. Fewer grain yield losses were reported when drought was experienced at early vegetative stages of sorghum **(OGUZ et al., 2022)**. After evaluating the effects of moisture stress induced throughout the season on several sorghum genotypes, **WENZEL (1999)** concluded that moisture stress reduced biomass more than grain yield. Empirical evidence on which of the two stresses in question has a greater impact on yield formation seems to be inconclusive, but certainly, their combined effect has a far-reaching impact.

The formation of yield components is adversely affected under combined heat and drought stresses, which results in significant yield losses. A reduced number of florets leading to a reduced number of grains and loss of grain size has been reported in sorghum crops exposed to both stresses before and/or during flowering **(FAHAD et al., 2017; LOHANI et al., 2020; CHADALAVADA et al., 2021a)**. Tillering in sorghum increases yield per unit area, thus, the reported negative effects of heat and drought stresses on tillering of the crop certainly reduce its yield potential **(BORRELL et al., 2014; CAMARGO and AUGHAN, 2021)**. Yield formation involves assimilation and partitioning of dry matter to the sink components. Thus, excessive temperatures and limited water supply to the photosynthetic apparatus negatively impact the photosynthetic capacity and partitioning of assimilates **(KUMAR et al., 2017; AZZOUZ-OLDEN et al., 2020; COSTA et al., 2021)**.

Enzyme activity is one of the delicate aspects related to yield formation that is negatively affected. Decreased activity of enzymes involved in grain filling, namely, sucrose synthase, starch synthase and adenosine diphosphate glucose pyrophosphorlase, results in serious grain yield losses **(QAZI et al., 2014; NARAYANAN, 2018)**. The reduced net assimilation rate (NAR) equates to reduced sucrose levels in the photosynthetic apparatus, hence reducing the movement of assimilates from the source to the sink **(POUR-ABOUGHADAREH et al., 2019)**. Average grain yield losses in sorghum of up to 10% has been attributed to the combined effects of heat and drought stresses **(MOHAMMED and TARPLEY, 2009)**. At maturity a black spot emerges where the seed connects to the plant and the grain is said to be physiologically mature **(STEDUTO et al., 2012; GETNET et al., 2015)**. At this time, the seed has reached its full maturity and will not receive any more nutrients or moisture from the plant and moisture level is usually approximately 30% at this time **(STEDUTO et al., 2012)**.

2.2. Morpho-physiological resistance mechanisms of sorghum to drought stress

Episodes of heat and drought stresses are unpredictable in terms of timing, frequency and duration. Thus, hardy crops such as sorghum are recommended in regions prone to such environmental stresses since they are able to survive harsh conditions **(MAVHURA et al., 2015; HADEBE et al., 2017; JABERELDAR et al., 2017)**. The crop is able to maintain cellular water balance and physiological and metabolic reactions to reach reproductive phases and produce reasonable yields under DS by employing various mechanisms of drought resistance **(MAITI and SINGH, 2019; AHMAD et al., 2021)**. Both heat and drought resistance mechanisms are commonly divided into three distinct categories: escape, avoidance and tolerance **(PARK and ANGELES, 1992; ABDEL-GHANY et al., 2020; FUKAI AND MITCHELL, 2022; OGUZ et al., 2022)**. In some recent schools of thought, resistance mechanisms are classified into two categories: drought avoidance and tolerance **(BIJU et al., 2018a; LAMAOUI et al., 2018; NADEEM et al., 2018)**. Resistance mechanisms can be temporal or long-lasting depending on the crop genotype and stage of growth, intensity and duration of stress **(CARCEDO and GAMBIN, 2019; HAVRLENTOVÁ et al., 2021)**. In mild stress, drought avoidance mechanisms that are aimed at achieving stable cellular and intercellular water status through matching water uptake to water loss are sufficient for the survival of plants **(STODDARD et al., 2006; MOSHELION, 2020)**. Under worsening stress conditions, plants develop drought tolerance mechanisms that help sustain metabolic reactions and physiological processes that contribute to yield formation even under stressed conditions **(BEYENE et al., 2015; GOBU et al., 2017; KAPOOR et al., 2020)**. Prolonged and more intense DS may warrant phenological and physiological adjustments in the life cycle of plants to avoid stress coinciding with critical growth stages through drought escape mechanisms **(LAMAOUI et al., 2018; SHAAR-MOSHE et al., 2019)**. Under extreme drought, some genotypes are able to adopt a quiescent adaptive mechanism called ‘drought recovery’, where crop plants are able to resume growth and grain yield after exposure to a severe drought **(GOSAVI et al., 2014; FERRADINI et al., 2015; SAHA et al., 2018)**. All three common mechanisms can be physiological, morphological or phenological **(KRUPA et al., 2017)**. Drought escape mechanisms are mainly phenological and physiological, avoidance mechanisms involve both morphological and physiological adjustments **(GOUFO et al., 2017)**, and tolerance is characterized by physiological and a few morphological adjustments **(MOSHELION, 2020; BALBAA et al., 2022)**.

2.2.1. Drought escape mechanisms

Crop plants naturally adjust the timing of events in their life cycle to evade abiotic stress coinciding with critical stages such as flowering and grain filling **(TACK et al., 2017; COSTA et al., 2021)**. Early flowering and increased early vigor are the most salient phenological drought escape mechanisms that allow shortened growing seasons, thereby enabling the crop to reach yield formation and grain filling stages well before episodes of limited soil water and excessive atmospheric temperatures to counteract significant drought-induced yield reductions **(ABDEL-GHANY et al., 2020)**. This kind of phenological plasticity applies to grain sorghum genotypes that are photoperiod insensitive and have less complex genetic control on the timing of flowering **(YANG et al., 2014; SHAAR-MOSHE et al., 2019)**, unlike forage and sweet sorghum **(SARSHAD et al., 2021)**. Three known alleles of the maturity Ma_3 gene known as ma_3^R codes for early flowering, insensitivity to photoperiod, rapid elongation and less tillering in sorghum **(YANG et al., 2014; PERRIER et al., 2017)**. Sorghum is also known for its flowering at night and early in the morning to escape the effects of DS during the day **(LOHANI et al., 2020; PRASAD et al., 2021b)**. It is noteworthy that breeding for early flowering can only be successful as a drought escape mechanism in agro-ecological zones (AEZ), where the timing of drought occurrence has been certified to be predictable and consistent over many years, which of late is becoming more unlikely in many regions due to increasing climate variability.

2.2.2. Drought avoidance mechanisms

Morphological and physiological adjustments enabling the plant to maintain a high water content by reducing water loss from the shoot and/or increasing water uptake by the roots are discussed in this section.

2.2.2.1. Osmotic adjustment

This is an energy-driven physiological mechanism that enables plants to accumulate more cellular compatible solutes in response to low cellular water potential induced by DS, thus conserving moisture within their cells **(GETNET et al., 2015; OSMOLOVSKAYA et al., 2018)**. In many references, it is classified as a drought tolerance mechanism instead of a commonly pronounced avoidance mechanism **(BLUM, 2017)**, probably due to its overlapping roles and interaction with several mechanisms in DS adaptation. Drought-tolerant sorghum genotypes and varieties have been reported to maintain high RWC even under DS **(TARI et al., 2013)**. Increased cellular solute concentration (reduced osmotic potential) maintains cellular water potential below that of its surroundings, thus keeping water within cells and maintaining high turgor pressure **(HAVRLENTOVÁ et al., 2021; OGUZ et al., 2022)** to avoid the effects of DS. A decrease in water potential can be passive when water exits plant cells freely to their surroundings under soil water deficit, therefore, osmotic adjustment is a counteractive mechanism **(PRASAD et al., 2021c)**. Osmotic adjustment has proven to be an effective and beneficial physiological drought avoidance strategy, particularly during post-flowering DS, as it delays or even inhibits some drastic drought avoidance mechanisms, such as stomatal closure and leaf rolling, which are detrimental to photosynthetic capacity **(NADEEM et al., 2018)** The capacity of osmotic adjustment in a plant can be determined by measuring the change in the amount of compatible solutes in its cells without any change in the amount of water, which is termed 'osmotic potential' (ψ_s) at 100% RWC **(VERSLUES et al., 2006**; **BLUM, 2017)**. Thus, high RWC in drought-stressed plants is used as drought tolerance screening **(GETNET et al., 2015)**. Osmotic adjustment is reportedly strongly related to a deep root system in sorghum **KRUPA et al., 2017)**, thus providing moisture for critical stages such as at grain filling to achieve high water use efficiency and photosynthetic capacity for enhanced crop productivity under drought-stressed conditions, especially in rainfed farming systems. A positive correlation between osmotic adjustment and grain yield has been noted in sorghum **(KHANNA-CHOPRA and SINGH, 2011; MAITI and SINGH, 2019)**. Existing empirical evidence on tolerant sorghum genotypes indicates increased osmotic adjustment capacity during post-anthesis to attain grain yield advantage over their susceptible counterparts **(DEVNARAIN et al., 2016; WAGAW, 2019)**. High biomass accumulation in sorghum is said to be sustained by osmotic adjustment during DS and thus transpiration efficiency, hence, in addition to grain-type sorghum, some fodder types seem to be adapted to DS in some way **(VADEZ et al., 2014)**. However, **GOUFO et al. (2017)** argued that high osmotic adjustment capacity may slow growth due to energy investment in the maintenance of osmotic potential at the expense of cell division that encourages leaf expansion and stem elongation.

In sorghum, a wide array of compatible metabolites of low molecular weight and high solubility accumulate in leaves and roots during osmotic adjustment, which include the main effectors free proline and proteins **(DEVNARAIN et al., 2016; OGBAGA et al., 2016a; ZANDALINAS et al., 2016)**, inorganic ions **(GETNET et al., 2015)**, glycine betaine **(ZULFIQAR et al., 2020)** and soluble sugars **(COELHO et al., 2018)**. The accumulation of various soluble sugars (glucose, fructose and lactose), complex sugars (cellobiose, sedoheptulose, arabinose, sorbose) and sugar alcohols (ribitol, myo-inositol and xylitol) in drought-tolerant sorghum varieties under drought-stressed conditions indicates their role as osmoprotectants **(OGBAGA et al., 2016b)**. More solutes are stored in the stem, which maintains a higher RWC than leaves even under DS **(GETNET et al., 2015)**. Their accumulation is induced by abscisic acid to maintain low water potential, osmotic potential, and higher turgor pressure but does not interfere with metabolic activities **(ZANDALINAS et al., 2016)**. **LAMAOUI et al. (2018)** reviewed that unlike proline and sugars, glycine betadine (GB) plays more of a protective role than an osmoticum. Since all metabolic reactions are genetically controlled, it follows that there is some genetic arm behind osmotic adjustment, though it is not yet fully understood **(BOKSZCZANIN and FRAGKOSTEFANAKIS, 2013)**.

2.2.2.2. Stomatal regulation

Stomatal closure is an immediate but temporal DS avoidance mechanism in most plants triggered by a combination of severe HS and DS to avoid further loss of water **(VERSLUES et al., 2006)**. Hydropassive and hydroactive are the mechanisms reported to be responsible for stomatal regulation. Excessive loss of water through evaporation from open stomatal pores leads to loss of osmotic pressure and flaccidity in leaf cells, in particular, guard cells lose turgor pressure and hence collapse. Closing the openings provides a hydropassive mechanism **(PIRASTEH-ANOSHEH et al., 2016)**. Reportedly, stomatal closure is more responsive to low root water status than leaf water levels **(BEYENE et al., 2015)**. Low soil water potential triggers the synthesis of a

DS hormone by root and leaf cells, its transportation into guard cells by the ATP binding cassette (ABC) found in plasma membranes and the activation of signaling pathways then result in stomatal closure, providing a hydroactive mechanism **(XIONG et al., 2019)**. The ability of plants to maintain closed stomata for a longer period to avoid further loss of water under low leaf water potential is termed 'stomatal resistance' **(AL-SALMAN et al., 2023; WANG et al., 2023)**, while the reciprocal response is termed 'stomatal conductance' **(CAL et al., 2019; KHANTHAVONG et al., 2022; WANG et al., 2023)**. However, stomatal sensitivity in sorghum varies with stages of growth and genotypes, indicating genotypic differences in drought tolerance **(STARK et al., 2019)**. During pre-flowering drought (water deficit occurring at the vegetative stage), when the demand for leaf expansion and stem elongation is at its peak, tolerant genotypes exhibit high stomatal resistance to avoid DS **(DEVAIAH et al., 2011)**. At the post-flowering stage, tolerant genotypes classified as non-senescent **KRUPA et al., 2017)** are able to maintain open stomata mainly for CO_2 uptake and limited transpiration under very low soil and leaf water potential and vapour pressure deficit due to their high osmotic adjustment capabilities **(AL-SALMAN et al., 2023)**, thus increasing stomatal conductance. **ASSEFA et al. (2010)** noted stomatal closure occurring at low leaf water potentials between -14 and -15 bars in sorghum genotypes they studied. Thus, in the semi-arid tropics, under rainfed cropping systems, low but reasonable yields in sorghum are still obtained with minimum water used by the crop **(ABBATTE et al., 2004)**. This explains the high water use efficiency of the crop under drought-stressed conditions compared to most other cereals **(ALLEN et al., 2011; GETNET et al., 2015; KHANTHAVONG et al., 2022)**. Water use efficiency (WUE), as defined by **VADEZ et al. (2014**) and **HADEBE et al. (2017)**, is the total above ground yield output produced by crops per unit evapotranspiration. This also equates to its efficiency in water uptake by roots and reduced loss in leaves **(PERRIER et al., 2017)**, which makes it a good candidate for regions prone to HS and DS.

Genetic and physiological associations of stomatal conductance and deep root systems have also been established in sorghum genotypes **(TSUJI et al., 2005; LOPEZ et al., 2017)**. This is another trait that delays stomatal closure or allows quick recovery from DS, as water is drawn from deeper soil zones to raise leaf turgor. Increased stomatal resistance, to avoid further water loss, is a trade-off between the loss of transpiration cooling effects, nutrient uptake, CO_2 uptake and photosynthesis **(VERSLUES et al., 2006)**. Stomatal conductance has been successfully used as an indicator or measurement of plant water status and a reliable screening tool for drought tolerance in sorghum **(STODDARD et al., 2006)**.

2.2.2.3. Root architecture

Sorghum is widely viewed as a drought-resistant crop due to the morphological plasticity of its roots **(MAGALHÃES et al., 2016; LIANG et al., 2017; ADAMS et al., 2020; PRASAD et al., 2021c)**. There is a consensus in several research findings on the strong correlation between drought avoidance and root traits that enables tapping of water from deeper soil zones to maintain high water content in sorghum grown under severe drought stressed conditions **(TSUJI et al., 2005; BLUM, 2017; KHALIFA and ELTAHIR, 2023)**. Roots play a critical role in water uptake from the soil; as such, they are the first organs in plants to detect any stress related to soil moisture. Thus, water uptake is affected by the anatomy of roots in addition to other soil factors, such as the availability of water and solute concentrations **(BIBI et al., 2012)**.

Several root traits that have been reported to successfully enhance the avoidance of dehydration in sorghum including an increased number of roots, length, volume, dry weight and root length density (RLD) **(BEYENE et al., 2015; WAGAW, 2019)** to provide a vigorous root system **(RAUF et al., 2016; KRUPA et al., 2017; WASAYA et al., 2018)**. Inhibition of brace rooting, root branching, nodal root emergence by steeper brace and crown root angles has been noted in drought-tolerant sorghum genotypes **(TSUJI et al., 2005; LIANG et al., 2017; LOPEZ et al., 2017)**. The high root density reported by several researchers is in agreement with the high root: shoot ratio **(BIBI et al., 2012; BASHA, 2017; ABREHA et al., 2022)**. As noted by **LAMAOUI et al. (2018)**, all the aforementioned traits encourage increased rates of water extraction from the soil by roots from deeper root zones under water deficit conditions. **HADEBE et al. (2017)**, indicating that pre-flowering DS suppresses several desirable root traits and the success of this mechanism. This was supported earlier by **BORRELL et al. (2014)** that maximum root growth in senescent sorghum genotypes is attained at the flowering stage. In view of these facts, it can safely be concluded that the morphological plasticity of roots is a post-flowering drought avoidance mechanism **(AHMADI et al., 2018b)**. Similar root traits have been noted in rice **(FORSTER et al., 2005; WASAYA et al., 2018)**, maize **(ÁLVAREZ-IGLESIAS et al., 2017)** and wheat **(AHMADI et al., 2018b)**, although to varying degrees.

Genotypic and growth stage variations have been noted in terms of root characteristics as a drought avoidance mechanism in sorghum **(BIBI et al., 2012)**. Although drought resistance is reportedly controlled by so many genes **(ABDEL-GHANY et al., 2020)**, the identification and mapping of genes related to root characteristics is overwhelming. Root characteristics are reportedly controlled by both dominant, additive and linked genetic effects **(ARYA et al., 2014)**. Eight (quantitative trait loci) QTLs for root volume and fresh and dry weight, four for root angles and three for root weight, have been mapped in sorghum **(LOPEZ et al., 2017)**. **CHADALAVADA et al. (2021b)** further reported linkages in the genetic expression of root angle, stay green and grain yield in sorghum.

2.2.2.4. Cuticular biosynthesis

Leaves are the main organs through which water exits plants, thus, major anatomical modifications associated with reducing water loss and maintaining high levels of water content occur in leaves. Increased cuticular biosynthesis during soil water deficit has been noted in tolerant varieties of sorghum **(MUTAVA et al., 2012)**. Thick waxy cuticles on leaves physically reduce water loss and increase the reflection of direct radiation on leaves. Glossy leaves at the seedling stage in drought-tolerant genotypes of sorghum were mentioned by **WAGAW (2019),** who further described the protective waxy covering of cuticles that gives the sheath covering the head at the booting stage a dull-white cast as glaucous. **TAKELE and FARRANT (2013)** reported enhanced water use efficiency in genotypes with thick waxy cuticles on leaves.

2.2.2.5. Leaf size

Long narrow leaves with decreased leaf area reduce direct radiation, thereby reducing transpiration **(HARIPRASANNA and PATIL, 2015)**. Genotypes that have thick leaves with small surface areas tend to have a higher chlorophyll density, which enhances their photosynthetic capacity and minimizes the rate of transpiration to achieve high water use efficiency under DS **(WRIGHT et al., 2005)**. Thus, a relatively high specific leaf weight (SLW) and low specific leaf area (SLA) have been used as indicators of morphological drought tolerance **(ZHANG et al., 2019a)**.

2.2.2.6. Leaf rolling

Leaf rolling is a common morphological drought avoidance mechanism in many cereal crops **(GOBU et al., 2017; AL-SALMAN et al., 2023; WANG et al., 2023)**. It is often mistakenly classified as a physiological mechanism (**BEYENE et al., 2015)**, due to its close association with osmotic adjustment, but it serves as a morphological mechanism, as indicated in many reviews and research articles **(RAUF et al., 2016)**. Leaf blades or lamina transversely roll up along the mid-rib in response to a severe decline in leaf water potential to reduce transpiration **(GOUFO et al., 2017)**. More often, it starts with the flag leaf **(DEERY et al., 2019)**. A strong correlation between leaf rolling and low water potential has been established **(WHEELER et al., 2015; BARET et al., 2018)**. Direct irradiance on the upper leaf surface is reduced, thereby curbing transpiration. Furthermore, a humid microclimate is created inside rolled leaves, which reduces the humidity deviation between the opened stomata and atmospheric air around the leaves, thus further reducing the exit of water from stomata on the upper surface of leaves **(WAGAW, 2019)**. However, **HECKATHORN and DELUCIA (1991)** argued that stomatal closure accounts for a 70-80% decrease in the transpiration rate, while leaf rolling contributes only 2%, rendering the contribution of the latter very small in reducing transpiration when compared to stomatal closure. Nevertheless, the argument on the extent, of its positive role as a drought avoidance mechanism cannot be totally dismissed.

The extent of leaf rolling, also termed the ‘degree of curvature’, is related to osmotic adjustment in leaves or the level of osmotic stress **(BEYENE et al., 2015)**. The increased ability of a plant to adjust osmotically will reduce leaf rolling to improve photosynthesis **(BEYENE et al., 2015)**. In addition to DS, it is reportedly dependent on several leaf-related factors, such as morphology, age and position **(GAVUZZI et al., 1997)**. **O’TOOLE and CRUZ (1980)** further hypothesized that the extent is also due to differences in stomatal resistance of upper and lower leaf surfaces. The genetic control of the trait is not well documented and understood, although some authors have classified some genotypes of crops, such as sorghum, rice and wheat, into non-leaf rollers, leaf rollers and high leaf-rollers, implying the existence of genetic variations. This trait has become popular as a quick method of screening cereals for drought tolerance due to its low cost and ease, although visual assessment is subjective.

2.2.3. Drought tolerance mechanisms

2.2.3.1. Stay green

Leaf senescence is a maturity stage in the life cycle of plants where leaves undergo chlorosis as a result of various factors, such as ageing and biotic and abiotic stresses. In senescent sorghum genotypes, post-flowering DS results in premature leaf senescence and abortion and consequently reduces grain yield **(BEGNA et al., 2022)**. Only genotypes that possess an integrated drought adaptation trait known as stay green, that delays leaf senescence, are able to tolerate post-flowering DS **(THOMAS and OUGHAM, 2014)**. There are two major types of stay green traits: cosmetics, which show delayed leaf senescence without any known physiological merit, and functional traits, which integrate delayed leaf senescence and photosynthetic capacity during grain filling **(THOMAS and HOWARTH, 2000)**. The latter with two major components are delayed onset of leaf senescence and the reduced rate of senescence, which can occur together or independently **(THOMAS and OUGHAM, 2014)**. Over the years, the functional stay green character has gained much interest in the improvement of crops such as sorghum, which are grown in drought-prone regions **(KRUPA et al., 2017)**, triggering vast genetic exploration of the trait. Making use of chlorophyll quantification using both quantitative and qualitative techniques as part of drought tolerance screening in cereal crops over the years **(BURKE et al., 2010)**. Several major stay green QTLs have been identified over the years and are associated with several desirable characteristics for drought adaptation that include the following: reduced tillering, green leaf area at anthesis (GLAA), stem lodging, increased carbohydrate content in stems, reduced stomatal density, long vertical and lateral rooting systems **(HARRIS et al., 2007; BORRELL et al., 2014; DE LUCHE et al., 2015; TRACHSEL et al., 2016)**. All the aforementioned physiological mechanisms are aimed towards reduced water expenditure during anthesis, thus saving water for an extended critical grain filling phase to achieve increased grain yield associated with the stay green character even under post-flowering DS **(BURKE et al., 2015)**. The success of staying green as a drought tolerance strategy is anchored to prolonged photosynthesis activity through a delayed transition from carbon assimilation to nitrogen remobilization to achieve high water use efficiency **(THOMAS and OUGHAM, 2014)**. Mechanisms such as channeling late season moisture to the production of photosynthates for grain filling instead of biomass have been highlighted by

HARRIS et al. (2007). An apparent yield advantage has been reported even in stay green genotypes grown under non-water limiting environments **(KAMAL et al., 2019)**. Furthermore, protection of the photosynthetic apparatus from ROS is enhanced by the reported high cytokinin levels associated with delayed senescence **(ABREHA et al., 2022)**. Stay green expression is affected by flowering time, sink strength and the environment **(HARRIS et al., 2007)**. According to **VAN OOSTEROM et al. (1996)** the time of onset of senescence, rate of senescence and green leaf area duration (GLAD) are independently inherited in sorghum genotypes. Expression of senescence rate has been observed to be dominant or GLAD partially dominant. The onset has been found to be additive **(KRUPA et al., 2017)**.

2.2.3.2. Canopy temperature

Leaf water status is one of the key indicators of the physiological status of a crop plant under abiotic stress, such as DS **(ZHANG et al., 2019a)**. Most of the traits that indicate DS tolerance in plants mostly affect the water relations of roots and/or leaves **(ZHANG et al., 2019b).** Canopy temperature is one of the traits that has been used over many years to determine leaf water content/status, especially under abiotic stresses such as heat, drought, waterlogging and salinity **(FISCHER et al., 1998; PURUSHOTHAMAN and KRISHNAMURTHY, 2014; BIJU et al., 2018b)**. Much research work related to canopy temperature has been done on cereal crops, legumes, orchard trees, vegetables and forests, covering aspects of diurnal variations **(MUTAVA et al., 2012; IVANOVA et al., 2016; MESEKA et al., 2018; FUKAI and MITCHELL, 2022).** Effects of environmental factors **(BALOTA et al., 2007; ZHANG et al., 2019a)**, crop water management such as irrigation scheduling, relationships with morphological and physiological traits **(BAZZAZ et al., 2015; KUMAR et al., 2017; AL-GHZAWI et al., 2018)**, sampling time, techniques and instrumentation **(GARNIER et al., 2001; PURUSHOTHAMAN and KRISHNAMURTHY, 2014; DEERY et al., 2019)**. Further explorations on the use of the techniques established, that if canopy temperature is to be used as an effective indicator of leaf water status, ambient temperature should also be considered since it affects canopy temperature **(FAHAD et al., 2017; SRIVASTAVA et al., 2017)**. Apparently, canopy temperature has been found to be very sensitive to changes in irradiance, hence, measurements should be performed within a short space of time to avoid large errors **(BALOTA et al., 2007)**. This led to the advent of canopy temperature deficit (CTD) which is a deviation between ambient and canopy temperature **(LEPEKHOV, 2022)**. The use of CTD is now a common rapid technique in the determination of plant/leaf water status **(BIJU et al., 2018b)**. More importantly, it is a screening tool for HS and DS tolerance in various crops. High positive CTD values are associated with cooler canopies in drought-tolerant crop genotypes that are able to keep their stomata open to allow exit of water vapor through transpiration (low stomatal resistance), hence maintaining the cooling effect of leaves and carbon dioxide uptake (high stomatal conductance) for photosynthesis under water-limiting conditions **(STODDARD et al., 2006; BAZZAZ et al., 2015; SRIVASTAVA et al., 2017)**. This is very handy for a crop like sorghum known for its low stomatal resistance and high stomatal conductance. The use of CTD has proven to be very efficient in screening drought- and heat-tolerant varieties in many crops. Cooler canopies (High CTD) have been found to be related to several physiological indicators of drought tolerance, such as increased grain yield **(OGUZ et al., 2022),** high transpiration rate **(FAHAD et al., 2017),** high chlorophyll content **(TOJO and AYMAN, 2013)**, root depth, water usage and stomatal conductance **(COTRINA CABELLO et al., 2023)**. **ZHANG et al. (2019b)** noted that the use of the technique in sorghum research is still limited, however, valuable insights have been documented.

Mid-day to late-noon canopy temperature measurements taken just before flowering up to the post grain filling stage were observed to yield maximum CTD values that positively correlated with grain yield in sorghum under drought stressed conditions **(OGDEN et al., 2020)**. Inconclusive results still exist on the most appropriate daytime for taking measurements, the required number of sampling units, readings and appropriate instrumentation. Many canopy temperature readings within a short period of time evade the effects of abrupt changes in weather conditions, and the use of wireless infrared thermometers (IRTs) is viewed as more efficient and reliable **(BALOTA et al., 2007)**. Generally, there is consensus in literature that canopy temperature is a real-time response that can be determined rapidly using appropriate instrumentation to yield an accurate and precise indication of plant/leaf water status and tolerance to abiotic stress.

2.2.3.3. Cellular membrane stability

Heat and drought stress occurring independently or combined, when they coincide, more often than not, have detrimental effects on damaging components of plasma membranes and their destabilization in various plants **(COHEN et al., 2021; SINHA et al., 2021)**. The accumulation of ROS and its derivatives in plant cells induced by abiotic stresses poses a serious threat to the integrity of cell membranes **(NXELE et al., 2017)**. The extent may vary with genotype, duration, intensity and timing of stress, similar as to any other physiological response **(KAPOOR et al., 2020)**. The common symptom of cell membrane permeability due to cell membrane injury is electrolyte leakage, hence, it is used as an indicator of cell membrane injury in many crops, including sorghum **(HOWARTH et al., 1997; GONG et al., 2001)**. Tolerant species are able to maintain cellular membrane stability (CMS) under the aforementioned abiotic stresses. Genotypic differences in CMS have been reported in sorghum, hence its adoption as a tool in screening for HS and DS resistance **(VERSLUES et al., 2006; BEGNA, 2022)**. High genetic correlations between cellular membrane stability and other desirable traits of HS and DS tolerance, such as grain yield reported in sorghum, have strengthened the reliability and validity of the CMS as a screening tool **(ABREHA et al., 2022)**. Compatible metabolites play a role in the protection of various components of the cell membranes under heat and drought stresses **(ZANDALINAS et al., 2018)**. Denhydrins and late embryogenesis abundant (LEA) proteins induced by HS and DS are known for their thermostability and hence play an important role in stabilizing membrane structures in tolerant sorghum **(OGBAGA et al., 2016a; ABREHA et al., 2022)**.

2.2.3.4. Antioxidant capacity

Oxidative stress is considered one of the most harmful effects of heat and drought stresses in plants due to the increased production of ROS and derivatives, which are very toxic to cellular components such as proteins, lipids, sugars, DNA, organelles, membranes and are disruptive to key physiological processes such as photosynthesis **(LAMAOUI et al., 2018; CHATURVEDI et al., 2021; DOS SANTOS et al., 2022)**. Tolerance to these abiotic stresses is conferred by antioxidant capacity, which is the ability of plants to control the levels of ROS and their derivatives and ameliorate their effects to avoid any significant disruption of metabolic activities and integrity of cells **(WU et al., 2017)**. Enzymatic and non-enzymatic antioxidants are produced to scavenge and detoxify ROS **(AHMAD et al., 2021)**. In sorghum, proline is one of the metabolites produced in response to DS that offers antioxidant action, among other functions **(DEVNARAIN et al., 2016; OGUZ et al., 2022)**. Its role in scavenging ROS and its derivatives and preventing membrane disruption **(BHATNAGAR-MATHUR et al., 2008)**. Denhydrins and LEA proteins rich in amino acids such as glycine, threonine, asparagine, glutamine, serine, aspartic and glutamic acid were found to be synthesized in response to DS in tolerant sorghum genotypes in protection of hydrophobic components of enzymes from denaturing solvent derivatives of ROS **(ZANDALINAS et al., 2016; OGBAGA et al., 2016b)**. These act as chaperones that protect proteins and membrane denaturation under HS and DS **(GRAY and BRADY, 2016)**. **MASZKOWSKA et al. (2019)** mentioned two specific chaperones, ERD10 and ERD14, that play a critical role in providing protection to an array of enzymes known for their cellular protective roles during heat and drought stresses, such as lysosomes, alcohol dehydrogenase, luciferase and citrate synthase. Protection of the photosynthetic apparatus from free radicals and their active derivatives of oxygen, such as superoxide, singlet oxygen and hydrogen peroxide, is induced by increased cytokinin levels to offer a series of detoxification processes with the aid of the antioxidants superoxide dismutase, ascorbate peroxide and glutathione peroxidase **(CHAVARRIA and DOS SANTOS, 2012; PAUDEL et al., 2016; NADEEM et al., 2018; ANDR, 2020)**. Signaling molecules such as cytokinin and ABA play a major role in inducing antioxidant activities **(KESKIN et al., 2010; RIVERO et al., 2022)**. Thus, antioxidant capacity is a genetically controlled, responsive and timeous defense mechanism that results in the synthesis of relevant antioxidants and chaperones to protect plants against oxidative stress.

2.2.3.5. Tillering

Some sorghum genotypes, especially landraces grown mostly by subsistence farmers in marginal areas under rainfed farming systems, are reported to exhibit tillering ability, although the trait is more prominent in cereals such as wheat and rice **(MAKUVARO et al., 2014; COTRINA CABELLO et al., 2023)**. In a few studies, this phenological plasticity has been associated with drought-tolerant varieties, especially under pre-flowering moisture stress. Tillering is thought to be triggered by DS in some sorghum genotypes but does not significantly contribute to increased grain yield **(KONG et al., 2014)**.

Tillering is likely to provide an increased leaf surface area, resulting in an elevated transpiration rate and photosynthesis, which can only be supported by non-moisture-limiting environments without a compromise in grain yield **(ALAM et al., 2014)**. Thus, there is a widely reported negative correlation between tillering ability and leaf size **(BORRELL et al., 2014)**. Furthermore, the expression of a confirmed drought tolerance stay green trait in sorghum genotypes has been associated with reduced tillering **(KIM et al., 2010)**. Therefore, the conclusion that tillering cannot be regarded as a drought tolerance mechanism but as a survival mechanism under water-stressed conditions, is more appealing **(WENZEL, 1999)**. Understanding the regulation of tillering is still limited, but there is increasing evidence of genetic and environmental interactions at play **(KIM et al., 2010)**. **ALAM et al. (2014)** further pointed out that genetic and environmental interactions on tillering regulation are mostly a non-crossover type that it is controlled by multiple loci and multiple alleles at each locus.

2.3. Modern tools in heat and drought stress research

The last two decades have seen some notable transformations and advancements in tools used in research that has aided understanding of biotic and abiotic factors that affect crop productivity **(YANG et al., 2021)**. Research on drought, heat and combined stresses which are the leading abiotic factors were not left out of the 21st century technology revolution. High throughput phenotyping for heat, drought and combined stresses in field and laboratory based research have been revolutionized by the ‘omics’ and sensor technology in plant science research **(KUMAR et al., 2017; HEIN et al., 2021)**. ‘Omics’ are a holistic approach of studying biological data arrays with more focus on their changes at specific sites and times in organisms under external stimuli such as DS and HS **(MEHTA et al., 2019; SINGH et al., 2021)**.

Remote sensing is a pool of techniques used to collect data from objects without any physical contact **(BIJU et al., 2018; AHMAD et al., 2021)**. Furthermore, quantification and characterization of RNA, protein, DNA and various metabolites pathways has been made easier, accurate, precise and even cheaper through various ‘omics’ tools **(MEHTA et al., 2019; RAZA et al., 2021)**. Similarly, remote sensing has matched the pace in phenotyping of agro-morphological traits in field based research generating massive data critical in informing HS and DS research **(WASAYA et al., 2018; COSTA et al., 2021)**.

Sorghum is one of the C_4 plants whose gene products, specifically in HS and DS related research, have been explored through ‘omics’ techniques due to the availability of its complete genome sequence (**MAGALHÃES et al., 2016)**. It has been used as a model crop given its proven resistance to these abiotic stresses and devotion in mainstream research in recent years **(KHATON et al., 2016; CHADALAVADA et al., 2021; KUMAR et al., 2022)**. The major 'omics' approaches are genomics, transcriptomic, proteomics, phenomics and metabolomics **(AHMAD et al., 2021; ZARGAR et al., 2022; MUKHERJEE et al., 2024)**. The use of these 'omics' approaches and other related tools in understanding heat and drought stresses in sorghum will be briefly discussed in the subsequent sections highlighting their achievements, challenges and future opportunities.

2.3.1. Genomics

Genomics is an elementary and leading approach of 'omics' that deals with identification, sequencing and noting of the entire genome for specific organisms **(IBRAHEEM et al., 2018; BABELE et al., 2022)**. It is divided into structural, functional and epigenomics **(MUTHAMILARASAN and KUMAR, 2019; DOS SANTOS et al., 2022)**. It has been easy to use functional genomics to understand heat and drought stresses in sorghum due to its comparatively small genome of about 730MB **(KUMAR et al., 2011)**. Genomics has facilitated the understanding of genetics and molecular basis in response to stress through various tools **(HAO et al., 2021)**. Understanding the genome has been made simple and efficient through Next Generation Sequencing (NGS) technique using tools like Illumina or Solexa **(MUKHERJEE et al., 2024)**. Genome Wide Association Studies (GWAS) have unmasked some desirable traits responsible for HS tolerance at vegetative stages in sorghum using Single Nucleotide Polymorphism techniques **(YE et al., 2018; BABELE et al., 2022)**. This has provided better insights on marker trait association hence potential DNA markers have been used in selecting for HS tolerance in sorghum. Advancement in mapping tools of quantitative trait loci such as Multi-Parent Advanced Generation Inter-Cross have aided dissection of genetic structure for numerous quantitative trait loci in sorghum population **(HAO et al., 2021; SINGH et al., 2021)**. Genomics has revived the knowledge based approaches ahead of hypothesis based methods in plant biotechnology **(MEHTA et al., 2019)**. However, limited tissue culture, genetic transformation and mutant resources for sorghum has constrained this novel approach **(TU et al., 2023)**. There is a need for functional validation of potential genes in order to support molecular breeding efforts in sorghum **(LOPEZ et al., 2017)**.

2.3.2. Transcriptomics

Transcriptomic approach is another very robust tool of ‘omics’ that seeks to study the entire set of mRNA or transcriptome focusing more on non-coding regions **(YANG et al., 2021)**. It has enabled understanding of crucial molecular processes, biological pathways involved in plant abiotic stresses response using sequencing based techniques and microarray technologies **(MEHTA et al., 2019)**. The major sequence based approaches are serial analysis of gene expression, parallel signature sequencing and cap analysis of gene expression **(SINGH et al., 2021)**. These have been used to analyze changes at transcript level, multiple genes and interactions. Transcriptomic approaches are also very handy in understanding the dynamics of heat and drought stresses as they show active gene expression in specific organs and plant ontogeny **(IBRAHEEM et al., 2018)**. Accordingly, prediction of timing of expression of unique transcripts with specific roles such HSPs has been achieved in sorghum abiotic research studies **(TU et al., 2023)**. Exploration of defense mechanisms of sorghum to combinatory stresses such as HS and DS has been made easier through transcriptomic techniques like Illumina’s Real Time Analysis and Next Generation Sequencing **(ÁLVAREZ-IGLESIAS et al., 2017; SATYAVATHI et al., 2021)**.

Visualization of variations in gene expression in seed for large populations of sorghum accessions has been enabled by Mapman which is a specialized software for profiling that is tagged along micro arraying technology **(SHAAR-MOSHE et al., 2019; ABDEL-GHANY et al., 2020)**. The mapping technologies have been successfully used to validate the expression of genes under different environments **(WOLDESEMAYAT et al., 2018)**. Through micro array techniques over 28 000 genes have been probed in order to establish gene expression changes on exposure to the combinatory stresses **(TARI et al., 2013; HASSAN et al., 2022)**.

The understanding of small non-coding RNA networks mainly used in endogenous post transcriptional regulation has led to the discovery of miRNA associated with heat and drought stresses **(LIU et al., 2023; YANG et al., 2021)**. Omics techniques, termed ‘miRNA’, have aided identification of reconfiguration of lipid profiles in cell membranes of plants subjected to heat and drought stresses using mass spectrometry under lipidomics approach **(DAS et al., 2023)**. Lipidomics is related to transcriptomic which has improved the understanding of correlation in changes of lipid levels and gene expression **(TU et al., 2023)**. The obvious circumstance that genes are not always expressed in the entire lifecycle of a plant means some classes of RNA can still not be studied using this genomic tool **(MUTHAMILARASAN and KUMAR, 2019)**. Studies have revealed that the greatest proportion of differentially expressed gene changes were attributed to DS or HS occurring in isolation **(WANG et al., 2016; OGUZ et al., 2022; TU et al., 2023)**. Above all lack of coverage, false positive and negative results and low resolution in some of the transcriptomic techniques remain a challenge in solving the HS and DS problems especially the complex combined stresses **(PARK et al., 2007; HAO et al., 2021)**.

2.3.3. Proteomics

Proteomics is an equally useful omics technology that enables the profiling of proteins associated with heat and drought stresses **(WANG et al., 2016; SINGH et al., 2021)**. Under this technology proteogenome deals with the cell wall proteome **(MEHTA et al., 2019)**. Phosphoproteome deals with phosphorylated proteins which both play a significant role in signaling and maintenance of membrane integrity during heat and drought stresses **(WANG et al., 2016; KAUR et al., 2021)**. The technology has been very orchestral in unravelling protein composition in different organs under drought stressed, optimal conditions and stress recovery patterns **(BHARDWAJ et al., 2023; LIU et al., 2023)**. Matrix Assisted Laser Detection of Desorption-Time of Flight (MALDI-ToF) is a very accurate proteomics tool used in determination of molecular weight of proteins **(ZULFIQAR et al., 2020)**. It has offered high resolution in identification of proteins that are organelle specific in DS tolerant genotypes. MALDI-ToF mass spectrophotometer and two-dimensional gel electrophoresis (2-DE) proved the increased production of HSP_{70} in roots of drought stressed sorghum plants **(SCARPECI et al., 2008; WANG et al., 2016; LIU et al., 2023)**. Similarly profiling of protein composition patterns associated with root response to *in vitro* induced osmotic stress in sorghum seedlings has been achieved thorough the 2-DE **(IBRAHEEM et al., 2018; PHUONG et al., 2019)**. Phenotyping of root plasticity in sorghum is one outstanding achievement of proteomics **(MAGALHÃES et al., 2016)**. The variability and reproducibility limitations of gel-based proteomics analysis has been eased by the Difference Gel Electrophoresis

(DIGE) which can detect very minute proteins and requires less number of gels **(WASAYA et al., 2018; KAUR et al., 2021)**. However, DIGE is expensive in terms of equipment and software. One major drawback of proteomics despite its well celebrated strides in unmasking a wide array of proteins related to plant stress is lack of capacity to identify isoforms which is a common phenomenon in polypeptides particularly enzymes **(AZZOUZ-OLDEN et al., 2020; JEYASRI, et al., 2021)**.

2.3.4. Metabolomics

Metabolites play a key role in tolerance of abiotic stresses like HS and DS hence the birth of metabolomics which specializes in identification, detection, quantification and characterization of metabolites and metabolic profiles under given environmental conditions **(GEORGII et al., 2017; ZANDALINAS et al., 2018; MUKHERJEE et al., 2024)**. The technology has provided vast opportunities in understanding signaling molecules, regulating products and metabolites that are key in mechanisms underlying HS and DS resistance in hardy crops such as sorghum **(PIRASTEH-ANOSHEH et al., 2016; OGUZ et al., 2022)**. Mass Spectrometer, High Performance Liquid Chromatography (HPLC), and Nuclear Magnetic Resonance Spectroscopy are reckoned tools in metabolomics that have been used in profiling metabolites with high accuracy, precision and sensitivity **(ALI et al., 2019)**. Accumulation of osmoprotectants such as proline and ROS scavenging factors that render drought tolerance in sorghum have come to light through these throughput techniques **(NXELE et al., 2017; ZULFIQAR et al., 2020)**. Elucidation of overlapping responses is one hurdle that still needs to be overcome by metabolomics despite its illustrious strides **(LIU et al., 2023)**.

2.3.5. Phenomics

Remote sensing techniques have added a rather unique and different approach to the omics technology called phenomics that is widely used in phenotyping agro-morphological traits related to heat and drought stresses. Modern indicators of HS and DS tolerance that include stay green **(TOVIGNAN et al., 2020; ENYEW et al., 2022)**, relative leaf water content **(AZARI et al., 2016)**, canopy temperature **(CARVALHO et al., 2020)** and even traditional traits like yield can now be quickly and conveniently assessed using various sensor based tools. Field scanalyzers, farm machinery mounted, fixed mounted and hand held sensors have been used to assess various parameters with high resolution in field studies **(THAPA et al., 2018; HEIN et al., 2021)**. These include digital cameras in measuring time of day flowering **(HEIN et al., 2021)**, Soil and Plant Analyzing Devices (SPAD) for chlorophyll content **(AL-GHZAWI et al., 2018)**, Infrared thermometers for canopy temperature **(KUMAR et al., 2017)**, hyperspectral radiometers for translocation of water soluble carbohydrates **(HEIN et al., 2021)** and hyperspectral radiometers for photosynthetic efficiency amongst many other functional traits **(AHMAD et al., 2021)**. These tools have enabled simultaneous measurements with a high degree of accuracy and precision. However, with less temporal variability and reduced labor requirements **(MEHTA et al., 2019)**. Future research should take advantage of this technology in phenotyping of diverse accessions using large scale techniques to decode plasticity of stress tolerance in sorghum across **(BHARDWAJ et al., 2023)**.

2.3.6. Bioinformatics

Integration of all the omics approaches has been possible through bioinformatics which has facilitated generation, analysis, visualization, storage and exchange of huge amounts of biological data **(JEYASRI et al., 2021)**. Rapidity, accuracy and precision of generation and analysis of high throughput data are possible through computational algorithms, packages and platforms such as Cytoscape web, peppy and Integrated Genome Browser **(RAZA et al., 2021; LIU et al., 2023)**. Bioinformatics has generally improved accessibility and management of biological data **(ZARGAR et al., 2022)**. Generalization and relying on assumptions due to the complex nature and large volumes of data generated through this technology still remains an impediment in complete understanding of some phenomenon in plant abiotic stresses **(VELAZCO et al., 2019)**. Prospects are now focusing on collation and integration of data from multi-omic platforms in order to pave way for data mining and re-use **(FINOTELLO et al., 2020)**. Lack of skilled personnel, high cost and unavailability of these tools in most laboratories located in underprivileged regions of the world where research that seeks to examine traits for neglected crops like sorghum is urgent and most needed, remains a hindrance **(HAO et al., 2021)**.

2.4. Sectional conclusion

Heat and drought stresses are primary abiotic restraints on the production and productivity of sorghum, especially in rain-dependent smallholder farming systems of the SATs, where it is recommended. Their combined effects are even more detrimental to germination, seedling, pre- and post-flowering stages. It results in stunted growth, injury and abortion of reproductive organs, subsequently leading to decreased yield prospectives. However, sorghum tolerates drought better than most cereals due to its superior ability in osmotic adjustment and stomatal regulation. Furthermore, its leaf characteristics and behaviour reduce water loss, while root plasticity increases water uptake. Resistance to drought, heat and combined stresses is often assessed using various morpho-physiological traits such as the extent and speed of germination, thermotolerance in seedlings, plant stem height, leaf area, CTD, RLWC, CMS and yield indices. Both heat and drought resistance mechanisms are commonly divided into three distinct categories: escape, avoidance and tolerance mechanisms. The presence of genetic variation in most traits that indicate drought resistance in sorghum provides a source for screening of genotypes and informed plant breeding. In addition, omics approaches provide an effective and capable high throughput technique and tools in providing insights of genetic make-up, proteins, metabolites, interactions, and transcription factors involved in HS and DS research. The major approaches are genomics, transcriptomics, metabolomics, proteomics and phenomics. The sensor technology has also emerged to provide high throughput phenotyping of agro-morphological traits in field-based research. Lack of skilled personnel, high cost and unavailability of omics tools limit their use in research, particularly in under developed countries.

CHAPTER 3: *IN VITRO* INDUCED HEAT, AND DROUGHT STRESSES AND THEIR COMBINATORY EFFECT ON SEED GERMINATION OF DIVERSE TROPICAL SORGHUM GERMPLASM

3.1. Introduction

The three salient stages of early crop growth that contribute to the attainment of optimum yield are germination, emergence and seedling establishment. Thus, farmers who target high yields invest a lot of time and resources in land preparation, securing and planting of quality seeds to ensure these critical stages are optimal. Irrefutably, seed quality is one of the most essential inputs that determines crop productivity. Seed viability, optimum edaphic and environmental factors are important prerequisites for germination, a weighty and incipient process that spans from seed imbibition to radicle emergence to early crop growth and establishment **(KHAEIM, et al., 2022)**. Insufficient soil moisture and excessive temperatures in seeding zones at planting are notable hindrances in crop production in SATs **(BAYU et al., 2005; KAPOOR et al., 2020)**. Accordingly, these areas are characterized by soils with poor water holding capacity, high evaporative demands due to excessive atmospheric temperatures and erratic rainfall. However, changes and variability in climate characterized by excessive atmospheric temperatures, reduced uneven spatial and temporal distributions of rainfall, coupled with porous soils renders concern on germination in rainfed crop production systems **(GEILBYO et al., 2018)**. It is more pressing for a small grain crop like sorghum, which is climate “smart” and suitable to aforementioned conditions of proven HS and DS tolerance **(PHIRI et al., 2020; NCIIZAH et al., 2021)**.

Critical and incipient stages like germination have been targeted by plant breeders, physiologists and botanists especially in screening germplasm for tolerance to abiotic stresses like heat, drought and salinity **(KRASENKY and JONAK, 2012; TSAGO et al., 2014)**. However, most studies have evaluated the effects of heat and drought stresses on sorghum seed germination separately, leading to inconclusive results **(ABREHA et al., 2022)**. Time and resources are saved in such studies when large populations of germplasm are evaluated for tolerance under combined conditions like heat and drought stresses, which often coincide **(FAHAD et al., 2017)**. Soil moisture deficit and salinity have always been singled out as the most severe inhibitors of seed germination **(NDLOVU et al., 2021)**. Imbibition marks the beginning of the germination process, though it is a physical process driven by the water potential gradient between dry seed and soil. Ideal soil moisture content is estimated at 35-45% for germinating seeds **(QUEIROZ et al., 2019),** and the optimum germination temperature range for sorghum is 20-28 ºC **(ADAMCIK et al., 2016)**. Low soil osmotic potential of 0-0.8 MPa has been proven to reduce final germination percentage and germination index by inhibiting seed imbibition **(SHAHRIARI et al., 2014; QUEIROZ et al., 2019)**. Mean germination time is prolonged by a lower osmotic potential **(AHMAD et al., 2009; ABREHA et al., 2022)**. Delayed germination increases the risk of losing seed to desiccation, predators like birds, rats and other pests as well as pathogens.

Combined heat and drought stresses are very detrimental to germination with far reaching implications as they often coincide during early and late plantings **(FAHAD et al., 2017)**. In SATs, soil temperatures are likely to be higher which results in high evaporation of moisture especially in the uppermost soil layer (in the range of 0-5 cm) which is the planting depth for small seeded cereals like sorghum **(BAYU et al., 2005)**. Despite the increasing incidences of combined heat and drought stresses which has been proven to be very detrimental to all stages of most crops, the two factors are often studied separately **(BHEEMANAHALLI, et al., 2022)**. Furthermore, most research work has focused on flowering and grain filling stages. This study is one of the few that seeks to investigate the two factors combined at germination of sorghum landraces, breeding lines and check varieties selected from a large pool of world and national gene banks which have not been tested. This study provides new insights on the response of the selected high potential tropical sorghum genotypes to heat and drought stresses either combined or occurring independently at germination. This offers a quick and cheap screening technique of the germplasm that will inform breeders and plant physiologists in crop improvement programs.

Field evaluation of emergence tends to be complicated by complex interactions of soil properties and other field conditions, thus, the widespread adoption of an *in vitro* technique using polyethylene glycol (PEG) inducing osmotic stress **(BOBADE et al., 2019)**. The technique has proven to be effective, low cost, easy to handle and less laborious for screening large pools of genetic material for abiotic stress tolerance **(TSAGO et al., 2014)**, to aid crop improvement programs. This study was aimed at assessing the response of 50 sorghum accessions to drought, heat and combined heat and drought stresses at germination.

3.2. Materials and Methods

3.2.1. Experimental site and research material

A laboratory experiment was conducted at Lupane State University, Seed Science Laboratory. 48 sorghum genotypes acquired from the Genetic Resources and Biotechnology Institute of Zimbabwe and ICRISAT Matopo were selected in a baseline study conducted from the previous season (2019/20) on a pool of 300 genotypes based on plant morpho-physiological characteristics that included stem height, days to maturity, susceptibility to lodging and 1000 grain weight measured at physiological maturity when a black layer formed a dark spot visible on the kernel at the base of the seed. Two commercial sorghum varieties known for their high grain yield and resilience to various abiotic stresses namely “Macia” and “SV4” were included as check varieties to bring the total to 50 genotypes. The seed for the genotypes used in the study was stored for 12 months after harvesting at Lupane State University Gene Bank in a temperature controlled and monitored room at temperatures of 0-10ºC and low relative humidity of 25-35% to maintain their viability.

3.2.2. Experimental design and management of the experiment

Seeds of 50 selected sorghum accessions **(Table S1)** were germinated in four artificially created stress conditions in a laboratory as follows: DS alone (20% (m/v) PEG 6000 solution induced osmotic potential of -0.85 MPa according to a procedure by **FOTI et al. (2002)**, HS alone (45 ℃ day and 25 ℃ night incubation temperatures in deionized water), combined heat and drought stresses (a combination of afore-mentioned HS and DS treatments) and no stress, optimum temperatures of 25 ℃ day and 22 ℃ night incubation in deionized water as a control.

Fifty seeds of homogenous size and age for each genotype were surface-sterilized by immersion in 1.5% sodium hypochlorite solution for five min and then thoroughly rinsed five times in distilled water. Thereafter, they were placed in sterilized Petri dishes lined with double Whatman No. 2 filter papers moistened with 7 ml of either de-ionized water for non-drought stressed treatments or 20% (w/v) PEG 6000 solution of -0.85 MPa osmotic potential for drought stressed treatments. The Petri dishes were sealed with parafilm to reduce water loss and kept in dark cupboards to exclude light. All the Petri dishes were replenished with 3ml of fresh PEG solutions and de-ionized water after four days of incubation and no fungi development was observed.

The experiment was laid out as a split-plot following a two-factorial completely randomized design with three replicates. The main factor for splitting was the induced stress at four levels, i.e. no stress, DS alone, HS alone and the two combined stresses. The sub-factor was the 50 genotypes which were randomly allocated in each stress condition. The resultant was 200 treatment combinations i.e. 50 genotypes × 4 stress conditions each replicated three times to give 600 experimental units. The experiment was kept in two separate incubators under dark conditions following the temperature treatments for seven days. The experiment was repeated in order to increase the sample size and the two sets were done in tandem.

3.2.3. Data collection

Count data on the germinated seeds was collected every 24 h for five days. Seeds that had between 1 - 2 mm protruding coleorhizae were considered germinated. The following four germination parameters were derived from the daily seed count:

i) Final germination percentage (FGP)

$$\text{FGP} = \frac{Number\ of\ normally\ germinated\ seeds}{total\ number\ of\ sown\ seeds} \times 100\% \qquad (1)$$

ii) Mean germination time (day)

$$\text{MGT} = \frac{Ti \times Ni}{N}, \qquad (2)$$

Where N_i is the number of newly germinated seeds at time T_i

iii) Germination index

$$\text{GI} = \frac{Gt}{Tt} \qquad (3)$$

Where Gt is the number of seeds germinated on day t, and Tt is the number of days

iv) Coefficient of variation of germination time (% seed day-^{1})

$$CVt = \left(\frac{St}{t}\right)100, \qquad (4)$$

Where S_t is the standard deviation of germination time and t is mean germination time.

3.2.3. Data analysis

Response of sorghum genotypes to the four most important germination parameters i.e. FGP, MGT, GI and CVt to the *in vitro* induced conditions was modelled using generalized linear models with either Poisson or negative binomial log-link functions and '*multcomp*' package to separate the means in R studio packages **(R Core Team, 2021)**. Principal Component Analysis of pooled means of the assessed germination parameters for 50 sorghum genotypes was conducted using Multivariate analysis /PCA function in Minitab version 14. Furthermore, the hierarchical agglomerative cluster analysis was performed using dissimilarity coefficients and unweighted pair-group method arithmetic average (UPGMA) of clustering for tree construction using DARwin 6.0.21 software. Principal coordinate analysis (PCoA) was also performed using DARwin to illustrate a two-dimensional map of the distribution of genotypes based on their performance in the treatments.

3.3. Results

3.3.1. Multivariate results of the influence of genotypes, stress and their interaction on germination parameters

The generalized linear model results revealed highly significant (< 0.001) effects of genotype and stress conditions on the four assessed germination parameters (FGP, MGT, Cvt and GI). Highly significant interaction of genotype and stress was only observed in GI. The PCA was used to indicate the variation that existed among the germination parameters **(Table 1)**. GI as a representative indicator of the extent and speed of germination was used to illustrate the effect of stress and the interaction of stress and genotype on germination of sorghum **(Table 2)**. Hierarchical cluster analysis based on unweighted pair group method analysis (UPGMA) **(Figure 4)** and Principal Coordinate Analysis (PCA) **(Figure 5)** plots confirmed the genotypic effects on the number of germinating seeds and their speed of germination under the stress conditions. The two complementary plots classified the genotypes into six distinct groups, according to their overall performance in all four germination parameters that were assessed.

3.3.2. Principal component analysis of the germination parameters

Diversity in tolerance combined HS and DS during germination in 50 sorghum genotypes that were assessed was expressed in some of the four germination parameters that were measured. The first two significant components expressed a cumulative variation of 80% in the assessed germination variables **(Table 1)** as shown by their Eigen values > 1. The first and the second components accounted for 62.5 and 17.3% respectively, of the variation that existed among the germination parameters **(Table 1)**.

Most of the variation attributed to the first component was contributed by GI which had the highest positive factor loading value of 0.51. Final germination percentage also contributed positively in the first component. The significant relationship to diversity in germination of the genotypes in the second component was due to the highest negative loading value of -0.63 observed in mean germination time and final germination percentage with a loading value of -0.424. Some positive contribution by the coefficient variation of germination time loading values of 0.41 was observed in the second component.

Table 1. Principal component analysis in 50 diverse sorghum genotypes for five germination parameters under combined HS and DS.

Variable	PC 1	PC 2	PC3
FGP	0.461	- 0.424	- 0.046
MGT	- 0.293	- 0.625	- 0.458
GI	0.509	0.002	0.087
CVt	0.213	0.410	- 0.878
Eigen values	3.752	1.037	0.847
Proportion of Variance	0.625	0.173	0.141
Cumulative proportion	0.63	0.80	0.94

FGP= Final germination percentage; MGT= Mean germination time; GI= Germination index; CVt= Coefficient of variation time.

3.3.3. Evaluation of the response of genotypes to the four stress treatments during germination using germination index

Germination index was significantly influenced by the main effects of stress, genotype and their interactions as revealed by the GLM analysis. GI as an indicator of the extent and speed of germination was used to illustrate the effect of stress and to screen the genotypes under these stresses. The performance of genotypes on the extent and speed of germination under the four test conditions as indicated by GI revealed that in over 70% of the genotypes, the combinatory effect of heat and drought stresses was the most detrimental **(Figure 2)**. Heat stress had the least negative effect on germination in almost all the genotypes except IS30015 which had a higher GI value under combined stresses than separately induced HS and DS **(Figure 2)**. At least 70% of the accessions under the heat stressed treatment had a GI mean higher than the overall GI mean of 20 and showed no significant differences to about 60% of the accessions germinated under non-stressed conditions **(Figure 2)**. Less than 50% of the accessions under the drought stressed treatment had a GI mean higher than the overall GI mean of 20 **(Figure 2)**.

In addition to genotype IS30015, genotypes NPGRC1593 and NPGRC1782 showed superiority under combined stresses with a GI higher than that of DS alone **(Figure 2)**. Separate HS and combined HS and DS had an equal impact on genotypes IS24426 and IS6944 as indicated by their equal GI values in the afore-mentioned treatments. Two genotypes namely IS9405 and IS26191, had GI means less than 10 under all the three stress treatments **(Figure 2)**, “Macia” and “SV4” check varieties were amongst the poorest performed varieties under combined stresses. However, the two check varieties moderately performers under DS and HS induced in solitary conditions.

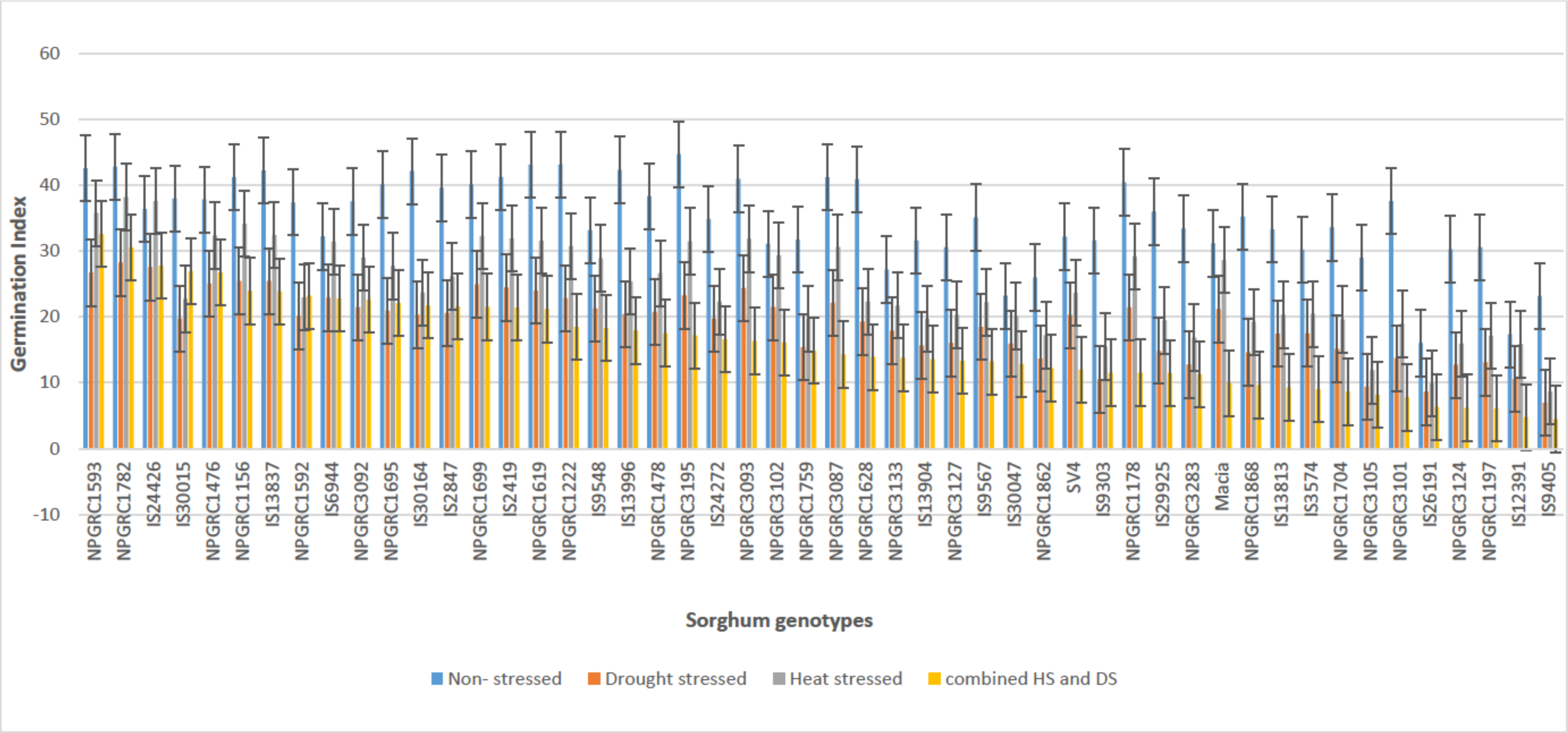

Figure 3. Mean germination indices of sorghum genotypes germinated under four *in vitro* induced stress conditions. Genotypes have been arranged in descending order of their observed GI means under combined heat and drought stresses. The vertical bars at the apex of each bar denote standard error of differences in means and non-overlapping error bars indicate statistical differences in means at $p<0.05$.

3.3.4. Clustering 50 sorghum genotypes based on their performance in four assessed germination traits under combined HS and DS

Agglomerative clustering of the 50 sorghum genotypes based on their performance in all the germination traits under combined HS and DS grouped the genotypes into six distinct Clusters. Cluster 1 and 3 were the largest groups with 15 and 13 genotypes respectively. While Clusters 5 and 6 had the least number of genotypes of 2 and 1, respectively **(Figure 3)**. The performance of genotypes in each group are detailed in **Table 2**.

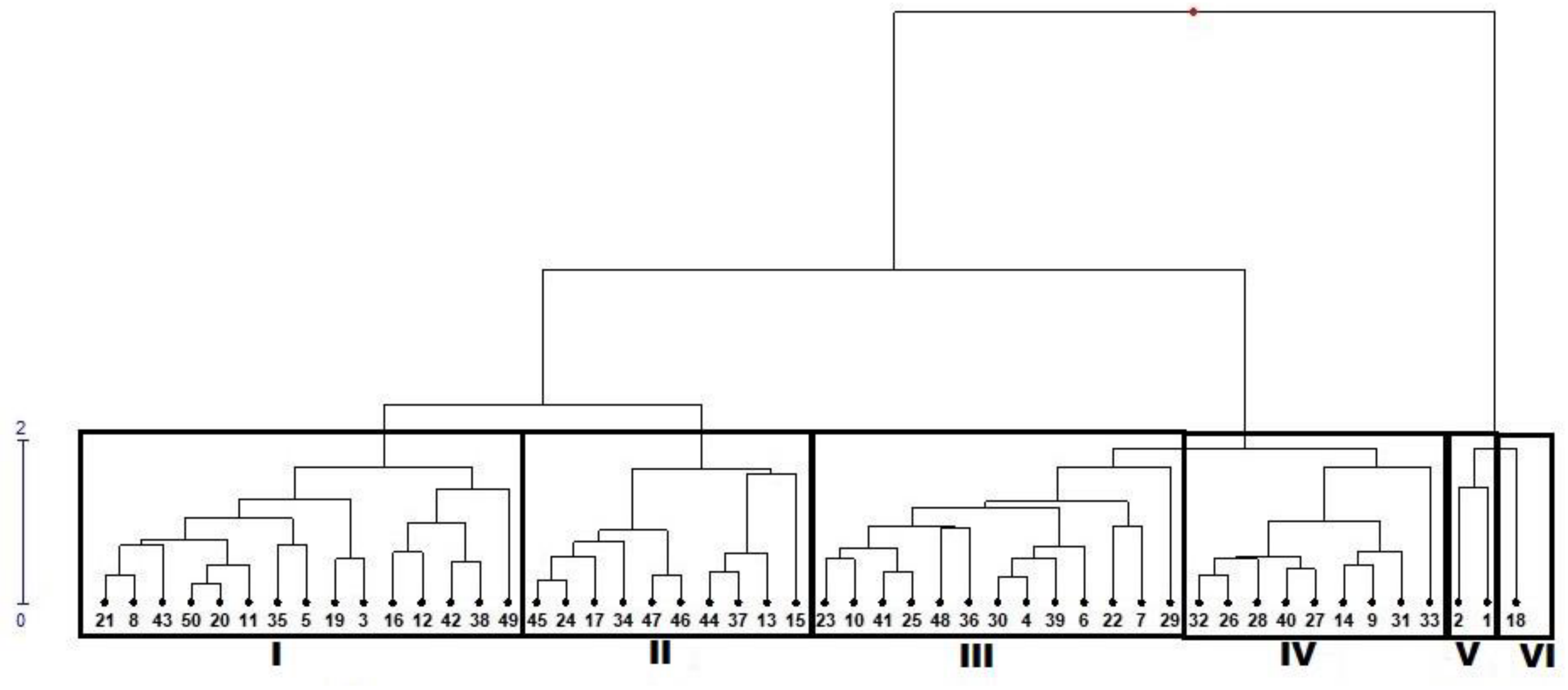

Figure 4. Dendrogram by hierarchical agglomerate cluster analysis of the 50 assessed sorghum genotypes under combined HS and DS. Genotypes grouped into six Clusters shown using text wrappings of different colors. The numbers denote the entry codes for genotype entries whose actual names are provided in supplementary **Table S1** in appendices.

The clustering was also confirmed by the Principal Coordinate Analysis (PcoA) plot **(Figure 4)** which produced a map for visualization of the genotypes based on their dis/similarity in germination parameters under combined HS and DS. The scattered genotypes in all the four quarters of the PcoA plot show a high degree of genetic variation among the assessed genotypes. Furthermore, the two-dimensional map gives a clear indication on the proximity of the genotypes in each Cluster based on the distance computed using the variations in performance in PCA. The closer the genotypes the more similar they are e.g. IS9405 and NPGRC1868 in Cluster 2, IS2419 and IS9303 in Cluster 3.

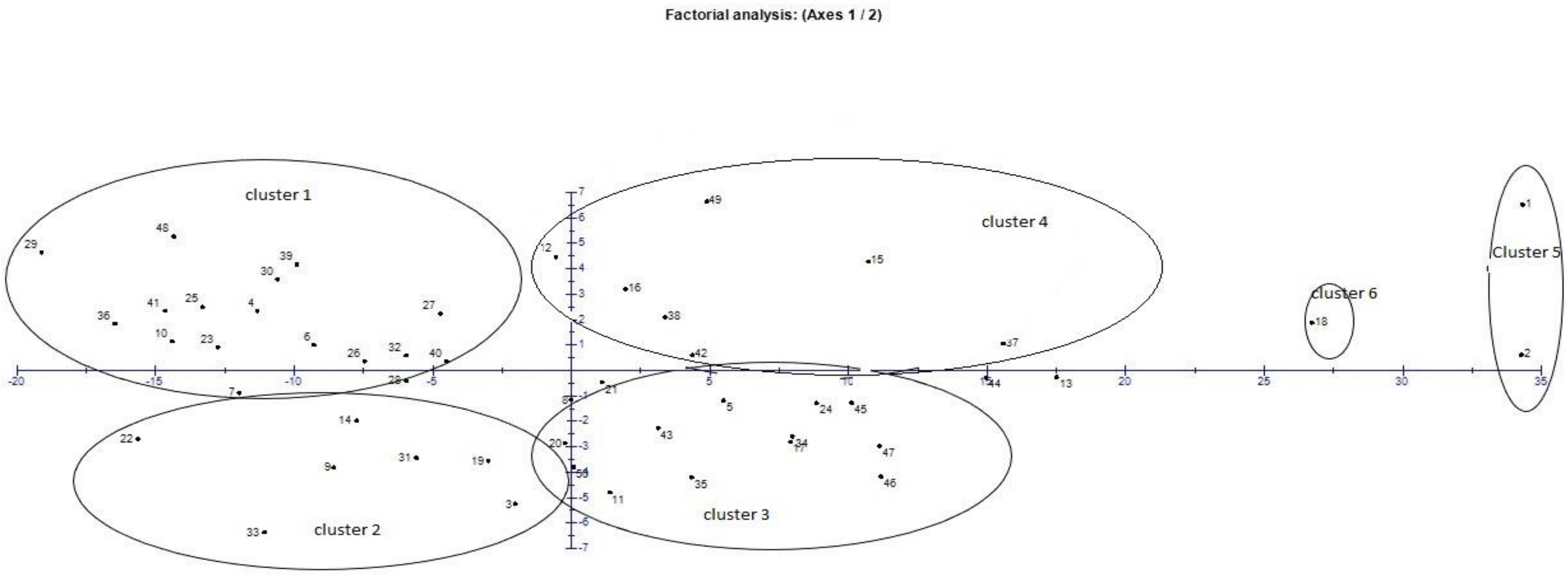

Figure 5. Principal Coordinate Analysis (PCoA) for 50 genotypes based on their performance in the 5 assessed germination parameters under combined HS and DS. Similar genotypes in terms of overall performance in assessed germination parameters grouped into clusters that are circled. The numbers denote the entry codes for genotype entries whose actual names are provided in Supplementary **Table S1** in appendices.

3.3.5. Comparison of the clusters under selected key germination parameters

Comparative analysis of performance of the six clusters under the four selected germination parameters indicated that Cluster 2 had the highest number of genotypes that exhibited tolerance to the effects of combined HS and DS followed by Cluster 1 **(Table 2)**. Genotypes in Cluster 2 had the highest mean final germination percentage of 75.6%, germination index of 30.26 and the lowest MGT of 1.48 days. The genotypes that showed the least resistance combined stresses, i.e. IS26191, IS9405, IS12391, NPGRC3124, NPGRC1197 and NPGRC3105 as presented previously **(Figure 2)** were found in Clusters 3 and 4. Cluster 4 had the lowest GI mean of 23.86 and as expected the highest MGT of 1.67 days. The check varieties fell into different groups with "SV4" in Cluster 1 and "Macia" in Cluster 5.

Table 2. Mean performance of clusters in different germination parameters of 50 sorghum genotypes under combined HS and DS.

Cluster	Germination parameters					
	FG%	MGT	GI	CVt	Number of Genotypes	Names of Genotypes
I	71.49	1.58	27.50	32.06	15	**IS24426**;NPGRC1222;NPGRC1862;IS2427;IS13996;NPGRC1592;IS9405;IS3574;NPGRC1695;"SV4";NPGRC1178;NPGRC3127;IS9548;NPGRC1619;IS2487
II	75.6	1.48	30.26	31.18	10	**NPGRC1782**;**NPGRC1476**;NPGRC1699;IS13837;NPGRC3087;NPGRC3093;NPGRC1868;IS30047;NGB1478;IS30164
III	68.77	1.62	26.17	31.94	13	NPGRC3195;IS9405;IS9567;NPGRC3133;NPGRC1704;**IS30015**;IS9303;NPGRC3092;IS2419;NPGRC3283;IS13904;**NPGRC1593**
IV	64.72	1.67	23.86	32.80	9	NPGRC3101;NPGRC1156;NPGRC1759;IS6944;NPGRC3105;IS26191;NPGRC3124;NPGRC1628;NPGRC1197
V	71.6	1.7	25.7	34.8	2	"Macia"; IS13813
VI	67.33	1.57	26.26	34.69	1	NPGRC3102

Genotypes in bold showed most resilience to combined heat and osmotic stresses during germination.

3.4. Discussion

Differential responses of genotypes in seed physiological tests like germination under limiting factors such as heat and drought stresses has been established in an array of crops including sorghum **(SHAHRIARI et al., 2014; MOYO et al., 2015; NYONI et al., 2020)**. Diversity in germination related parameters under combined HS and DS conditions which was found to be the most detrimental was confirmed by the cluster analysis results which grouped the genotypes into six distinct groups **(Table 2)**. Most of the tolerant genotypes were landraces from semi-arid areas of Zimbabwe like Chiredzi and KwaZulu-Natal Province of South Africa. This is suggestive of co-evolution of these landraces in these hot, dry environments which allows them to adapt to such unfavorable conditions.

The two check varieties "Macia" and "SV4" were grouped with genotypes that showed less resistance to combined DS and HS during germination but performed moderately under HS but showed lack of resistance to DS, indicating that the major target of their improvement was probably HS more than DS. Recent studies have also confirmed the existence of genotypic differences in tolerance of heat and drought stresses in sorghum **(MASETTO et al., 2017; GOVINDARAJ et al., 2021)**. The results of this study followed suit in exhibiting some variations in response of genotypes to heat and drought stresses occurring separately and to the dual effect of two stresses on genotypes as hypothesized. This means the genotypes respond differently to the two stresses when presented separately and even when the combined. Actually, in the field, they often occur together and are likely to interact significantly to delay and reduce germination as observed in this study. Occurrence of different genotypes with tolerance to each of the factors separately could be indicative of independent genetic mechanisms controlling both. This is also attributed to the instant effect of *in vitro* induced stresses as compared to the gradual effect of the stresses under natural conditions.

The complex responses of genotypes to combined HS and DS during germination is not conclusively understood **(ABREHA et al., 2022)**. Evidence points to elevated osmotic pressure in hot soils due to depletion of moisture, more so in saline conditions **(DEHNAVI et al., 2020)**. Maize is reported to be more sensitive to both heat and drought stresses than sorghum, a study by **KHAEIM et al. (2022)** observed that maize seeds failed to germinate at 40 ºC. In a separate study by **MINGLI et al. (2015)** of two maize varieties on the effects of DS of almost the same intensity with the one used in the present study revealed that germination percentage was reduced by a margin of between 28-50%.

Germination index was selected to represent the germination parameters in screening for tolerance in genotypes due to its highest contribution to the variation in the first component observed in the PCA. Germination index, a function of final germination percentage and mean germination time, is a very accurate and comprehensive measure which takes into account the extent and speed of germination **(KADER, 2005)**. A higher GI indicates a higher final germination percentage and a lower MGT, i.e. more seeds germinating within the shortest period of time. The energy and time taken for germinating seeds to osmotically adjust under high osmotic pressure renders the differential response to speed and extent of germination signified by the variations in the GI **(SHAHRIARI et al., 2014)**. Generally, in this study, DS suppressed germination more than HS as shown by a difference of 10 units between the highest values recorded for mean germination index for the two factors induced separately. Accordingly, genotypes such as NPGRC1782, IS24426, NPGRC1593, NPGRC1156 and NPGRC1476 that exhibited high GI under the combined effects of the two stresses have a high potential of resistance, and further studies on the genetic basis of their resistance mechanisms could benefit plant breeding efforts that intend to exploit the material as parents.

Supra-optimal temperatures above 25 ºC have been proven to inhibit germination even when soil moisture conditions are favorable **(PATANE et al., 2012)**. The antagonistic effects of DS on germinating seeds is reportedly amplified by imbibition at high temperatures **(WEN, 2015)**. This is attributed to the inhibition of enzyme dependent reactions and even their total shutdown due to changes at supra-optimal temperatures. Though not significantly different to over 10% of the assessed genotypes, three genotypes appeared at the top of the list in all three tested stress treatments, namely genotypes NPGRC1782, NPGRC1593 and IS24426.

3.5. Conclusions and future perspectives

The combinatory effect of heat and drought stresses was the most detrimental in almost all the sorghum genotypes that were assessed in this study an exception is genotype IS30015. Supra-optimal temperatures of 45 ºC induced as a solitary stress treatment was less detrimental to most genotypes than induced DS. Double the number of genotypes showed resistance to HS compared to DS when benchmarked using the observed overall mean germination index of 20. A huge diversity in HS and DS tolerance was exhibited in the assessed germplasm. The genotypes were grouped into six classes based on their performance in terms of extent and time taken to germinate under the three tested stress treatments. Genotypes NPGRC1782, NPGRC1593, IS24426, IS30015 and NPGRC1476 were the top five, and NPGRC9405, IS12391, NPGRC1197, NPGRC3124 and NPGRC3101 were the five least performing genotypes under the combinatory effects of induced HS and DS. The results can be used in selecting the tolerant genotypes for either HS or DS or combined stresses during germination for crop improvement purposes depending on the trait of interest. Further screening of the genotypes that showed potential in this study is recommended under field conditions.

CHAPTER 4: UNLOCKING BASAL AND ACQUIRED THERMOTOLERANCE POTENTIAL IN TROPICAL SORGHUM

4.1. Introduction

The recent drastic changes in climatic patterns, especially recurrent heat waves in sub-Saharan regions, calls for a revolutionized focus on crop improvement. Temperature and rainfall are the two most important environmental factors which affect growth and development of plants **(GALICIA-JUÁREZ et al., 2020; LI and HOWELL, 2021)**. All plant species have specific temperature thresholds for different phases of growth and development **(HATFIELD and PRUEGER, 2015; CABELLO et al., 2023)**. Increase in temperature beyond critical threshold levels stimulates the production of ROS in plant cells **(SONG et al., 2012)**. The resultant imbalance in the level of production and removal of the ROS in plant cells results in oxidative stress, thus the emanation of HS **(SCARPECI et al., 2008)**. Heat stress destabilizes biological molecules, structural stability and enzymatic reactions which are key for normal functioning of most biochemical and physiological reactions in plant cells **(GALICIA-JUÁREZ et al., 2020)**. The accumulation of ROS induced by extreme temperatures is more detrimental to delicate stages of growth and development of plants, particularly germination and seedling establishment **(NADEEM et al., 2018)**.

In the wake of reports that soil temperatures can reach 50 ºC during midday in semi-arid and arid regions **(SCARPECI et al., 2008)**, it is becoming clear that crop establishment and growth in such areas are at risk. This will worsen the already dire situation for resource-poor farmers whose agricultural practices and means are climate dependent. Of late these have, however, proved variable and unreliable **(YADAV et al., 2016)**. In field crops, the only way to reduce soil temperatures is through irrigation which is beyond reach for many smallholder farmers in marginal areas in the SATs of Africa. This calls for stepped up efforts in identifying resilient genotypes of known robust graniferous crops like sorghum, so that their production can continue under sub-optimal conditions to meet the ever-increasing food demand.

The widely accepted range of optimum temperatures for sorghum growth and development is 20-32 ºC **(SARI and JUNIARTI, 2023)**. In a study by **PEACOCK (1982)**, seedling emergence in sorghum failed at 45 ºC. Similarly, in maize, coleoptile growth came to a halt at 45 ºC **(AKMAN, 2009)**, thus coleoptile elongation has been used as an indicator for HS tolerance **(BASHA, 2017)**. Even pearl millet, a known hardy crop that is a good candidate to complement and/or substitute sorghum, is equally affected by HS at germination and seedling establishment **(HOWARTH et al., 1997; YADAV et al., 2016)**. However, genotypic differences on inherent tolerance and natural acclimation to temperature extremes and fluctuations exist for sorghum **(PAVLI et al., 2011)**, hence efforts can be directed at promising genotypes among the existing large populations. Thus, the need for rapid and low cost but effective techniques for evaluating large populations of genotypes for acquired and basal thermotolerance **(HOWARTH et al., 1997)**.

Basal thermotolerance is the inherent ability of a plant to survive HS due to evolutionary adaptation **(SONG et al., 2012; BOKSZCZANIN and FRAGKOSTEFANAKIS, 2013)**. While the ability to survive potentially lethal extreme temperatures through prior exposure to mild HS is termed ‘acquired thermotolerance’ **(SELVARAJ et al., 2011)**. Acquired thermotolerance is measured in the laboratory by comparing growth of seedlings that have received prior exposure to supra-optimal temperatures to those that are directly exposed to high temperatures without acclimatization **(CHOINSKI, 1999)**. Controlled heat induction in the laboratory can be used to mimic field shocks or gradual increase in soil temperature **(HOWARTH et al., 1997)**. Some genotypes possess higher basal than acquired thermotolerance **(NARAYANAN, 2018)**. Acquired thermotolerance is controlled by genes that code for principal regulatory factors called HSP **(PAVLI et al., 2011; FERRADINI et al., 2015; BERNFUR et al., 2017)**. HSPs are molecular chaperones that are involved in reduction of ROS, repairing and reconfiguration of proteins which offers some level of protection to HS in plants **(KUMAR and RAI, 2014; AHMAD et al., 2021)**. Activation of such genes when plants are gradually exposed to HS result in increased synthesis of several metabolites and proteins which confers some level of protection **(SONG et al., 2012)**. Accordingly, this study aimed at determining sorghum genotypes with potential basal and acquired thermotolerance with a view of using them as a tool to minimize sorghum losses due to HS.

4.2. Materials and Methods

4.2.1. Planting material

As indicated in Chapter 3.

4.2.2. Germination procedure and experimental design

Twenty seeds for each genotype were surface sterilized for 5 min using 1% sodium hypochlorite, thoroughly rinsed three times in deionized water and germinated in Petri dishes lined with double Whatman No. 2 filter papers moistened with deionized water in a growth chamber at 30-35 ºC. Petri dishes were placed in a completely randomized design with three replicates in an incubator. Two sets of the experiment were done in tandem.

4.2.3. Basal thermotolerance of sorghum germplasm

Five healthy two-day-old seedlings were randomly selected and exposed to heat shock treatment at 50 ºC for 10 min in an incubator and then allowed to recover at 30-35 ºC for 36 h in a growth chamber. Control treatments were not exposed to the heat shock treatment. The experiment was arranged following a split plot in Completely Randomised Design (CRD 50 × 2 × 3); where 50 genotypes were replicated three times in two separate HS treatments. Changes in coleoptile length after 36 h for the seedlings that were exposed to heat shock treatments as well as those not exposed to heat shock were measured using a digital Vernier caliper.

4.2.4. Acquired thermotolerance of sorghum germplasm

Five healthy two-day-old seedlings were exposed to HS treatment by gradually increasing the temperature in the growth chamber from 30 °C by 5 °C increments every hour until it reached 45 °C and was then kept constant at this temperature for one h. The seedlings were then allowed to recover at 30-35 °C for up to four days. Seedlings were then exposed to a second heat shock at 50 ºC for 10 min in a growth chamber and then allowed to recover again at 30-35 ºC for up to four days. Control treatments were not acclimatized through gradual heat shock treatments but were exposed to a heat shock treatment at 50 ºC. The experiment was again arranged following a split plot in Completely Randomised Design (CRD 50 × 2 × 3), where 50 genotypes were replicated three times in two separate HS treatments. Coleoptile length was measured before and after heat treatments using a digital Vernier caliper and the differences recorded **(Figure 5)**.

Figure 6. Taking of measurements for coleoptile length of sorghum seedlings using a digital Vernier Caliper as part of determination of thermotolerance in seedlings.

4.2.5. Statistical analysis

In assessment of basal and acquired thermotolerance, quantitative data on coleoptile length changes was subjected to a two-way analysis of variance following a CRD in GenStat Statistical Package 14th edition, to determine significant differences in means for the two heat treatments of 50 genotypes and their interactions. Means were compared at 95% level of significance and separated using Bonferroni's test where significant differences were observed. Data was subjected to the tests of assumptions of ANOVA prior to being subjected to the F-test.

4.3. Results

4.3.1. Response of seedlings of 50 sorghum genotypes to heat shock and acclimatization treatments

Assessment of 50 tropical sorghum accessions for basal and acquired thermotolerance using coleoptile elongation as an indicator revealed that heat shock of 50 ºC for a 10 min suppressed coleoptile elongation in sorghum seedlings, while prior exposure of seedlings to gradually increasing temperatures up to 45 ºC reduced the effects of heat shock as indicated by the degree of coleoptile elongation. Heat shocking of sorghum seedlings significantly reduced coleoptile elongation ($p<0.001$) as demonstrated by the F-test results for both sets of basal and acquired thermotolerance **(Table 3)**. Significant variability in sorghum genotypes that were tested for basal and acquired thermotolerance at germination was also observed in the two sets of experiments for each test. Differential response of the assessed genotypes to heat shock and acclimatization treatments was demonstrated by the highly significant interaction of genotypic factors and the HS treatments in both sets of basal and acquired thermotolerance tests **(Table 3)**. In both basal and acquired thermotolerance tests, temperature had the greatest effect as indicated by large mean of square errors **(Table 3)**.

Table 3. Analysis of Variance for basal and acquired thermotolerance in diverse tropical sorghum accessions done in two sets.

		Set 1		Set 2	
		Basal	Acquired	Basal	Acquired
Source of Variation	DF	Mean Squares	Mean Squares	Mean Squares	Mean squares
Temperature (T)	1	3987.4***	11556.2***	13700.5***	5441.1***
Genotype (G)	49	586.7***	30.9***	50.6***	139.9***
G ×T	49	282.2***	49.8***	32.5***	95.0***
Error	1400	119.2	6.5	6.7	16

DF= degrees of freedom; *** significant at <0.001.

In both sets of the basal thermotolerance assay heat shocking of sorghum seedlings significantly suppressed coleoptile elongation by 13.7 and 31.9%, respectively **(Table 4)**, when compared to their counterparts that were not exposed to heat shock. Acclimatization of sorghum seedlings reduced the effects of heat shock, as demonstrated by significantly lower coleoptile elongation in non-acclimatized sorghum seedlings in the two sets of the experiment by 62.1 and 52.5% respectively when compared to the acclimatized seedlings **(Table 4)**.

Table 4. Mean comparison of coleoptile changes for heat treatments of sorghum seedlings indicating basal thermotolerance in two sets of each experiment.

Treatments	Basal thermotolerance		Acquired thermotolerance	
	Set 1	Set 2	Set 1	Set 2
Heat shocked	20.54^b	12.91^b	3.40^b	3.45^b
Non-Heat shocked	23.80^a	18.96^a	8.96^a	7.26^a
LSD (5%)	1.11	0.26	0.26	0.41

Non-heat shock for Acquired thermotolerance means genotypes were acclimatized through incremental temperature increases. Means with similar superscripted letter(s) in the same column were significantly different at ($p<0.05$) measured as coleoptile length in millimeters.

4.3.2. Interaction of genotypes and temperature treatments on basal thermotolerance

Following significant interactions of genotype and heat treatments for coleoptile elongation in sorghum seedlings conferring differential expression of basal thermotolerance, their means were separated using the Bonferroni’s test. The top ten and five least performing genotypes in terms of coleoptile elongation were identified and are presented in **Table 3** for the two sets of the experiment. Genotypes NPGRC1704 and IS24426 were consistently amongst the top ten performers in terms of basal thermotolerance in the two sets of the experiment **(Table 5)**. “Macia” a commercial check variety was amongst the top ten performers. In the first set only the two least performing genotypes, IS30164 and NPGRC3127, were significantly different from the top two best performers in basal thermotolerance **(Table 5)**. In set two the five least performers were significantly different from the top three performers. Only one genotype, NPGRC3127, consistently appeared in the least five performers in both sets of basal thermotolerance test. “SV4” a commercial check variety had the lowest mean change in coleoptile length in set 2, indicating lack of basal thermotolerance when compared to the tropical sorghum genotypes that were assessed.

Table 5. Mean comparison of coleoptile changes for 50 sorghum genotypes indicating basal thermotolerance in seedlings in two sets.

SET 1			SET 2		
Genotype	Heat shocked	Non- heat shocked	Genotype	Heat shocked	Non heat shocked
Top 10			Top 10		
NPGRC1695	30.80^{abcdef}	36.06^{ab}	NPGRC1704	16.72^{g-x}	22.5^{ab}
IS24426	$30.56^{abcdefg}$	$28.86^{abcdefg}$	IS9567	16.57^{h-z}	22.13^{abc}
NPGRC1478	$29.24^{bcdefgh}$	33.28^{abcd}	IS24426	16.22^{i-z}	18.48^{b-n}
NPGRC1592	$22.77^{bcdefghi}$	$27.67^{bcdefghi}$	NPGRC1222	15.03^{l-D}	18.31^{c-n}
NPGRC3087	$22.95^{bcdefghi}$	$26.33^{bcdefghi}$	NPGRC1759	14.86^{m-D}	18.22^{c-o}
“MACIA”	$19.11^{bcdefghi}$	$26.20^{bcdefghi}$	NPGRC1628	$14.2O^{o-D}$	19.32^{b-j}
NPGRC1868	$22.80^{bcdefghi}$	$24.91^{bcdefghi}$	NPGRC1197	14.08^{o-D}	18.45^{b-n}
IS2867	$20.81^{bcdefghi}$	$24.75^{bcdefghi}$	NPGRC1868	14.07^{o-D}	21.0^{a-f}
IS26191	$13.47f^{ghi}$	$24.67^{bcd}f^{ghi}$	NPGRC1178	14.07^{o-D}	$20.24^{a-}i$
NPGRC1704	$24.57^{bcdefghi}$	$25.31^{bcdefghi}$	NPGRC1782	13.98^{p-D}	14.66^{n-D}
Bottom 5			Bottom 5		
IS9548	14.52^{fghi}	$18.34^{cdefghi}$	IS12391	11.56^{D}	17.97^{c-q}
IS9303	14.11^{fghi}	$19.09^{bcdefghi}$	NPGRC3087	11.33^{D}	18.63^{b-n}
IS6944	13.01^{ghi}	$18.81^{bcdefghi}$	NPGRC3127	11.20^{D}	15.94^{j-C}
IS30164	10.92^{hi}	$17.97^{cdefghi}$	NPGRC3092	11.13^{D}	19.02^{b-m}
NPGRC3127	9.65^{i}	$20.42^{bcdefghi}$	“SV4”	11.0^{D}	18.66^{b-m}
Overall mean	22.17		15.94		
LSD	7.82		1.85		

Means with similar superscripted letter(s) in the same column were significantly different at $p<0.05$. Only 10 top performing and five least performing genotypes are presented for each set.

4.3.3. Differential expression of acquired thermotolerance in tropical sorghum genotypes

Genotypes NPGRC3093 and IS24272 consistently demonstrated superiority in acquired thermotolerance in two sets of the experiment as confirmed by separation of means using the Bonferroni's test **(Table 6)**. No genotype showed consistency amongst the least performers for the two sets. Interestingly, several genotypes that were among the least performers in basal thermotolerance showed superiority in acquired thermotolerance. These genotypes are NPGRC1704, IS9567, NPGR3093, IS12391, and IS9548 **(Tables 3** and **4)**. Genotype NPGRC3127 was the only genotype that consistently showed lack of basal thermotolerance **(Table 5)**. It also showed lack of thermotolerance in one of the sets for the acquired thermotolerance experiment **(Table 6)**.

Table 6. Mean comparison of seedling coleoptile length changes for 50 sorghum genotypes in acquired thermotolerance.

SET 1			SET 2		
Genotype	Acclimatized	Non- acclimatized	Genotype	Acclimatized	Non -acclimatized
Top 10 performing genotypes					
IS13837	13.56^{a}	1.88^{B-D}	NPGRC3124	22.32^{a}	4.64^{d-j}
NPGRC1704	12.5^{ab}	3.24^{s-D}	IS12391	18.2^{ab}	7.18^{c-j}
NPGRC1619	12.09^{a-c}	1.63^{CD}	IS29925	18.18^{ab}	2.98^{g-j}
IS9567	11.99^{a-d}	2.8^{v-D}	IS24272	13.30^{bc}	2.32^{g-j}
NPGRC1699	11.65^{a-d}	2.9^{t-D}	NPGRC3093	10.72^{cd}	4.12^{e-j}
NPGRC1476	11.48^{a-d}	2.75^{w-D}	NPGRC1197	10.11^{c-f}	2.25^{g-j}
NPGRC3093	11.24^{a-e}	1.94^{B-D}	NPGRC1868	10.02^{c-f}	3.31^{g-j}
IS24272	11.21^{a-e}	2.64^{y-D}	NPGRC1478	8.71^{c-g}	2.76^{g-j}
NPGRC3195	11.13^{a-e}	4.42^{o-D}	NPGRC1782	8.48^{c-h}	5.8^{d-j}
IS9548	11.12^{a-e}	3.40^{s-D}	IS30164	8.42^{c-i}	4.98^{d-j}
Bottom five performing genotypes					
NPGRC3105	6.69^{g-y}	1.74^{CD}	NPGRC3195	4.22^{e-j}	4.55^{d-j}
IS13813	6.43^{i-z}	5.07^{m-C}	NPGRC1862	3.78^{e-j}	2.86^{g-j}
NPGRC3127	5.94^{k-B}	3.51^{s-D}	NPGRC3105	3.6^{f-j}	1.55^{j}
NPGRC1593	5.08^{m-C}	2.89^{t-D}	IS9548	2.67^{g-j}	3.15^{g-j}
NPGRC1782	4.66^{n-D}	3.41^{s-D}	IS6944	2.45^{g-j}	3.8^{e-j}
Overall mean	6.18		5.35		
LSD	1.83		2.87		

Means with similar superscripted letter(s) in the same column were significantly different at $p<0.05$. Only 10 top performing and 5 least performing genotypes are presented.

4.4. Discussion

The study established existence of basal and acquired thermotolerance in sorghum as previously observed in several other studies with other plants **(HOWARTH et al., 1997; PAVLI et al., 2011; GALICIA-JUÁREZ et al., 2020)**. Coleoptile length was successfully established as an indicator for both basal and acquired thermotolerance in heat shocked and non-heat shocked treatments, and between acclimatized and non-acclimatized treatments respectively. In a similar study by **ARYA et al. (2014)**, germination and coleoptile elongation was reduced at temperatures above 35 ºC in pearl millet which is a hardy crop that can match or surpass sorghum. High temperatures that were used to heat shock seedlings at 50 ℃ in this study mimic the soil temperatures in agronomic habitats of crop plants in semi-arid and arid tropics which may exceed 50 ℃ as indicated by **YADAV and ARYA, (2013)**. It is noteworthy that the effects of HS are organ and stage of growth specific **(BOKSZCZANIN and FRAGKOSTEFANAKIS, 2013)**. Temperatures that exceed the threshold lead to HS which is associated with accumulation of ROS manifesting itself as oxidative stress **(GONG et al., 2001; MITTLER, 2006; KUMAR and RAI, 2014; YADAV et al., 2016; DOS SANTOS et al., 2022)**. Reduced coleoptile elongation that was noted is a result of direct and indirect consequences of oxidative stress on cellular homeostasis, which include inhibition of protein synthesis, denaturation of proteins and other macromolecules, disintegration of membrane lipids and loss of membrane integrity **(NAHAR et al., 2016; BERNFUR et al., 2017; KHAN et al., 2021)**. The prime indirect effect is retardation of cell division and cell elongation which are critical physiological processes in growth and development of all seedlings **(PAUPIÈRE et al., 2014; HATFIELD and PRUEGER, 2015; DOS SANTOS et al., 2022).**

At the peak of reported unfavorable effects of HS, sorghum has been proven to be among crops that tolerate and adapt to excessive temperatures above their threshold even at early stages of growth **(SANKAR and SATYAVATHI, 2014; GALICIA-JUÁREZ et al., 2020)**. This study confirmed the existence of genetic variability within sorghum genotypes for basal and acquired thermotolerance, as previously shown in several studies on sorghum and other related hardy crops like pearl and finger millet **(SANKAR and SATYAVATHI, 2014; YADAV et al., 2016; TACK et al., 2017)**. These observations in this study, together with the earlier assertions, results supports notion by **CRAUFURD and PEACOCK (1993)**, that survival and crop establishment of these aforementioned thermophilic crops is mainly hinged on thermotolerance rather than DS tolerance and is associated with genetic variation. Genotypes like NPGRC1704 and IS24426 that consistently exhibited inherent superiority in the current study, showing relatively less inhibition of coleoptile elongation. This lends support for the existence of basal thermotolerance in sorghum. Supporting the claims that some genotypes are able to emerge and continue to grow at temperatures above optimal **(YADAV et al., 2016)**.

Basal thermotolerance is conceivably dependent on the intrinsic expression of transcripts for heat shock proteins, ROS scavenging enzyme like catalase, osmoprotectants, secondary metabolites and antioxidant factors that offer protection against oxidative stress without prior exposure to lethal temperatures **(PAVLI et al., 2011; AHMAD et al., 2021; NDLOVU et al., 2021)**. It is associated with a swift response and protection against acute heat episodes that are becoming frequent with climate change in SATs **(BOKSZCZANIN and FRAGKOSTEFANAKIS, 2013)**.

Some genotypes, such as NPGRC3093 and IS24272, consistently exhibited resilience to heat shock but after their prior exposure to gradual increases of temperatures up to suboptimal temperatures, is suggestive of the existence of acquired thermotolerance. Acquired thermotolerance is uniquely achieved through stimulation of specific defense pathways in response to gradually increasing temperatures **(LARKINDALE et al., 2005; YEH et al., 2012; AHMAD et al., 2021)**. During the “priming” period there is accumulation of certain transcripts that code for molecular chaperones such as HSPs that offer protein folding, osmolytes like polyamines, proteins, secondary metabolites, including ROS quenching enzymes like CAT and APX that offer protection against oxidative stress **(NAHAR et al., 2016; BERNFUR et al., 2017; AHMAD et al., 2021)**. The heat shock based protection mechanism is complemented by a highly conserved sensory and signaling network that triggers heat shock regulation pathways thereby enhancing acquired thermotolerance **(MITTLER et al., 2012; NAHAR et al., 2016)**. If we are to go by the mechanisms that confer acquired thermotolerance in crop plants, adaptation and evolution are also more likely possibilities that render this kind of thermotolerance **(YADAV et al., 2014; HADEBE et al., 2017; PRASAD et al., 2021; RAD et al., 2023)**. Supporting the well documented claim that sorghum evolved in SATs characterized by high temperatures, hence their resilience to extreme temperatures **(CHAKRABARTY et al., 2022; KHALIFA and ELTAHIR, 2023)**.

Interestingly, two genotypes that were consistent in acquired thermotolerance in the current study, are research materials originating from Tanzania and Chiredzi in Zimbabwe, respectively. Though the exact location of origin of the Tanzanian genotypes is not known, most of the country is semi-arid **(WORKU et al., 2020)**. Chiredzi in Zimbabwe is in the lowveld region characterized by high temperatures with average maximum temperature ranging between 28 and 32 °C **(CAIRNS et al., 2013; NHIWATIWA et al., 2017; CHANZA and MUSAKWA, 2022)**. Chiredzi is a sugar cane area which is a typical C_4 plant like sorghum hence the convincing possibility of adaptation of these genotypes to high temperatures that rendered them the observed acquired thermotolerance. This could be a co-evolutionary adaptation mechanism of germplasm to HS in these areas. In the current study some genotypes displayed one type of resilience and not the other while some exhibited both. This signifies that the differences in the type of thermotolerance displayed by crop plants is determined by the quality and quantity of the heat shock proteins that are produced prior or during HS stimuli **(YADAV et al., 2016)**. Whereas quite a number of genotypes in the present study exhibited traits of both basal and acquired thermotolerance, featuring in at least one of the sets of each assay. These were identified as NPGRC1704, IS9567, NPGRC1197, NPGRC1868 and NPGRC1782 **(Tables 3 and 4)**. This indicates thermotolerance diversity where certain subsets of HSR genes overlap rendering the genotype both types of thermotolerance **(YEH et al., 2012)**. This is also shown in expression of HSR genes across plant tissues, organs and growth stages, thus hypocotyl, roots and many more organs have been used as indicators of thermotolerance **(LARKINDALE et al., 2005; SONG et al., 2012; AHMAD et al., 2021)**. Significant positive correlation between acquired thermotolerance, expression of heat shock proteins and yield was established in wheat and is a possibility in sorghum as well **(JOSHI et al., 1997; SUD and BHAGWAT, 2010)**.

Significance of G×E implies that genotypes can be specifically deployed to areas prone to HS. Popular varieties like “Macia” and “SV4” may not perform in the near future given the increased severity and recurrence of drought. This is of great concern given their large-scale production by subsistence farmers in Southern Eastern and Central Africa. According to **IPCC (2014)**, global atmospheric temperature is likely to increase by 2.5 to 5.8 ºC in the current century exacerbating the likelihood of HS. It was also interesting to note that differences in coleoptile elongation between the acclimatized and non-acclimatized sorghum seedlings was greater than that observed between the heat shocked and non-heat shocked seedlings in the basal thermotolerance assay. This posits acclimation as a more superior form of thermotolerance and an adaptive mechanism that enables crop plants to survive HS levels that would otherwise be lethal in its absence **(BOKSZCZANIN and FRAGKOSTEFANAKIS, 2013)**.

4.5. Conclusions and future perspectives

Sorghum genotypes showed differential response to heat shock in both basal and acquired thermotolerance indicating great diversity. Amongst the top ten performers for basal thermotolerance, genotypes NPGRC1704 and IS24426 emerged as the most consistent in the two sets of the assay while for acquired thermotolerance assay, genotypes NPGRC3093 and IS24272 consistently demonstrated superiority. Genotypes NPGRC1704, IS9567, NPGRC1197, NPGRC1868 and NPGRC1782 exhibited potential traits of both basal and acquired thermotolerance. Expression profiling will also be necessary to understand the diversity of elicited proteins while phenotyping and genotyping of the identified potential genotypes may be extended to other growth stages to enhance selection efficiency. Identified genotypes are potential donors in crop improvement programs that seek to improve thermotolerance in sorghum.

CHAPTER 5: MORPHOLOGICAL RESPONSES OF SORGHUM SEEDLINGS TO HEAT, AND DROUGHT AND COMBINED STRESSES

5.1. Introduction

There is substantial agreement among botanists and agronomists that a combination of soil-water deficit and extreme atmospheric temperatures is the most undesirable conditions for crop production **(MITTLER, 2006**; **VILE et al., 2012**; **CHANIAGO et al., 2017**; **KHANTHAVONG et al., 2022)**. Early establishment and survival of crops in the field is largely dependent on rainfall distribution rather than the total amount in a season **(RIZHSKY et al., 2004)**. Similarly, extreme temperatures negatively affect crop growth and development particularly at the seedling stage. Heat waves are characterized by great variability in intensity, duration and timing, more often than not coinciding with dry spells **(JHA et al., 2014; EGGEN et al., 2019a)**. Prolonged soil-water deficit and extremely high temperatures that cause damage to normal physiological functioning in crops are termed HS and DS respectively **(CAIRNS et al., 2013; CVIKROVÁ et al., 2013; MESEKA et al., 2018)**. Temperatures above 35 ℃ are considered extreme in semi-arid tropics and lead to HS in crops **(AHMAD et al., 2021)**. It is quite disturbing to note that frequency and magnitude of the two stresses, separately or in combination, has escalated in recent years due to climate change and variability **(IVANOVA et al., 2016; EGGEN et al., 2019; HASSAN et al., 2022)**. The recent developments present a great threat to cropping and food production, especially in the semi-arid and arid regions of the world and particularly in much of Africa **(HADEBE et al., 2017; COTRINA CABELLO et al., 2023; KHALIFA and ELTAHIR, 2023)**.

S*orghum bicolor* (L.) Moench (sorghum) is a well-documented thermophilic, drought tolerant and hardy cereal crop that is grown and serves as a source of livelihoods particularly in marginal areas **(PENNISI, 2009; YAHAYA et al., 2023)**. The crop plays a major role in food security, livestock feed, biofuel and brewing is very salient world over **(BIBI et al., 2012; CHANIAGO et al., 2017; KHALIFA and ELTAHIR, 2023)**. Despite its perceived adaptation to most abiotic stresses **(PAVLI et al., 2011; GALICIA-JUÁREZ et al., 2020; HASSAN et al., 2022)**, heat and drought stresses occurring separately or in combination at various critical ontogenetic stages negatively affect its productivity **(JAGTAP et al., 1998; MITLER et al., 2016; KHALIFA and ELTAHIR, 2023)**. The situation is likely to worsen in the next few years as predicted by **MACKAY (2008)**, thus, the rising interest in resilient crops such as sorghum. Germination, emergence and seedling stages of any crop are critical growth phases vulnerable to the dual abiotic stresses, sorghum is no exception **(HOWARTH et al., 1997; WU et al., 2009; YUAN et al., 2011; ABRO et al., 2022)**. Empirical evidence of detrimental effects of water deficit at seedling establishment and growth in various crops like maize, millets, barley and sorghum is well documented **(IVANOVA et al., 2016; ÁLVAREZ-IGLESIAS et al., 2017; AHMADI et al., 2018)**. Drought stress is known to inhibit cell division and elongation which impedes growth of both roots and shoots in seedlings **(CRAUFURD et al., 1996; HATFIELD and PRUEGER, 2015; QUEIROZ et al., 2019)**. On the other hand, HS has been shown to destabilize membranes and most temperature dependent metabolic reactions in plants **(PAUPIÈRE et al., 2014; ZHAO et al., 2016; BERNFUR et al., 2017)**. Accordingly, the adverse effects of HS and DS in seedlings is topical, especially given the drive to promote sorghum to build resilience in areas with limited capacity to adapt to climate change and variability **(MOSHELION, 2020; CHADALAVADA et al., 2021)**. Vast genetic resources are available for sorghum **(CHAKRABARTY et al., 2022)**, which is a starting point for identifying promising genotypes which can cope with HS and DS.

Over the years, mainstream research focused on pre- and post-anthesis DS for cereals like maize and wheat **(RIZHSKY et al., 2004; FAROOQ et al., 2009)**. Recent interest in resilient crops such as sorghum, that thrive in marginal areas characterized by extreme temperature and erratic rainfall, has shifted focus to studying the effects of a combination of stresses (HS and DS) rather than occurring singly **(ZEGADA-LIZARAZU and MONTI, 2013; PRASAD et al., 2021)**. The alteration of growth parameters by each factor separately and in combination is certainly unique and diverse in genotypes for each crop **(RIZHSKY et al., 2002; MITTLER, 2006; YAHAYA et al., 2023)**. Thus, it is important to understand traits that are directly and indirectly related to tolerance to HS and DS individually and in combination. In isolated cases it has been reported that for plant breeding purposes measurement and screening for tolerance to HS and DS as separate factors is preferred **(HANDAYANI and WATANABE, 2020)**. Tolerance of plant species to abiotic stress is measured by their ability to establish with vigor under limiting environmental conditions **(YE et al., 2018)**. Long seedling roots is assumed to be associated with early vigor that ultimately determine the final grain and biomass yield **(MAGALHÃES et al., 2016; AHMADI**

et al., 2018). This information is very crucial to physiologists and plant breeders, offering great potential in producing varieties that cope with problematic abiotic stresses at critical stages of growth. Furthermore, a positive correlation has been established between root and coleoptile length in seedlings and grain yield in sorghum **(ALI et al., 2009; ALI et al., 2011)**. This makes seedling root and coleoptile characters critical when evaluating its superior germplasm for HS and DS.

Laboratory or pot assays reduce cost, labor requirements and have less complexities compared to field trials **(AHMADI et al., 2018; OSMOLOVSKAYA et al., 2018; WASAYA et al., 2018)**. Spatial and temporal constraints in replicated field experiments that seek to assess tolerance to DS and HS often renders the results inconclusive **(ÁLVAREZ-IGLESIAS et al., 2017)**. Use of PEG to induce osmotic stress offers a good short term assessment tool at emergence to early seedling stages to mimic drought **(ÁLVAREZ-IGLESIAS et al., 2017; SURBHAIYYA et al., 2018; ABRO et al., 2020; ABRO et al., 2022; RAD et al., 2023)**. Furthermore, HS is easier to induce in controlled environments hence in some instances it has been used as a screening protocol for both HS and DS **(PEACOCK, 1982)**. However, the current need for conclusive empirical evidence on their combined and individual effects in various crops of importance such as sorghum compels the current study. Thus, this study is aimed at characterizing selected high potential African sorghum genotypes for heat, drought and combined stress responses at early and late seedling stages based on our hypothesis that elongation of root and shoot of sorghum will not be significantly influenced by the type of stress and their genotypic interactions at early and late seedling stages.

5.2. Materials and Methods

5.2.1. Description of the study site

The study was carried out at an experimental plot and Seed Science Research Laboratory in the Faculty of Agricultural Sciences at Lupane State University which is located 18°93’00S, 27°75’93E and altitude of 1016 meters above sea level in Zimbabwe. The study site is situated in Agro-ecological Region IV and has a climate characterised by hot and dry summers and cold winters with temperatures ranging from 10-41 °C. The rainy season usually begins in November ending in April and an average of 450-650 mm is received annually. The rainy season is usually short as rains begin late and terminate early. The rainy season is characterised by early and mid-season dry spells. Soils are dominantly Kalahari sands with low water holding capacity and are inherently infertile **(MAKUVARO et al., 2014)**.

5.2.2. Planting material

As stated in Chapter 3.

5.2.3. Study type

The study consisted of two separate experiments; a laboratory experiment that assessed effects of stress on 7-day-old seedlings of sorghum and a pot experiment that assessed the effects of induced stress on 21-day-old seedlings carried out in an open field and temperature-controlled rooms. This represented early and late seedling stages respectively for the 48 uncharacterized genotypes and two check varieties bringing the total to 50 diverse genotypes.

5.2.3.1. Determination of the effects of *in vitro* induced drought, heat and combined stresses at early seedling stage (7-day-old)

5.2.3.1.1. Experimental design

A two-factorial laboratory experiment was laid out following a split-plot arrangement in a completely randomized design with two replicates. The two factors were stress and genotype. The main factor was stress at four levels i.e. HS alone (seedlings placed in distilled water and kept at controlled atmospheric temperature of 40 °C), DS alone (20% PEG solution at 25 °C atmospheric temperatures), combined heat and drought stresses (20% PEG solution and 40 °C), and non-stressed (distilled water and atmospheric temperature of 25 °C) as a control. Drought stress was artificially induced by placing seedlings in 20% PEG solution with a low osmotic potential of 0.85 Mpa. Heat stress was induced by placing seedlings in a temperature-controlled room at 40 °C.

Combined heat and drought stresses were created by placing seedlings in 20% PEG solution in a controlled room at 40 °C atmospheric temperature. The non-stressed condition (control) was induced by placing seedlings in 250 ml plastic containers containing 20 ml of de-ionized water with a high osmotic potential of 0 Mpa at a controlled temperature of 25 °C. The second factor was genotype at 50 levels i.e. 48 sorghum genotypes and two check varieties i.e. "Macia" and "SV4". Treatment combinations were as follows; a total of 50 genotypes × 4 artificially created growing conditions which gave a total of 200 treatment combinations replicated twice. An experimental unit was a 250 ml plastic container with two sorghum seedlings (sampling units) placed in 20 ml of either distilled water or 20% PEG solution. Treatments were induced 7 days after germination and represented as 'early seedling stage' in this study.

5.2.3.1.2. Laboratory procedures and management

Sorghum seeds of similar size for each genotype were sterilized using 1% hypochlorite for 5 min and rinsed thrice using distilled water. Thereafter, 20 seeds for each genotype were germinated in Petri dishes lined with double Whatman No. 2 filter papers moistened with 7 ml of distilled water and placed in an incubator at 25 °C. Measurements for seedling hypocotyl and root lengths were taken 7 days after germination before placing two seedlings in 250 ml plastic containers completely immersing the roots in 20 ml of either 20% PEG solution for DS treatments or distilled water for the control. Seedlings were then left to acclimatize in a room for 24 h under artificial light provided by cool white compact fluorescent lamp (Amberlite, China) at light intensity of 672 mmol.m^2.s-^{1} at room temperatures of 22-25 ºC. Subsequently they were placed randomly in temperature-controlled rooms with artificial lighting for the respective temperature treatments for 96 h before taking final measurements.

5.2.3.2 Pot experiment: Determination of drought, heat and combined stresses at late seedling stage (21-day-old)

5.2.3.2.1. Treatments and experimental design

The experiment was laid out as a split-plot following a randomized completely block design with three replications. Treatments were as follows; T_1 - no stress (seedlings placed in a temperature-controlled room **(Figure 6)** at temperatures of 25-35 ℃ for 1 h between 12h00 and 13h00 midday under artificial light provided by cool white compact fluorescent lamp (Amberlite, China) at a light intensity of 672 $mmol.m^2.s\text{-}^1$ to mimic the maximum optimum temperatures for sorghum at the vegetative stage **(PRASAD and STAGGENBORG, 2008)**, and watered to field capacity whenever seedlings showed signs of rolling and folding leaves at 06h00 in the morning to avoid DS as a control, following a method of visually determining DS by **SANCHEZ-DIAZ and KRAMER (1971)**, T_2- DS alone (seedlings placed under optimum atmospheric temperatures as explained in T_1 and watered to 50% field capacity when seedlings showed signs rolling or folding leaves at 06h00 in the morning) **(ENGELBRECHT et al., 2007)**, T_3 - HS alone (seedlings placed in a temperature-controlled room **(Figure 6)** with temperatures between 35-40 ℃ for 1 h between 12h00 and 13h00 midday, to mimic environmental HS, and watered to field capacity (when seedlings showed signs of rolling or folding leaves at 06h00 in the morning to avoid DS) and T_4 - combined HS and DS (induced using procedures T_2 and T_3 for DS and HS treatments respectively). During the induction of the above-mentioned treatments kaylite float trays for each treatment were placed randomly. Seedlings were assessed at 21 days after emergence referred to as 'late seedling stage' in this study and treatments were induced for 14 consecutive days. For the rest of the experimentation period outside treatment induction the kaylite trays with growing seedlings were kept in an open field under natural light, watered to field capacity and the prevailing atmospheric temperatures were 26-35 ℃ during the day and 18-22 ℃ at night.

5.2.3.2.2. Planting and management

Kaylite float trays (250 celled) were filled with the same amount of Kalahari sand premixed with the recommended compound D (7N:14P:7K + 8.5%S) fertilizer at a rate of 5 g per cell which equates to 200 kg/ha^{-1}. Seedlings for each genotype were germinated in rows with 10 planting cells in kaylite trays with 25 rows, hence two trays to make a block that can handle all 50 genotypes. Watering to field capacity (until water started dripping from holes at the bottom of trays) was done using a fine rose head every two days before emergence, and after emergence it was done when more than 50% of seedlings showed signs rolling or folding leaves at 06h00 in the morning **(SANCHEZ-DIAZ and KRAMER 1971; ENGELBRECHT et al., 2007)**. The seedlings were grown in natural sunlight and temperature in an open field for 21 DAE. Thereafter, treatments were administered for 14 consecutive days. HS treatments were induced for 1 h between 15h00 and 16h00 every day when temperatures are usually at its highest. The temperature-controlled rooms were used to ensure standardized temperatures for the 14 consecutive days which would otherwise vary and be unpredictable if natural conditions were used. Planting was done in the third week of September 2022.

Figure 7. Induction of HS to 21-day-old seedlings (late seedling stage) in a temperature-controlled room.

5.2.3. Data collection

5.2.3.1. Laboratory (*in vitro*) experiment at early seedling stage

Seedlings for each experimental unit were measured for root and hypocotyl (germinating seedling stem) length in mm using a digital Vernier caliper (INCCO HDCO01150, China) before and after treatments. Changes in hypocotyl and root length were determined. Measurements before the treatments were subtracted from the measurements after the four days of being exposed to the treatments.

5.2.3.2. Pot experiment (late seedling stage)

After 14 days of treatment induction, five seedlings for each genotype were randomly selected from each replicate of each treatment, carefully uprooted by pushing through the hole at the bottom of each cell, then the soil washed off and dissected into roots and shoots. Shoot length and root length were then measured.

5.2.4. Data analysis

Data for hypocotyl and root length at early seedling stage for the *in vitro* experiment **(NDLOVU et al., 2023)**, was analyzed using a two-way ANOVA test following a completely randomized design with stress and genotype as the two main factors in GenStat 13th Edition **(PAYNE et al., 2012)**, to determine any significant differences in means of stress treatments, genotypes and their interactions. All the data were subjected to tests of the assumptions of ANOVA prior to analysis. Where significant differences were found at $p \leq 0.05$, the means were separated using the Bonferroni's test.

Data on shoot and root length changes at late seedling stage assessed in a pot experiment **(NDLOVU et al., 2023)**, were subjected to the Additive main Effects and multiplicative interaction (AMMI) in GenStat 13th edition. The AMMI analysis was performed to fit the additive effects of the factors i.e. genotype (G), environments (E) and their multiplicative effects for genotype × environment interaction (GEI). AMMI, genotype main effects by genotype × interaction (GGE) biplots and which-won-where graphical display were also plotted in GenStat software for the pot experiment and laboratory experiment respectively, to qualitatively assess and visualize genotypes on root and shoot growth performance, their adaptability and stability in the four different artificial created test environments that were studied **(YAN and TINKER, 2006)**.

5.3. Results

5.3.1. Main effects of stress (S), genotype (G) and their interactions (G×S) on root and hypocotyl lengths of sorghum at early seedling stage

The analysis of variance of root and hypocotyl length of seedlings for 50 sorghum genotypes showed highly significant ($p< 0.001$) main effects of stress, genotype and their interactions **(Table 7)**. Stress contributed the greatest variation (51.2%) on shoot length while root length was chiefly influenced by the genotype × stress interaction (30.7%) at early seedling stage.

Table 7. Two-way Analysis of Variance of the main effects of *in vitro* induced stress (S), genotypes (G) and their interactions (G × S) on root and hypocotyl length of 7-day-old sorghum seedlings.

SOV	Mean of squares		
	D.f	Root length	Hypocotyl (shoot)
Stress	3	2400.4***	2829.7***
Genotype	49	119.0***	35.2***
Genotype × Stress	147	64.49***	17.57***
Error	600	14.0	6.29
Total	799	30913.2	16570

SOV= sources of variation; D.f = degrees of freedom; ***significant differences at $p<0.001$.

5.3.2 Main effects of *in vitro* induced stress on root and hypocotyl elongation of sorghum at early seedling stage

Combined HS and DS had the most adverse effect on growth of sorghum seedlings according to hypocotyl elongation, though it was not significantly different from that of HS applied separately **(Table 8)**. Combined stresses reduced hypocotyl length by 77.5%. No significant differences were observed between the three stresses that were assessed i.e. HS alone, DS alone, and the combined stresses on root elongation at an early seedling stage. Seedlings that were exposed to combined stresses had the lowest mean length **(Table 8)**.

Table 8. Analysis of Variance and mean comparisons of roots and hypocotyl lengths of 7-day-old seedlings of sorghum genotypes assessed in four *in vitro* induced test environments.

	Hypocotyl	Roots
Stress	Mean length change	Mean length change
No stress	9.99^{a}	10.35^{a}
Drought stress	2.96^{b}	3.79^{b}
Heat stress	2.28^{c}	3.33^{b}
Combined stress	2.25^{c}	3.18^{b}
Significance	***	***
LSD	0.49	0.74

Means superscripted with different letter(s) were significantly different at $p<0.05$, *** = significant at $p<0.001$.

5.3.3. Influence of interaction of genotype and stress on root and hypocotyl elongation of sorghum at early growth stage of seedlings

Following the significant interaction of the genotype and environment on both root and hypocotyl length in sorghum seedlings, the means were compared using Bonferroni test. The top five performing i.e. potential resilient genotypes in each stress level were selected for presentation

(Table 9). Genotypes IS13904, IS3474, IS9405, IS13813 and NPGRC3283 showed resilience to combined stresses. Genotypes IS13904, IS3574, IS9405, IS24272, and NPGRC3124 were identified as the top performers under independently applied HS while genotype IS3574 also showed resilience to DS applied separately along with genotypes NPGRC1699, IS30047, NPGRC3097 and NPGRC1197 **(Table 9)**. It is noteworthy that genotype IS3574 was among the top five performers in terms of hypocotyl length in all three stressed environments and genotype IS13904 in all three test environments for root length at early seedling stage. Genotype IS13904 exhibited an outstanding performance in both root and hypocotyl length under combined stresses applied separately.

Table 9. Analysis of Variance and mean comparison of root and hypocotyl lengths for 7-day-old sorghum seedlings as influenced by the interaction between four different *in vitro* induced environments and genotypes.

Environment	Top 5 performing genotypes	Mean hypocotyl length changes (mm)	Top performing genotypes	Mean root length changes (mm)
Combined stresses	IS13904	6.33^{c}	NPGRC1475	18.42^{cd}
	IS3574	6.13^{c}	NPGRC1478	14.94^{de}
	IS9405	5.51^{c}	IS30164	11.45^{ef}
	IS13813	5.49^{c}	IS9549	9.07^{ef}
	NPGRC3283	4.73^{c}	IS13996	6.82^{f}
Drought stress	IS3574	7.58^{bc}	IS13813	17.95^{cd}
	NPGRC1699	6.97^{bc}	IS30164	14.53^{de}
	IS30047	6.61^{bc}	IS13996	14.51^{de}
	NPGRC3092	6.43^{bc}	NPGRC1699	6.85^{f}
	NPGRC1197	4.91^{c}	NPGRC1476	6.83^{f}
Heat stress	IS13904	9.83^{b}	IS13813	19.80^{bc}
	IS3574	7.87^{bc}	IS3574	13.96^{de}
	IS24272	6.62^{bc}	IS30015	12.81^{de}
	NPGRC3124	5.04^{c}	IS2847	10.05^{ef}
	IS9405	4.73^{c}	IS30164	9.02^{ef}
No stress (Control)	IS2419	17.39^{a}	IS3904	33.5^{a}
	IS13904	17.29^{a}	IS3574	29.42^{a}
	IS24272	16.85^{a}	IS24272	26.16^{b}
	IS9548	15.8^{a}	NPGRC3092	22.54^{bc}
	NPGRC3092	15.74^{a}	IS13996	16.24^{d}
Significance at $p<0.05$		***		***
LSD		3.4		5.2

Genotype means with similar superscripted letter(s) in the same column were not significantly different at $p<0.05$. *** means significant different at $p< 0.001$.

5.3.4. Performance of the sorghum genotypes in terms of hypocotyl length of 7-day-old seedlings under four *in vitro* induced environments in a laboratory

Following the noted differential response of genotypes to the different stress levels, particularly in hypocotyl elongation of seedlings at early seedling stage noted in ANOVA, data was further plotted into a which-won-where graphical display of the GGE biplot **(Figure 7)**, which visually displays the genotypes that performed exceptionally well in a certain mega environment at an early seedling stage. The first two principal components accounted for 89% of the total variation of the G×E interaction for hypocotyl length. The which-won-where polygon view of the GGE biplot shows that for hypocotyl length the vertex genotypes were IS3574 and IS13904 **(Figure 7)**. The two genotypes fell inside a sector containing HS and DS and were regarded as top performers in terms of hypocotyl elongation under the two environments **(Figure 7)**. Most genotypes were clustered close to the origin of the biplot, this indicates that these genotypes were not specifically adapted to any of the stress environments.

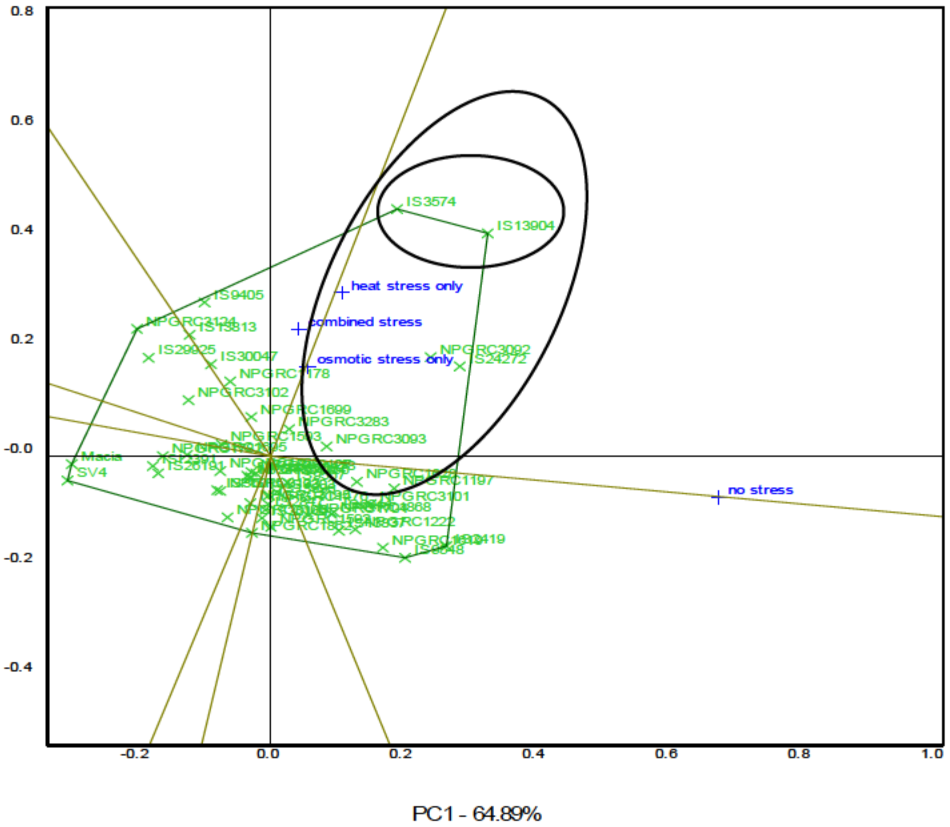

Figure 8. The GGE biplot which-won-where polygon of 50 sorghum genotypes for shoot length at early seedling stage. Genotypes at the vertex of the polygon had an outstanding performance in the stress levels that are within the sector containing that particular vertex.

5.3.5. Effects of genotype (G), induced test environments (E) and G×E interactions on shoot and root length at late seedling stages of sorghum in a pot experiment

AMMI revealed that genotype, an artificially induced growing environment, and G×E interaction effects were all highly significant ($p<0.001$) for root and shoot length growth at late seedling stage **(Table 10)**. AMMI analysis indicated the highest proportion of total sum of squares attributed to the induced stress levels at late seedling stage was on shoot length (25.6%), with 6.5% attributed to genotypic effects, and 13.5% to the G×E interaction effects **(Table 10)**. A weaker contribution of the genotype and environmental effects were noted on root length i.e. 5.9 and 2.3% respectively, while G ×E interaction contribution was higher at 17.1%.

Table 10. AMMI analysis for root and shoot length at late stage seedling stage of 50 sorghum accessions evaluated in four simulated environments in a pot experiment.

	Root (mm)			Shoot (mm)		
Source of Variation	DF	Mean Square	TSS Explained (%)	DF	Mean Square	TSS Explained (%)
Genotype (G)	49	1118***	5.9	49	12586***	6.5
Environments (E)	3	7031***	2.3	3	807864***	25.6
Block	8	288^{ns}		8	6954^{ns}	
Interaction (G×E)	147	1072***	17.1	147	8715***	13.5
IPCA$_1$	51	2015***	11.1	51	12017***	6.5
IPCA $_2$	49	677***	3.6	49	10 124***	5.2
Residuals	47	461		47	3663	

DF= degrees of freedom; ***= significant at p<0.001; ns = not significant p>0.05; TSS= Sum of Squares; IPCA= Interaction principal component axes.

5.3.6. Mean performance of sorghum genotypes in four environments for root and shoot elongation at late seedling stage (pot experiment)

Four models for the two parameters of the AMMI model family indicate the best-performing sorghum genotypes under drought, heat and combined stresses **(Table 11)**. Seedlings exposed to combined HS and DS had the lowest shoot length mean of 108.0 mm, followed by 115.6 mm for the DS. Mean root length for seedlings exposed to DS alone had a lower mean of 62.6 mm when compared to HS (65.67 mm) and combined stress treatments (66.62 mm) **(Table 11)**. Shoot length means showed a higher variability than the root length with the greatest variability for the two parameters under study observed in heat stressed treatments while combined stress environments had the least variance across **(Table 11)**.

Table 11. Best performing sorghum genotypes in four stress levels in terms of seedling root and shoot elongation according to the AMMI model families in a pot experiment.

Parameter	Stress	Mean (mm)	Variance	Ranking of top four genotypes			
				1	2	3	4
Root length	Heat stress	66.62	307.9	NPGRC1704	IS9548	**IS6944**	NPGRC1593
	Drought stress	62.60	261.0	**IS26191**	1S9567	**IS6944**	**NPGRC1478**
	Combined stresses	65.67	242.3	NPGRC1628	NPGRC1619	NPGRC3127	**NPGRC1478**
	No stress	70.08	269.5	NPGRC3127	NPGRC1628	IS29925	NPGRC1592
Shoot length	Heat stress	166.4	2586	**IS9567**	IS24426	**IS30015**	NPGRC1868
	Drought stress	115.6	2287	**IS26191**	NPGRC1782	**IS30015**	**IS30164**
	Combined stress	108.0	1247	NPGRC1476	IS2847	IS30164	IS9567
	No stress	170.2	3294	IS9567	NPGRC3102	NPGRC1476	NPGRC1699

Genotypes in bold appeared as the top four performers in more than one stress.

Best performers under the drought stressed environment on root length were genotypes IS29191, IS9567, IS6944 and NPGRC1478. Genotypes IS9527, IS30164 and IS30015 exhibited resilience across the three stressed environments in terms of shoot elongation, while IS6944 and NPGRC1478 were amongst the best performers in root length growth in at least two stressed environments that were tested. Genotype IS30164 showed good performance in both shoot and root length in HS alone and in combined stresses. Genotypes NPGRC1476, IS2847, IS30164, and IS9567 were the best performers for shoot length under the most detrimental condition of combined stress **(Table 11)**. Drought stress applied independently was observed as the second most suppressing test environment on shoot length as confirmed by the visual assessment shown in **Figure 8**. The following genotypes IS26191, NPGRC1782, IS30015 and IS30164 showed resilience to DS in terms of shoot elongation. Good performers for shoots were not necessarily the best for the roots except for IS26191 under DS conditions.

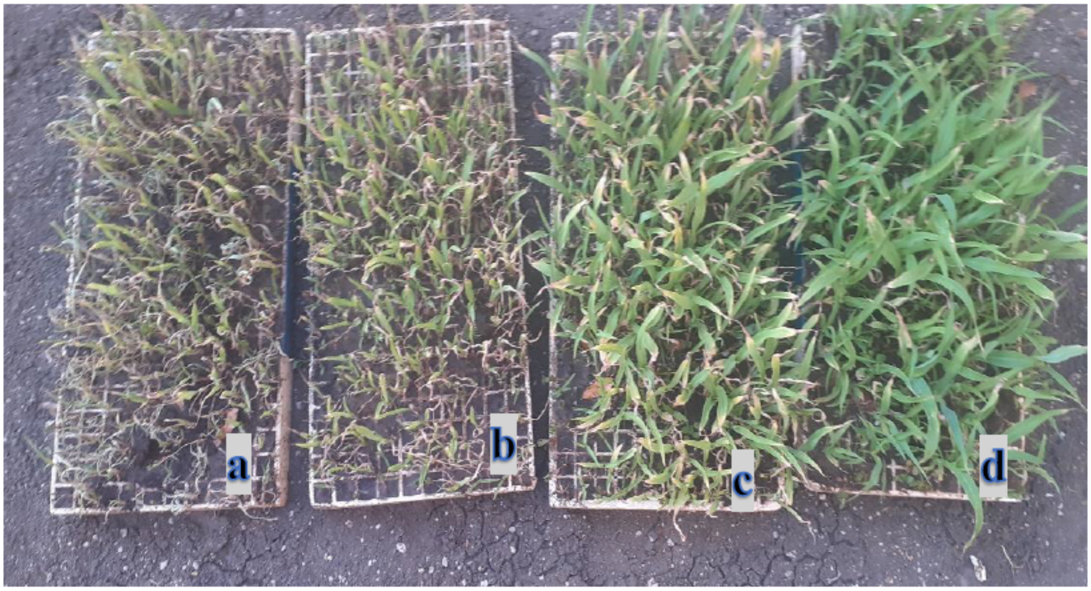

Figure 9. Qualitative comparison of sorghum seedlings exposed to the four test environments (a) combined stresses (b) drought stress (c) heat stress and (d) no stress at 21 days after emergence.

5.3.7. Interaction of stress and genotypes on shoot length for sorghum seedlings at late stage in a pot experiment

In a similar manner as in the early seedling growth stage, shoot length was the most affected by stress levels, hence the AMMI biplot was used to display the interaction of stress environments and genotypes in terms of shoot length. AMMI biplot for shoot length of 50 sorghum genotypes tested under four environments at late seedling stage clearly show that combined stress and DS alone had an adverse effect on shoot length. Heat stress induced separately had a less adverse effect on shoot length. Furthermore, the plot discriminated against performance and stability of genotypes in the test environments. A significant number of genotypes, including the check variety "SV4", showed average performance and static stability in shoot growth under the four environments due to their proximity to the origin of the IPCA. The range of the mean shoot length was between 100 and 180 mm showing less variability. Best performing and genotypes with dynamic stability are NPGRC1478, NPGRC1476, IS30015 and IS30164. Genotypes close to the origin of axis show statistic performance and these are IS13904, IS9405, IS12391 and the check variety "SV4". Genotypes NPGRC3093 and IS24272 showed a distinct positive association with combined DS and HS as indicated by their proximity **(Figure 9)**.

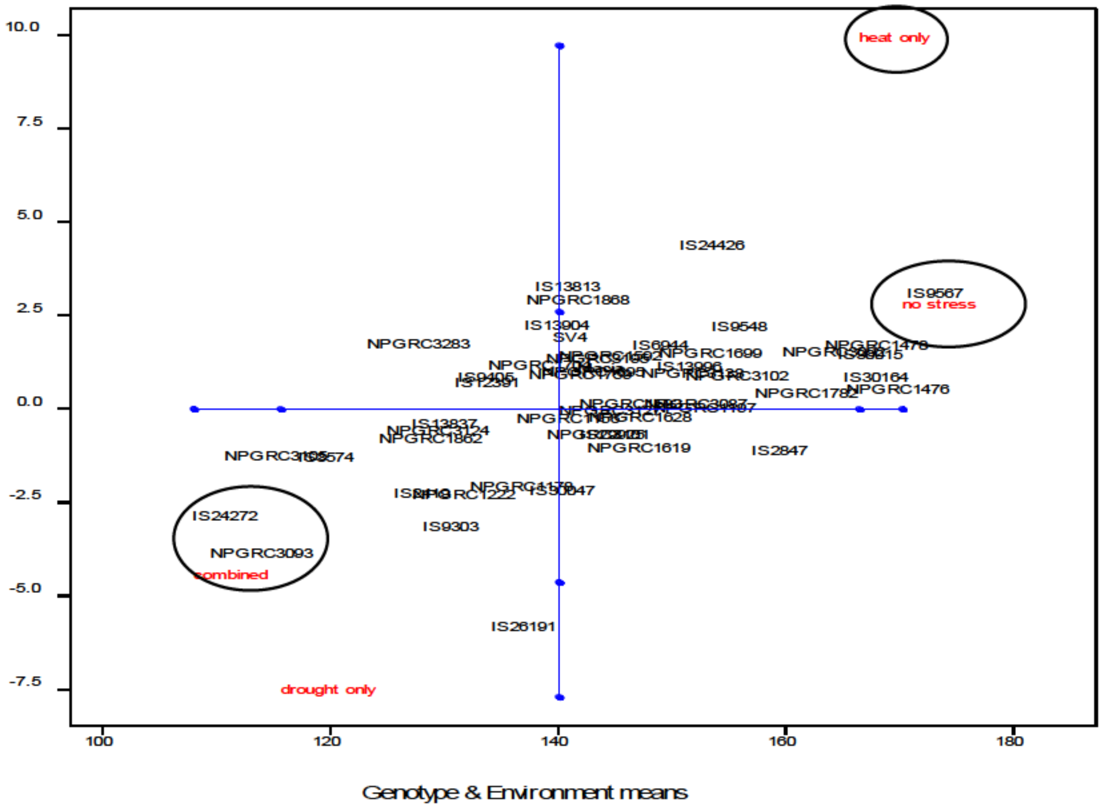

Figure 10. AMMI biplots of shoot length at late seedling stage for the 50 sorghum genotypes within four test environments studied in a pot experiment.

5.4. Discussion

The observed significant influence of genotype, environment and G×E interactions on root and hypocotyl (shoot) elongation at both early and late seedling stages in the current study is in line with several reports that root and shoot growth in seedlings at different growth stages for various crops is regulated by genetics but also with notable environmental effects **(BIBI et al., 2012; EHDAIE et al., 2012; YE et al., 2018; ABRO et al., 2020)**. The observed adverse effects of HS and combined stresses on shoot elongation, more than that of DS, is attributed to increased levels of ROS that has been reported damage useful biomolecules which then affects mostly the foliage in delicate seedlings **(GOSAVI et al., 2014)**. Thus, indicating the sensitivity of the shoot, due to emergence of leaf structures where several biochemical reactions occur, particularly photosynthesis **(PYNGROPE et al., 2013; TRIPATHI et al., 2020)**. However, the three stresses that were assessed had a more or less similar suppressive effect on root length though DS occurring alone seems to be less injurious than HS and the combination of the two stresses.

In most studies DS was found to be less detrimental on root elongation in seedlings probably because at early growth stages plants tend to prioritize water and nutrient uptake hence invest more in root systems than in shoots **(EHDAIE et al., 2012; AHMADI et al., 2018)**. In accordance with our findings, other studies in sorghum **(CRAUFURD and PEACOCK, 1993; JAGTAP et al., 1998; WIPF et al., 2021; KHALIFA and ELTAHIR, 2023)**, pearl millet **(YADAV et al., 2016; GOLHARE and LATA, 2019)**, wheat **(MACHADO and PAULSEN, 2001; KELES and ONCEL, 2002)** and maize **(CAIRNS et al., 2013; MESEKA et al., 2018; TANDZI et al., 2018)**, reported more injurious effects of combined HS and DS on shoot height.

The differences in the degree of stress detection, signal transduction and gene expression by different species lead to differences in dealing with the types of stress **(CONDE et al., 2011)**. Roots are understood to be the first organs to detect DS in the soil and defense mechanisms are signaled to enable adjustments of metabolic and physiological processes that conserve water and promote root elongation at the expense of plant stem growth, hence offering them some level of resistance to mild DS to the above ground parts **(ZAHRA, 2012)**. There are several metabolites like proline that are reportedly synthesized to induce osmotic adjustment in order to maintain water uptake and root elongation during DS in plants **(PATANÈ et al., 2013; SUZUKI et al., 2014; DOS SANTOS et al., 2022)**. Increased root elongation under limited soil water conditions enables the plant to reach deeper soil levels to absorb water which is expected to increase with plant growth stages as water requirements increase **(OSMOLOVSKAYA et al., 2018; YE et al., 2018)**. Thus root length has been pointed out as a good indicator of drought tolerance which becomes more efficacious in seedling establishment under mild drought stressed conditions **(QUEIROZ et al., 2019a; PRASAD et al., 2021)**. It is indeed common for certain traits to respond to one stress and not the other **(VILE et al., 2012)**. Variations in root and shoot morphological traits across DS, HS and combined stresses in sorghum seedlings have been recorded in several research findings (**MACHADO and PAULSEN, 2001; TSUJI et al., 2005**; **MAGALHÃES et al., 2016**; **WASAYA et al., 2018**; **AHMADI et al., 2018**; **PRASAD et al., 2021**).

AMMI confirmed the highly significant influence of genotype, induced environment and G×E interactions on root and shoot growth at the late seedling stage. Unlike at early seedling stage assessed *in vitro*, DS suppressed root elongation more than combined stresses and HS applied individually. The results are contrary to most research findings where root length has been identified as least sensitive to DS applied separately **(EHDAIE et al., 2012; CHANIAGO et al., 2017; ABRO et al., 2020)**. Perhaps the induced DS levels in the pot experiment of the current study was too severe given the limited amount of soil present in cells of kaylite trays used in the study which led to rapid cellular dehydration reducing cell division, expansion and elongation, eventually reducing growth of seedlings **(KESKIN et al., 2010; TSAGO et al., 2014)**. The observed differences in response of roots and shoots to different stresses at the two assessed seedling stages could be suggestive of independent shoot and root physiological responses to HS and DS which are also stage dependent. Thus, the assessed test environments were effective in segregating the genotypes in terms of shoot and root length. This implies that genotypes can be selected for specific environments and stages of seedling growth using one of the parameters depending on the objectives of crop improvement. Accordingly, specific genotypes need to be deployed depending on the prevalent stress factor which complicates recommendations. This will enable the selection of high potential sorghum genotypes with specific and broad adaptations to the environments in terms of root growth **(DE VITA et al., 2010)**.

Genotypes NPGRC1478 and IS30164 showed tolerance to DS applied separately and to combined HS and DS but not to HS applied separately in both shoot and root growth at both stages. From the AMMI biplots **(Figure 9)**, it can be inferred that the two genotypes also exhibited dynamic stability and specific adaptability (deviations from zero on the ordinate) that means consistently high performance above the mean to specific environments that encourages optimal performance **(DE VITA et al., 2010)**. This explains the existence of cross tolerance in sorghum genotypes **(WOLDESEMAYAT et al., 2018; PRASAD et al., 2021**; **WIPF et al., 2021; HASSAN et al., 2022)**, which is attributed to existence of synergistic relations and overlapping of defense mechanisms **(SUZUKI et al., 2014)**. Genotype NPGRC1476 that showed high performance to combined stress on shoot elongation **(Table 9)** and NPGRC3105 tolerance to HS on root elongation **(Table 9)** were among the dynamic stable genotypes to note. Such desirable genotypes show more contribution to the G×E interactions and are more responsive to improved environmental factors and changes in provision of a resource like irrigation **(SETIMELA et al., 2017)**.

Maintenance of an average and constant performance in terms of shoot and root length growth of seedlings in the four test environments, with less variability was shown by some genotypes like IS13904, NPGRC1156 and "SV4" which distinctly showed static stability and wide adaptation (close to the ordinate) in root length. Such genotypes are less responsive to the tested stresses and associated with consistently minimal performance in all the stresses **(DE VITA et al., 2010)**. Typical of the check varieties that were used in the study, which were certainly bred for biological stability from locally adapted lines to suit smallholder farmers with minimum resources in marginal areas. There were a few cases of high performing and erratic genotypes like IS9567, which can be explained by its adaptation to an unstressed environment displayed in the AMMI biplot for shoot length **(Figure 9)**, and of course some poor performing and erratic cases like genotypes IS24272 and NPGRC3093 **(Figure 9)**, which are less desirable.

The GGE biplot showed potential adaptation of genotypes IS3574 and IS13904 to heat and drought stresses applied separately at an early seedling stage **(Figure 7)**. However, deployment of such genotypes will be quite limited as the two stresses usually occur concurrently. Tolerance of sorghum genotypes to HS and DS is also attributed to adaptation to their places of origin **(ENYEW et al., 2022)**. A significant number of genotypes like NPGRC1478, IS3574 and IS13904 that exhibited combined HS and DS tolerance are local landraces of Zimbabwe and regional areas such as KwaZulu-Natal Province, a typical hot Province in neighboring South Africa **(Supplementary Table S1)**. **DUGAS et al. (2011)** explained the development of heritable morphological characteristics, like the rooting system, which enhances tolerance. Continuous exposure to an environmental stress alter some biological functioning of plants like time to sensing of external stimuli and activation of defense mechanisms rendering the differences in response and cross adaptation to abiotic stress like HS and DS in genotypes **(GONG et al., 2001; DOS SANTOS et al., 2022; GUIHUR et al., 2022)**. Given the diversity of agro-ecological regions, where the assessed germplasm was collected from, such varied responses to the stresses was expected.

5.5. Conclusions and future perspectives

The present results indicated highly significant effects of genotype, stress and their interaction on root and shoot length growth at both early and late seedling stages. Combined HS and DS was found to significantly suppress shoot length at both stages. Individual effects of HS, DS and their combination equally suppressed root length at an early seedling stage while DS applied separately were found to be more adverse on root elongation at a later stage of seedling growth in sorghum. Genotypes IS3574 and IS13904 exhibited the highest mean performance in shoot elongation and specific adaptation to HS and DS applied separately but not when combined at an early seedling stage. One genotype IS13813 performed exceptionally well and exhibited specific adaptations to all three assessed stressed conditions for root length at an early seedling stage. At late seedling stage genotypes NPGRC1478, NPGRC1476, IS30015 and IS30164 exhibited best performance and dynamic stability in shoot length while genotypes NPGRC1478, NPGRC1619 and NPGRC3105 were identified as best performers and dynamic stable genotypes in root elongation independent of root and shoot. These genotypes can be used as donors for tolerance, depending on the stage of seedling growth and trait that needs improvement. Overall, genotype IS30164 showed tolerance to DS applied separately and to combined stresses for both shoot and root length at both stages of seedling growth, while genotypes IS13904, NPGRC1156 and a commercial check variety distinctly showed a salient static stability and wide adaptation in both shoot and root growth at the late seedling stage. Genotypes NPGRC1478 and IS13904 are recommended for breeding programs that seek to improve sorghum tolerance to combined stresses at seedling stage and deployment in marginal areas of the arid and semi-arid tropics given the empirical evidence on positive correlation between final grain yields and root and shoot characteristics at seedling stages in sorghum.

CHAPTER 6: PRE-ANTHESIS MORPHO-PHYSIOLOGICAL RESPONSE OF TROPICAL SORGHUM TO COMBINED HEAT AND DROUGHT STRESS

6.1. Introduction

A combination of the ever-increasing global food demand driven by increasing population and recent recurrences of harsh environmental conditions, intensified by climate change and variability, is instigating huge anxiety to all patrons concerned with food production. Soil moisture deficit is regarded as the most limiting abiotic factor in rainfed agronomic habitats, and indications are, when in combination with excessive atmospheric temperatures its effects are aggravated **(MACHADO and PAULSEN, 2001; CHANIAGO et al., 2017; WOLDESEMAYAT et al., 2018; BEGNA et al., 2022; CABELLO et al., 2023)**. Atmospheric temperatures above the earth's surface are expected to increase by 3.7 to 4.8 ºC in the present century **(IPCC, 2014)**, meaning the effects of DS on crop production are yet to worsen and destabilize crop production especially in the semi-arid and arid regions of SSA **(MONGI et al., 2010; KIHARA et al., 2011; NAIM et al., 2017; HADEBE et al., 2017; BEGNA et al., 2022)**.

Over 500 million inhabitants of the SSA region are dependent on rainfed hardy millets as staple crops **(JAIN et al., 2010; MORRIS et al., 2013; BEGNA et al., 2022; KHALIFA and ELTAHIR, 2023)**. Sorghum (S*orghum bicolor* (L.) Moench) is one of the putative multipurpose hardy crops grown in dry and warm regions is facing more risk than other crops and has been pushed into the limelight **(KHATON et al., 2016; TACK et al., 2017; PRASAD et al., 2021; CHADALAVADA et al., 2021)**. The potential of "King of millets" (sorghum) in SATs to withstand the current and anticipated climatic conditions, and continue to be sustainably produced, is under threat. Sorghum ontogenesis is characterized by three distinguished periods. The first growth stage (GSI) between emergence and panicle initiation, secondly pre-flowering, between panicle initiation and anthesis, (GSII) and thirdly between anthesis and physiological maturity (GSIII) (**KAYALVIZHI and ANTONY, 2011)**. GSIII can also be further split into flowering and grain filling stages. All the aforementioned stages of growth in the life cycle of sorghum are undesirably affected by HS and DS occurring separately or in combination, impacting on growth and development.

Despite the noted simultaneous occurrence of HS and DS, especially in semi-arid and arid tropical regions, the effects of the two are mostly studied separately **(MITTLER, 2006; LAWAS et al., 2018; YOUSAF et al., 2022)**. This is partly because induction of combined heat and drought stresses is difficult and expensive as it requires specialized growth chambers or temperature regulating facilities and rain exclusion shelters. These which are costly unless planting dates are manipulated to coincide with the dry and very hot periods of the season with growth stages of interest. Furthermore, variations in response of sorghum to these abiotic factors is dependent on species variability, stage of growth, type, intensity and duration of stress **(BEYENE et al., 2015; GANO et al., 2021; DOS SANTOS et al., 2022; YOUSAF et al., 2022)**. Two distinct responses of sorghum to stress are known as pre- and post-flowering **(BEYENE et al., 2015; LOBELL et al., 2015)**. Much seminal research work has focused on response of sorghum species to DS at flowering and grain filling stages **(MUCHOW and CARBERRY, 1990; VAN OOSTEROM et al., 1996; TACK et al., 2017; CARCEDO AND GAMBIN, 2019; OGDEN et al., 2020; DE SOUZA et al., 2021)**. Water deficit during flowering and at grain filling stages affect morphological, physiological, biochemical processes and consequently yield components **(ASSEFA et al., 2010; TOVIGNAN et al., 2016; LAWAS et al., 2018; DE SOUZA et al., 2021)**. Yield losses to post-flowering DS in rainfed sorghum are estimated in the region of 50-55% **(KHATON et al., 2016)**. For a long time post-flowering DS has been viewed as the only threat, conversely the pre-flowering stage has been found to be equally affected by mid-season dry spells **(OGDEN et al., 2020)**. Pre-flowering DS contributes up to 36% grain yield losses in sorghum **(ASSEFA et al., 2010)**. Pre-flowering DS is also associated with leaf senescence in most crops **(GEORGE-JAEGGLI et al., 2017; BEGNA et al., 2022; ENYEW et al., 2022)**. Heat stress has been found to be equally detrimental at both pre- and post-flowering, while the effects of combined HS and DS remain inconclusive **(ANANDA et al., 2011; LAWAS et al., 2018; PRASAD et al., 2021b)**. Extreme temperatures exceeding a threshold of 32-33 ºC **(TACK et al., 2017)**, just before flowering, reportedly decreased plant stem height, number of flowers and panicle size in sorghum **(BEGNA et al., 2022)**. A combination of the two stresses during pre-flowering stage result in abortion of the florets, reduced panicle size, and significant reduction in seed size in rice **(AKMAN, 2009; LAWAS et al., 2018),** maize **(HU et al., 2010; MESEKA et al., 2018)** pearl millet **(YADAV et al., 2016)**, sorghum **(PRASAD and STAGGENBORG, 2008; MACHADO and PAULSEN, 2016; YAHAYA et al., 2023)** and wheat **(MACHADO and PAULSEN, 2001; QASEEM et al., 2019)**, all of which consequently decreased the yield potential **(NAIM et al., 2017)**. The harmful effects of the dual stresses on pollen viability and receptive ability of the stigma significantly impede pollination and fertilization during the reproductive stages of sorghum **(PRASAD and STAGGENBORG, 2008; TANG et al., 2008; JHA et al., 2014; HATFIELD and PRUEGER, 2015)**. As a result, formation of yield apparatus is severely affected leading to substantial yield losses **(WENZEL, 1999; KHATON et al., 2016; NAIM et al., 2017; AL-GHZAWI et al., 2018)**.

Resistance and tolerance of a crop to stress involves biochemical, physiological and morphological traits **(HASANUZZAMAN et al., 2013; NAHAR et al., 2016; UPADHYAY, 2019; SARSHAD et al., 2021)**. An array of morpho-physiological traits in sorghum, such as increased relative leaf water content, high cellular membrane stability and high canopy temperature depression under conditions of limited available soil water and excessive atmospheric temperatures, are indicative of its ability to resist and/or tolerate HS and DS that occur individually or concomitantly **(GAVUZZI et al., 1997; MACHADO and PAULSEN, 2001; KUMAR and RAI, 2014; MACHADO and PAULSEN, 2016; OGDEN et al., 2020; NDLOVU et al., 2021)**. If we are to achieve a holistic approach of minimizing yield losses to the dual stresses there is an urgent need to cascade scientific inquiries on the effects of combined heat and drought stresses using various indicators. The scope of sorghum cultivars needs to be expanded in order to sustain its productivity in the face of climate change and variability. For that reason, this study aimed to assess the morpho-physiological response of tropical sorghum genotypes to combined HS and DS at pre-flowering stage in order to identify potential donors of various desirable traits.

6.2. Materials and Methods

6.2.1. Planting material

As described in Chapter 3.

6.2.2. Experimental design

Performance of genotypes was evaluated in combined HS and DS induced 10 days before flowering i.e. at booting stage in a pot experiment for two consecutive seasons 2021/22 and 2022/23. The pot experiment was laid out in an open field following a split-plot in a randomized complete block design with three replicates **(Figure 10)**. The main factor was stress i.e. combined HS and DS and no stress as a control. Genotypes were the sub-factor at 50 levels allocated randomly in each condition. Combined HS and DS was induced by planting at the beginning of September under irrigation then watering was withheld at booting stage in mid-October, that was 10 days before flowering **(PRASAD et al. 2015)**. Watering was done at 50% of the predetermined field capacity of the soil only when the plants showed signs of rolling or folding leaves at 06h00 in the morning to keep them drought stressed but avoiding wilting **(SANCHEZ-DIAZ and KRAMER 1971; ENGELBRECHT et al., 2007)**. This period coincided with maximum temperatures of 35-40 ℃ **(Table 12)**, exceeding the optimum of 25-28 ºC hence also inducing heat stress which coincided the pre-flowering stage with a period of combined heat and drought stresses. The timing of induction of the combined stresses was genotype dependent since most had different booting times determined during preliminary studies, flowering started at 55-65 days after sowing (DAS) for most genotypes **(PRASAD et al., 2015)**. Combined HS and DS treatments were maintained for 10 consecutive days. Non-stressed treatments were planted at the beginning of December with supplementary irrigation to avoid HS and DS. Pots were watered to field capacity (until water started to drip from holes at the bottom of the pots) whenever the plants showed signs of rolling or folding leaves at 06h00 in the morning to avoid DS **(SANCHEZ-DIAZ and KRAMER, 1971)**. Thereafter the plants were watered equally and sufficiently until physiological maturity. During the rainy season treatments were rainfed, and supplementary watering was done on dry episodes until crop physiological maturity.

Table 12. Rainfall (mm) and temperature (℃) data during the trial period at Lupane State University experimental plots in seasons 2021/22 and 2022/23.

Season		Months					
		Oct	Nov	Dec	Jan	Feb	Mar
2021/22	Mean minimum temperature	26.4	25.6	25.2	22.4	22.3	23.6
	Mean maximum temperature	38.6	38.2	30.7	27.4	34.1	33.6
	Total rainfall	0	43	37.7	225.7	45.5	29.6
2022/23	Mean minimum temperature	26.6	26.1	24.4	19.8	21.4	21.02
	Mean maximum temperature	39.9	37.4	31.9	28.1	33.1	32.5
	Total rainfall	9.6	164	75	161	141	36

6.2.3. Agronomic practices

Cattle manure (250 g) was premixed with 3 kg of sandy loamy soils before filling each pot and one heaped bottle top (5 g) of Compound D basal fertilizer (NPK 10:20:10 + 6.5%S) manufactured by Superfert, Zimbabwe resulting in approximately 200 kg ha^{-1} applied at each planting station at a depth of 7.5 cm in the middle part of each pot and then covered with soil to avoid direct contact with the seeds. Three seeds were planted at 5 cm depth in each planting station. Thinning to two plants per pot was done at three weeks after emergence. The pots were kept weed free, and diseases and pests were controlled as per recommended growing practices.

Figure 11. Outlay of the pot experiment showing three blocks of sorghum plants at booting stage.

6.2.4. Data collection

6.2.4.1. Laboratory assessments

All laboratory assessments were performed within five days at the end of 10 days of combined HS and DS treatment. First fully expanded leaves from the flag leaf were detached from one of the two plants in each treatment, enclosed in a polythene bag and placed in a cooler box, then transported to the laboratory. Leaf discs measuring 1 cm in diameter were cut using a cork borer for use in the following laboratory procedures.

6.2.4.2. Cellular membrane stability (CMS)

To assess the cellular membrane stability of leaves, four leaf punches were randomly selected from a pool of cut leaf punches and washed separately in deionized water 3-4 times to remove electrolytes from the injured cells at cut edges **(SRIVASTAVA et al., 2017)**. The discs were put in test tubes then immersed in a water bath and kept at 25 ºC for 1 hr. This was followed by high-temperature induction in a water bath at 54 °C for 15 min followed by incubation at 4 °C for 24 h. Discs from control treatments were maintained at 25 °C. After incubation, conductivity was measured from temperature-induced discs and controls using a digital conductivity meter, and readings were recorded as T_1 and $C_{1,}$ respectively. Test tubes were then covered in aluminum foil and autoclaved at 120 °C for 20 min, and the final conductance was measured after autoclaving and recorded as T_2 and C_2. Cellular membrane stability, also termed the ‘percentage relative injury’ (RI %), was calculated using the following formula (**GAVUZZI et al., 1997)**.

$$\text{CMS \%} = \frac{1 - [1 - (T_1/T_2)]}{1 - (C_1/C_2)}$$

6.2.4.3. Relative leaf water content determination (RLWC)

Four leaf discs were randomly selected from a pool of cut discs, and their fresh weight (FW) determined. The weighed leaf discs were then floated overnight in Petri dishes containing distilled water **(Figure 11)**. Thereafter, water on the disc's surfaces were blotted off, and turgid weight (TW) was determined. Subsequently, leaf discs were dried in a forced-air oven at 80 °C for 48 h, and dry weight (DW) was determined. The percentage RLWC was calculated using the following formula **(WEATHERLEY, 1950)**.

$$\mathrm{RLWC} = \left[\frac{(FW-DW)}{(TW-DW)}\right] \times 100\%$$

Figure 12. Punched leaf discs immersed in water to determine turgid weight as part of the experiment to determine relative leaf water content.

6.2.5. Field assessments of combined heat and drought stress tolerance

6.2.5.1. Canopy temperature depression (CTD)

Canopy temperature depression, which is the difference between ambient air (T_a) and canopy (T_c) temperatures, was determined by measuring the canopy and ambient air using a hand-held infrared thermometer (Dikang DM300 model, China). Temperature readings were performed between 1500 and 1600hrs for four hot and cloudless days during the 10 days of the induction of the stress treatments. Canopy temperature readings was performed following a method described by **FISCHER et al. (1998)**. Four readings were taken from each plant using a hand-held infrared thermometer pointed to the foliage horizontally at an angle of approximately 30º standing a meter away from each plant. Ambient air temperatures were recorded as well, and the CTD of each plant was determined using the formula:

($CTD = T_a - T_c$).

6.2.5.2. Basal diameter (BD)

A digital Vernier caliper (INCCO HDCD001150, China) was used to measure stem basal diameter at physiological maturity on the first node just above the soil.

6.2.5.3. Plant stem height (PH)

Plant height was measured at physiological maturity from the soil level to the tip of the panicle head using a graduated meter stick.

6.2.5.4. Leaf area (LA)

For each genotype intact leaf positioned number two from the flag leaf (assumed to be fully expanded) of the second plant was selected at physiological maturity, and the leaf blade length, width and midpoint measured along the midrib. Leaf area was then calculated after determining the leaf length and maximum leaf width using the following formula **(STICKLER et al., 1961)**:

Leaf length × maximum leaf width × 0.75

6.2.6. Grain weight (GW) assessment and stress tolerance indices (STI)

1000 GW from non-stressed treatments (Y_p) and stressed-treatments (Y_s) was measured for each treatment at physiological maturity. Panicle length was also measured before thrashing. The 1000 GW of non-stressed ($\overline{Y_p}$) and stressed-treatments ($\overleftarrow{Y_s}$) were used to calculate various yield and stress tolerance indices as follows;

i) Yield stability index (YSI) = $\frac{Ys}{Y_p}$ **(BOUSLAMA and SCHAPAUGH, 1984)**

ii) Stress tolerance index (STI) = $\frac{Y_p\, Y_s}{(Y_p)^2}$ **(HAO et al., 2011)**

iii) Stress susceptibility index (SSI) = $\frac{1 - {}^{Y_s}\!/_{Y_p}}{1 - \frac{\overleftarrow{Y_s}}{\overline{Y_p}}}$ **(FISCHER and MAURER, 1978)**

iv) Mean productivity index (MPI) = $\frac{Y_p + Y_s}{2}$ **(HOSSAIN et al., 1990)**

v) Drought tolerance efficiency (DTE) = $\frac{Ys}{Yp} \times 100\%$

6.2.7. Statistical analysis

Quantitative data on all the assessed morpho-physiological traits, 1000 grain weight and yield based tolerance indices were subjected to one-way-ANOVA to evaluate the performance and tolerance of genotypes under combined HS and DS conditions. All data was first subjected to tests of assumptions of ANOVA. Means were separated using Fischer's Least Significant Differences method at the 5% significance level where significant differences were detected using the GenStat version 13th edition statistical package **(PAYNE, 2012)**. Principal component analysis was used to determine the amount of variation contributed by each variable to the data in Minitab 14th edition.

Pearson’s correlation and path coefficient analysis by **DEWEY and LU (1959**) were used to determine direct and indirect effects of the independent variables on grain yield. The hierarchical cluster analysis of sorghum genotypes was performed using dissimilarity coefficients and unweighted pair-group method arithmetic average (UPGMA) of clustering for ‘tree’ construction to group the genotypes according to their similarity in performance of all the assessed parameters using DARwin 6.0.21 software.

6.3. Results

6.3.1. Analysis of variance for the main effects of combined heat and drought stress, genotypes and their interaction effects on sorghum morphological and physiological traits at pre-flowering stage in 2021/22 and 2022/23 of the pot trial

The F-probability values indicated that stress had highly significant ($p<0.01$ to $p<0.001$) negative effects on almost all traits (CTD, CMS, RLWC, BD, PH, PL, 1000GW) in both seasons except for LA in the second season and RLWC in both seasons **(Table 13)**. Statistically significant ($p<0.05$) variability in sorghum genotypes that were evaluated for the same traits was also observed in both seasons except for BD and RLWC. No significant differences were observed in CMS means in the first season of the trial. The interaction between the genotype and stress (G×S) significantly affected CTD, BD, PL and grain weight in both seasons, while CMS, PH, and LA were significantly affected in one of the seasons. No significant influence of the interaction was observed on RLWC in both seasons **(Table 13)**.

Table 13. Analysis of Variance of sorghum morphological and physiological traits grown under combined heat and drought stress, and non-stressed conditions for 2021/22 and 2022/23.

			Mean square errors							
Season	SOV	D.f	CTD	CMS	RLWC	BD	PH	LA	PL	GW
2021/22	Stress (S)	1	8926.37***	13522.1***	0.399^{NS}	562.80***	2046***	7646092***	84303***	2.38**
	Genotype (G)	49	73***	307^{NS}	1.42^{NS}	22.17^{NS}	2047***	941493*	3477***	1.15***
	S×G	49	75.15***	221.3^{NS}	0.45^{NS}	24.90*	2103***	1154061***	2246***	0.41*
2022/23	Stress (S)	1	11302***	2179**	0.22^{NS}	1627.55***	3333.3*	984138^{NS}	19764.1***	0.89*
	Genotype (G)	49	28.1***	442.3**	0.35^{NS}	17.46^{NS}	4670.4***	727754***	5517.1***	1.25***
	S×G	49	13.47***	388.6*	0.31^{NS}	21.41*	1156.5^{NS}	520264^{NS}	4325.3***	0.49***

SOV= Sources of variation; D.f= Degrees of freedom; CTD= Canopy temperature depression; CMS= Cellular membrane stability; RLWC= Relative leaf water content; BD= Basal diameter; PH= Plant height; LA= Leaf area; PL= Panicle length; GW= 1000 Grain weight; * significant at $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; NS= Non-significant $p > 0.05$.

6.3.2. Influence of the main effects of combined heat and drought stress on morpho-physiological traits of sorghum for two consecutive seasons

Analysis of variance results indicated that most of the traits that were assessed were significantly suppressed by combined HS and DS treatments in both seasons except for basal diameter whose mean was conversely significantly lower under non-stressed treatments **(Table 14)**. Only RLWC showed no statistical differences between the two treatments in both seasons though the stressed plants had lower mean values. Season 2021/22 had the highest negative impact for all the traits with the greatest impact noted in CTD with a decrease of over 100% under stress when compared to non-stressed treatments. Plant stem height was reduced by 15.3% in 2021/22 and 4.4% in 2022/23 while CMS yielded a difference of 20.1 and 8.6% in the two respective seasons. The effect of stress on leaf area remained relatively low compared to other traits that were assessed, producing a reduction of 9.3% in 2021/22 and 4.2% in 2022/23 of the trial. The impact of stress was also relatively low on yield compared to the morphological and physiological traits with 7.2 and 5% reduction in 1000 grain weight yielded respectively in the two seasons. The panicle length yielded a 17.5 and 9% reduction respectively in the two consecutive seasons of the trial. RLWC and CTD had the highest variations as shown by their percentage coefficients of variation values ranging between 50 and 95%.

Table 14. Analysis of Variance and mean comparison of sorghum morphological and physiological traits under combined heat and drought stress, and no stress treatments at pre-flowering in 2021/22 and 2022/23.

Season	Stress	BD (mm)	PH (cm)	LA (mm^2)	CTD (ºC)	CMS (%)	RLWC (%)	PL (mm)	GW (g)
2021/22	Stressed	22.96^a	138.6^b	3121.9^b	-1.40^b	53.2^b	69	158.7^b	2.32^b
	Non-stressed	20.22^b	117.4^a	3441.2^a	1.04^a	66.6^a	82	192.3^a	2.50^a
	Significance	***	***	***	***	***	NS	***	**
	Grand mean	21.6	128.0	3281.2	-0.18	59.9	76	175.5	2.41
	LSD	0.94	7.24	178.2	0.28	3.83	7.7	7.72	0.11
	%CV	19.2	24.8	23.8	58.7	28.1	89.2	19.3	20.8
2022/23	Stressed	20.06^a	148.2^b	1924	-3.06^b	57.7^b	59	163.9^b	2.11^b
	Non-stressed	15.41^b	154.8^a	2038	-0.31^a	63.1^a	64	180.1^a	2.22^a
	Significance	***	*	NS	***	**	NS	***	*
	Grand mean	17.7	151.5	1981.0	-1.69	60.36	62	172	2.17
	LSD	0.82	6.64	140.2	0.13	3.52	13	7.02	0.09
	%CV	20.4	19.3	31.1	58.1	25.6	92.4	17.9	31.1

Means with similar superscripted letter(s) in the same column were significantly different at $p<0.05$. BD= Basal diameter; PH= Plant height; LA= Leaf area; CTD= Canopy temperature depression; CMS= Cellular membrane stability; RLWC= Relative leaf water content; PL= Panicle length; GW= Grain weight; *** $p<0.001$; **$p<0.01$; *$p<0.05$; $p>0.05$; NS= non-significant.

6.3.3. Influence of interaction of genotype and combined heat and drought stress on four selected morphological and physiological traits on sorghum at pre-flowering in 2021/22 and 2022/23

Genotypes showed significant differential responses to combined HS and DS and non-stress induced environments in the two seasons of the trial. Some traits were significantly affected in both seasons while some in only one of the seasons. The performance of the traits is detailed as follows:

6.3.3.1. Basal diameter

Basal diameter measured at plant physiological maturity was significantly influenced by an interaction between genotypes and combined HS and DS induced at pre-flowering stage in both seasons **(Table 15)**. In both seasons the plants grown under combined stresses had thicker stems than the unstressed plants though not statistically different. Differential performance was noted in the genotypes under the two test environments. In the first season of the trial (2021/22), the only significant difference ($p<0.05$) was noted for genotype NPGRC3093 which had the highest mean of 27.97 mm under the control, and genotype NPGRC1156 which had a mean of 13.93 mm under the same treatment **(Table 15)**. In the second season (2022/23), genotype IS24426 under the combined HS and DS treatment had the highest mean of 26.38 mm outperformed 10% of its counterparts under the same test condition, however, it was not significantly different from all the other treatment combinations. Genotype NPGRC3105 consistently performed as the best performing genotype in the two seasons **(Tables 15** and **16)**.

6.5.3.2. Plant stem height

In 2021/22 one genotype (NPGRC1592) had the highest mean stem height of 217.3 cm under non-stressed conditions and was significantly higher than about 20% of the genotypes that were grown under stressed conditions. Ten percent of the genotypes were outperformed by genotype NPGRC3097 which had the second highest plant stem height mean of 210 cm under the unstressed environment as well **(Table 15)**. In 2022/23 outstanding performance was noted in genotype

NPGRC3127 under the stress test environment and was significantly different from the lowest performing genotype NPGRC3283 also under the stress conditions **(Table 16)**.

6.3.3.3. Leaf area

Significant differential performance of genotypes under the two test environments was only noted in the first season. Genotypes NPGRC1222 with a mean of 3966 mm^2 and IS9405 with a mean of 3955 mm^2 showcased superior performance under the stressed treatments, both significantly outperformed 10% of the genotypes under non-stressed treatment **(Table 15)**.

6.3.3.4. Canopy temperature depression

Canopy temperature depression was significantly ($p<001$) affected by an interaction between genotype and combined HS and DS induced at pre-flowering stage in both seasons. The check variety "Macia" had the coolest canopy recording 1.7 ℃, cooler than the ambient air under stressed conditions, outperforming over 10% of its counterparts under the same conditions in 2021/22. Genotype IS3574 was the second performing genotype though way lower than "Macia", significantly different from the least performing genotype NPGRC1476 which had a canopy temperature of 7.2 ℃ higher than the ambient air under stressed conditions **(Table 15)**. Some of the least performing genotypes were IS13813, NPGRC3124 and IS29925. In the second season only genotype NPGRC3092, which had the coolest canopy under the stressed environment, though its canopy temperature was 1.9 ℃ higher than the ambient air and was significantly different from only one genotype NPGRC1862 which was the least performing in terms of cooling its canopy under stressed conditions **(Table 16)**. Other superior genotypes were NPGRC1478, IS30015, IS24426 and IS12391 though not significantly different from their counterparts. CTD showed the highest variation in both seasons and no variety showed consistence in the two seasons.

6.3.3.5. Cellular membrane stability

Cellular membrane stability was significantly ($p<0.05$) affected by the interaction between genotypes and stress only in the second season. Genotype NPGRC3127 under combined heat and drought stressed conditions had the most stable cell membranes with a mean of 85.45% even higher than the unstressed treatments though it was only significantly different from NPGRC3283 under the same treatment. Other genotypes that exhibited stable membranes as noted by high CMS values are NPGRC1222, NPGRC1476, IS30015, IS29925 and NPGRC3124 though their CMS values were not significantly different from other genotypes in both test environments **(Table 16)**.

6.3.3.6. Panicle length

Highly significant ($p<0.001$) influence of the interaction between genotype and stress induced at pre-flowering on panicle length was observed in both seasons. Genotype NPGRC1704 with a mean of 233.3 mm displayed a superior performance under a stressed environment and significantly outclassed about 10% of its counterparts under the same treatment in 2021/22. In 2022/23, genotypes IS30015 and IS24426 managed to maintain longer panicles of 265 and 260 mm respectively showing no differences with unstressed plants and outperformed 10 and 20% of their un-stressed and stressed counterparts respectively.

6.3.3.7. Relative leaf water content

No statistical ($p>0.05$) differences in performance of the sorghum genotypes under stressed and non-stressed conditions were observed on RLWC in both seasons of the experiment.

Table 15. Analysis of Variance and mean comparison of morphological and physiological traits of sorghum genotypes under combined heat and drought stress and no stress treatments at pre-flowering in 2021/22.

	PH		BD		LA		CTD	
Genotypes	Stressed	Non-stressed	Stressed	Non-stressed	Stressed	Non-stressed	Stressed	Non-stressed
IS12391	134.3^{a-c}	151^{a-c}	23.97^{ab}	22.75^{ab}	2933^{i-r}	4610^{a-e}	-2.25^{a-i}	0.15^{a-i}
IS13813	126^{a-c}	171.7^{a-c}	24.56^{ab}	19.75^{ab}	3231^{g-r}	3333^{f-r}	-3.76^{ij}	-0.58^{a-i}
IS13837	117.7^{a-c}	141.7^{a-c}	25.27^{ab}	19.77^{ab}	2475^{o-r}	4142^{a-i}	-2.4^{c-i}	1.24^{a-g}
IS13904	138.3^{bc}	149^{a-c}	22.48^{ab}	17.65^{ab}	2935^{i-r}	3416^{d-q}	-2.04^{a-i}	0.06^{a-i}
IS13996	94.7^{c}	130^{a-c}	25.76^{ab}	22.01^{ab}	2730^{k-r}	2621^{m-r}	-1.87^{a-i}	2.06^{ab}
IS2419	90^{c}	117.3^{a-c}	23.81^{ab}	18.27^{ab}	2861^{j-r}	2721^{k-r}	-0.97^{a-i}	1.11^{a-g}
IS24272	147.7^{a-c}	91.7^{c}	20.66^{ab}	21.42^{ab}	2450^{p-r}	4793^{ab}	-0.36^{a-i}	-0.93^{a-i}
IS24426	96.7^{bc}	92^{c}	19.41^{ab}	22.28^{ab}	3240^{f-r}	3055^{h-r}	-0.47^{a-i}	0.70^{a-i}
IS26191	145.3^{a-c}	68.3^{c}	22.22^{ab}	24.98^{ab}	3343^{f-r}	4648^{a-d}	-1.14^{a-i}	-0.65^{a-i}
IS2847	74.3^{c}	160^{a-c}	23.68^{ab}	15.11^{ab}	2794^{j-r}	3011^{i-r}	-0.98^{a-i}	1.69^{a-i}
IS29925	127^{a-c}	152.7^{a-c}	25.46^{ab}	23.49^{ab}	3720^{a-o}	3883^{a-l}	-3.42^{h-j}	1.49^{a-d}
IS30015	71.3^{c}	143.3^{a-c}	25.51^{ab}	21.63^{ab}	2791^{j-r}	4936^{a}	-0.35^{a-i}	0.96^{a-h}
IS30047	129.3^{a-c}	135^{a-c}	20.42^{ab}	18.74^{ab}	2947^{i-r}	3481^{c-q}	-1.29^{a-i}	0.90^{a-h}
IS30164	74.7c	156.3^{a-c}	23.98^{ab}	22.63^{ab}	2560^{n-r}	3130^{h-r}	-0.82^{a-i}	1.46^{a-f}
IS3574	100.3^{a-c}	102.7^{a-c}	25.16^{ab}	22.95^{ab}	3302^{f-r}	4778^{ab}	0.28^{a-i}	0.26^{a-}i
IS6944	89^{c}	100.3^{a-c}	21.59^{ab}	18.47^{ab}	3299^{f-r}	3573^{b-q}	-2.07^{a-i}	1.97^{a-c}
IS9303	107.7^{a-c}	136.3^{a-c}	27.98^{ab}	18.58^{ab}	3801^{a-n}	3148^{h-r}	-1.12^{a-i}	1.37^{a-f}

IS9405	126.3^{a-c}	162^{a-c}	21.86^{ab}	19.09^{ab}	3955^{a-k}	2873^{j-r}	-2.43^{a-i}	1.58^{a-f}
IS9548	129.3^{a-c}	172.3^{a-c}	21.09^{ab}	19.19^{ab}	2911^{i-r}	3184^{g-r}	-0.97^{a-i}	-0.18^{a-i}
IS9567	85.7^{c}	146.7^{a-c}	27.67^{ab}	19.23^{ab}	2975^{i-r}	3321^{f-r}	-0.67^{a-i}	2.17^{ab}
“Macia”	111^{a-c}	106^{a-c}	18.63^{ab}	25.25^{ab}	2847^{j-}r	2461^{o-r}	1.71^{a-d}	1.19^{a-g}
NPGRC1156	112^{a-c}	91.3^{c}	20.01^{ab}	13.73^{b}	2620^{m-r}	3014^{i-r}	-1.63^{a-i}	1.66^{a-e}
NPGRC1178	111.7^{a-c}	177^{a-c}	22.05^{ab}	18.21^{ab}	2602^{m-r}	2858^{j-r}	-1.45^{a-i}	0.47^{a-i}
NPGRC1197	120.3^{a-c}	170^{a-c}	24.9^{ab}	19.44^{ab}	2905^{i-r}	3965^{a-k}	-2.8^{f-j}	0.33^{a-i}
NPGRC1222	99^{bc}	116.7^{a-c}	27.34^{ab}	19.92^{ab}	3966^{a-k}	3500^{c-q}	-0.11^{a-i}	1.80^{a-b}
NPGRC1476	76^{c}	120.3^{c}	20.36^{ab}	20.87^{ab}	2648^{l-r}	3494^{c-q}	-7.23^{j}	2.26^{a}
NPGRC1478	100^{a-c}	149.3^{a-c}	21.86^{ab}	18.50^{ab}	3086^{h-r}	2606^{m-r}	-2.77^{e-i}	1.45^{a-f}
NPGRC1592	119.7^{a-c}	217.3^{a-c}	22.49^{ab}	17.40^{ab}	3736^{f-r}	3329^{f-r}	-0.89^{a-i}	0.84^{a-h}
NPGRC1593	101.3^{a-c}	164.3^{a-c}	26.38^{ab}	25.69^{ab}	2996^{a-c}	4703^{a-c}	-1.59^{a-i}	1.38^{a-f}
NPGRC1619	121^{a-c}	136.3^{a-c}	25.46^{ab}	17.30^{ab}	3690^{qr}	2408^{qr}	-2.14^{a-i}	2.28^{a}
NPGRC1628	101^{a-c}	149.7^{a-c}	20.79^{ab}	19.76^{ab}	3195^{h-r}	3031^{h-r}	-2.01^{a-i}	1.20^{a-g}
NPGRC1695	138.7^{a-c}	95.3^{bc}	21.6^{ab}	14.38^{ab}	3380^{j-r}	2780^{j-r}	-0.21^{a-i}	1.45^{a-f}
NPGRC1699	145^{a-c}	148.3^{a-c}	24.13^{ab}	20.21^{ab}	2805^{a-p}	3698^{a-p}	-1.32^{a-i}	2.16^{ab}
NPGRC1704	122^{a-c}	140^{a-c}	24.6^{ab}	19.85^{ab}	2763^{a-p}	3708^{a-p}	-0.22^{a-i}	1.50^{a-f}
NPGRC1759	104.3^{a-c}	154^{a-c}	18.23^{ab}	18.56^{ab}	2927^{qr}	2383^{qr}	-1.62^{a-i}	0.60^{a-i}
NPGRC1782	102^{a-c}	129^{a-c}	22.93^{ab}	18.66^{ab}	2741^{g-r}	3222^{g-r}	-0.73^{a-i}	2.24^{a}
NPGRC1862	144^{a-c}	137.3^{a-c}	21.64^{ab}	17.09^{ab}	3373^{i-r}	2999^{i-r}	-0.54^{a-i}	0.27^{a-i}
NPGRC1868	99^{bc}	164^{a-c}	25.83^{ab}	25.08^{ab}	3537^{a-h}	4282^{a-h}	-1.48^{a-i}	0.18^{a-i}
NPGRC3087	135^{a-c}	210^{ab}	19.35^{ab}	18.94^{ab}	2913^{h-r}	3130^{h-r}	-0.58^{a-i}	1.94^{a-c}

NPGRC3092	123.3^{a-c}	142.7^{a-c}	21.64^{ab}	21.41^{ab}	2938^{i-r}	2940^{i-r}	-1.58^{a-i}	1.02^{a-h}
NPGRC3093	146^{a-c}	118.3^{a-c}	19.53^{ab}	29.27^{ab}	2673^{a-g}	4415^{a-g}	-0.07^{a-i}	1.96^{a-c}
NPGRC3101	126.3^{a-c}	109.3^{a-c}	21.97^{ab}	24.51^{ab}	3066^{a-p}	3690^{a-p}	-1.59^{a-i}	0.38^{a-i}
NPGRC3102	137.3^{a-c}	149.7^{a-c}	24.25^{ab}	20.69^{ab}	3643^{i-r}	2982^{i-r}	-0.16^{a-i}	0.40^{a-i}
NPGRC3105	112.3^{a-c}	145^{a-c}	28.77^{ab}	16.35^{ab}	3821^{a-f}	4493^{a-f}	-2.58^{d-i}	0.36^{a-i}
NPGRC3124	139.3^{a-c}	136.7^{a-c}	18.94^{ab}	23.52^{ab}	3200^{a-j}	4004^{a-j}	-3.19^{g-j}	0.29^{a-i}
NPGRC3127	143^{a-c}	139.3^{a-c}	21.69^{ab}	19.74^{ab}	3166^{a-g}	4409^{a-g}	-1.11^{a-i}	0.84^{a-i}
NPGRC3133	149.7^{a-c}	113.3^{a-c}	24.66^{ab}	18.08^{ab}	3639^{i-r}	3001^{i-r}	0.13^{a-i}	1.43^{a-f}
NPGRC3195	144.3^{a-c}	178.3^{a-c}	21.58^{ab}	19.17^{ab}	3691^{r}	2152^{r}	-0.48^{a-i}	2.08^{ab}
NPGRC3283	153.7^{a-c}	139.3^{a-c}	22.27^{ab}	19.79^{ab}	3491^{i-r}	2882^{i-r}	-0.53^{a-i}	0.93^{a-h}
“SV4”	127.7^{a-c}	102^{a-c}	21.48^{ab}	21.62^{ab}	2782^{j-r}	2866^{j-r}	-1.90^{a-i}	0.50^{a-i}
Overall mean	128		21.29		3281.18		-0.18	
LSD	51.19		6.66		1260.2		1.98	
%CV	24.8		19.2		23.8		58.7	

Means with similar superscripted letters in the same column were significantly different at $p < 0.05$.

PH= Plant height; BD= Basal diameter; LA= Leaf area; CTD= Canopy temperature depression.

Table 16. Analysis of Variance and mean comparison of morphological and physiological traits of sorghum genotypes under combined heat and drought stress, no stress treatments at pre-flowering in season 2022/23.

	Traits					
	BD		CMS		CTD	
Genotype	Stressed	Non-stressed	Stressed	Non-stressed	Stressed	Non-stressed
IS12391	19.36^{a-c}	20.1^{a-c}	52.45^{ab}	64.29^{ab}	-2.29^{g-x}	0.87^{a}
IS13813	19.64^{a-c}	18.61^{a-c}	49.72^{ab}	61.02^{ab}	-3.05^{q-x}	0.33^{a-d}
IS13837	20.89^{a-c}	15.33^{a-c}	40.38^{ab}	68.82^{ab}	-3.22^{s-x}	-0.86^{a-p}
IS13904	23.98^{a-c}	14.08^{a-c}	57.21^{ab}	72.86^{ab}	-2.38^{h-x}	0.73^{ab}
IS13996	18.72^{a-c}	11.43^{bc}	39.90^{ab}	53.1^{ab}	-2.97^{p-x}	0.31^{a-d}
IS2419	18.52^{a-c}	15.15^{a-c}	56.41^{ab}	54.44^{ab}	-3.26^{t-x}	-0.91^{a-p}
IS24272	21.34^{a-c}	16.59^{a-c}	65.41^{ab}	65.93^{ab}	-3.16^{r-x}	0.23^{a-e}
IS24426	26.38^{a}	17.26^{a-c}	51.59^{ab}	56.85^{ab}	-2.17^{f-x}	-0.27^{a-i}
IS26191	17.48^{a-c}	21.26^{a-c}	30.3^{ab}	68.23^{ab}	-3.15^{r-x}	-1.60^{d-v}
IS2847	17.66^{a-c}	13.12^{a-c}	59.25^{ab}	64.09^{ab}	-2.53^{k-x}	0.47^{a-d}
IS29925	21.3^{a-c}	14.91^{a-c}	80.5^{ab}	65.22^{ab}	-3.88^{wx}	-0.55^{a-l}
IS30015	19.93^{a-c}	16.74^{a-c}	80.63^{ab}	68.87^{ab}	-2.14^{f-x}	0.61^{a-c}
IS30047	16.92^{a-c}	16.67^{a-c}	32.7^{ab}	62.11^{ab}	-2.2^{f-x}	-0.26^{a-i}
IS30164	16.58^{a-c}	15.15^{a-c}	58.6^{ab}	64.59^{ab}	-2.98^{p-x}	-0.28^{a-i}
IS3574	21.82^{a-c}	16.69^{a-c}	62.67^{ab}	61.58^{ab}	-2.43^{j-x}	-0.08^{a-f}
IS6944	17.43^{a-c}	14.54^{a-c}	44.67^{ab}	56.69^{ab}	-2.89^{o-x}	-1.11^{a-s}
IS9303	22.21^{a-c}	15.85^{a-c}	59.74^{ab}	70.52^{ab}	-3.88^{wx}	-0.60^{a-m}

IS9405	21.49^{a-c}	12.78^{bc}	64.49^{ab}	64.27^{ab}	-3.32^{u-x}	-0.52^{a-l}
IS9548	21.37^{a-c}	13.47^{a-c}	58.3^{ab}	66.93^{ab}	-3.08^{r-x}	0.05^{a-e}
IS9567	21.66^{a-c}	14.54^{a-c}	64.65^{ab}	59.73^{ab}	-2.20^{p-x}	-0.32^{a-j}
“Macia”	18.75^{a-c}	13.08^{a-c}	64.49^{ab}	62.9^{ab}	-2.39^{i-x}	0.24^{a-e}
NPGRC1156	14.63^{a-c}	13.6^{a-c}	68.42^{ab}	43.16^{ab}	-3.18^{s-x}	-1.03^{a-r}
NPGRC1178	21.76^{a-c}	16.02^{a-c}	41.05^{ab}	65.94^{ab}	-3.31^{u-x}	0.23^{a-e}
NPGRC1197	21.89^{a-c}	14.35^{a-c}	67.51^{ab}	67.59^{ab}	-3.85^{w-x}	-0.23^{a-h}
NPGRC1222	18.98^{a-c}	15.74^{a-c}	82.63^{ab}	58.74^{ab}	-3.1^{r-x}	-0.92^{a-q}
NPGRC1476	19.39^{a-c}	14.35^{a-c}	82.63^{ab}	60.2^{ab}	-2.88^{o-x}	0.75^{a}
NPGRC1478	18.29^{a-c}	15.58^{a-c}	52.77^{ab}	52.36^{ab}	-2.13^{f-x}	-0.28^{a-i}
NPGRC1592	16.43^{a-c}	16.73^{a-c}	54.75^{ab}	63.81^{ab}	-3.21^{s-x}	0.46^{a-d}
NPGRC1593	21.46^{a-c}	14.93^{a-c}	60.87^{ab}	70.56^{ab}	-3.73^{v-x}	0.09^{a-e}
NPGRC1619	16.89^{a-c}	13.92^{a-c}	55.17^{ab}	75.58^{ab}	-3.60^{v-x}	-1.43^{c-u}
NPGRC1628	21.17^{a-c}	15.24^{a-c}	51.48^{ab}	70.22^{ab}	-3.55^{u-x}	0.46^{a-d}
NPGRC1695	24.26^{a-c}	11.01^{c}	46.92^{ab}	57.15^{ab}	-3.40^{u-x}	-0.45^{a-k}
NPGRC1699	20.18^{a-c}	14.27^{a-c}	49.82^{ab}	55.32^{ab}	-3.17^{r-x}	-0.08^{a-f}
NPGRC1704	14.41^{a-c}	19.94^{a-c}	28.85^{ab}	64.52^{ab}	-2.77^{n-x}	-0.8^{a-o}
NPGRC1759	19.21^{a-c}	13.78^{a-c}	58.48^{ab}	52.09^{ab}	-3.39^{u-x}	-1.12^{a-t}
NPGRC1782	18.77^{a-c}	11.78^{bc}	72.21^{ab}	67.63^{ab}	-2.72^{m-x}	0.39^{a-d}
NPGRC1862	22.99^{a-c}	13.27^{a-c}	74.92^{ab}	47.63^{ab}	-4.04^{x}	-0.71^{a-n}
NPGRC1868.	18.1^{a-c}	16.27^{a-c}	75.15^{ab}	70.55^{ab}	-3.41^{u-x}	0.06^{a-e}
NPGRC3087	17.32^{a-c}	13.04^{a-c}	60.5^{ab}	66.57^{ab}	-2.66^{l-x}	-0.21^{a-g}

NPGRC3092	20.81^{a-c}	19.71^{a-c}	69.84^{ab}	55.58^{ab}	-1.86^{e-w}	0.35^{a-d}
NPGRC3093	22.81^{a-c}	20.66^{a-c}	42.73^{ab}	68.46^{ab}	-2.77^{n-x}	-1.67^{d-v}
NPGRC3101	22.58^{a-c}	18.65^{a-c}	40.73^{ab}	69.18^{ab}	-3.98^{wx}	-0.42^{a-k}
NPGRC3102	18.15^{a-c}	20.81^{a-c}	48.73^{ab}	63.26^{ab}	-3.26^{s-x}	-0.92^{a-q}
NPGRC3105	24.34^{a-c}	14.51^{a-c}	67.95^{ab}	76.35^{ab}	-3.44^{u-x}	-1.41^{b-u}
NPGRC3124	23.47^{a-c}	14.86^{a-c}	76.59^{ab}	61.89^{ab}	-3.62^{v-x}	-0.37^{a-j}
NPGRC3127	17.15^{a-c}	15.33^{a-c}	85.45^{a}	68.99^{ab}	-2.77^{n-x}	-0.74^{a-o}
NPGRC3133	24.59^{a-c}	13.97^{a-c}	61.36^{ab}	56.98^{ab}	-3.34^{u-x}	-0.82^{a-o}
NPGRC3195	20.66^{a-c}	12.43^{bc}	33.58^{ab}	66.62^{ab}	-3.70^{v-x}	-0.28^{a-i}
NPGRC3283	19.5^{a-c}	14.4^{a-c}	26.75^{b}	49.7^{ab}	-3.08^{r-x}	-0.86^{a-p}
“SV4”	19.58^{a-c}	13.79^{a-c}	74.97^{ab}	73^{ab}	-2.62^{l-x}	-0.12^{a-f}
Overall mean	17.74		60.36		-1.69	
LSD	5.83		24.87		0.95	
%CV	20.4		25.6		58.1	

Means with similar superscripted letter(s) in the same column were significantly different at $p<0.05$. BD= Basal diameter; CMS= Cellular membrane stability; CTD= Canopy temperature depression.

6.3.4. Yield performance of fifty tropical sorghum genotypes assessed under combined heat and drought stress induced at pre-flowering in a pot experiment in two consecutive seasons

The assessed sorghum genotypes showed a significant ($p<0.05$) variation in 1000 grain weight under combined HS and DS and non-stressed treatments induced at pre-flowering stage in a pot experiment. Genotype IS2847 which yielded the highest mean 1000 grain weight of 3.55 g under stressed treatments outperformed about 10% of the genotypes under non-stressed treatments and a similar proportion of its counterparts under a stressed environment in 2021/22 **(Figure 12)**. Other genotypes that showed superior performance with a mean 1000 grain weight over 3 g under the dual stress in 2021/22 are IS24426, “Macia”, NPGRC3093, IS3574 and NPGRC1478 **(Figure 12)**. The successive season was dominated by genotype IS24426 yielding a mean 1000 grain weight of 3.57 g and outperforming more than 10% of its counterparts under the stressed environment **(Figure 12)**. Other genotypes that showcased superiority were IS12391 and IS30047 yielding the highest grain weight of 3.93 and 3.67 g for 1000 randomly selected grains respectively. The least grain weight was observed in genotype NPGRC1178 recording less than 1 g for 1000 grains under the stressed conditions against an overall mean of 2.44 g in season 2 **(Figure 13)**.

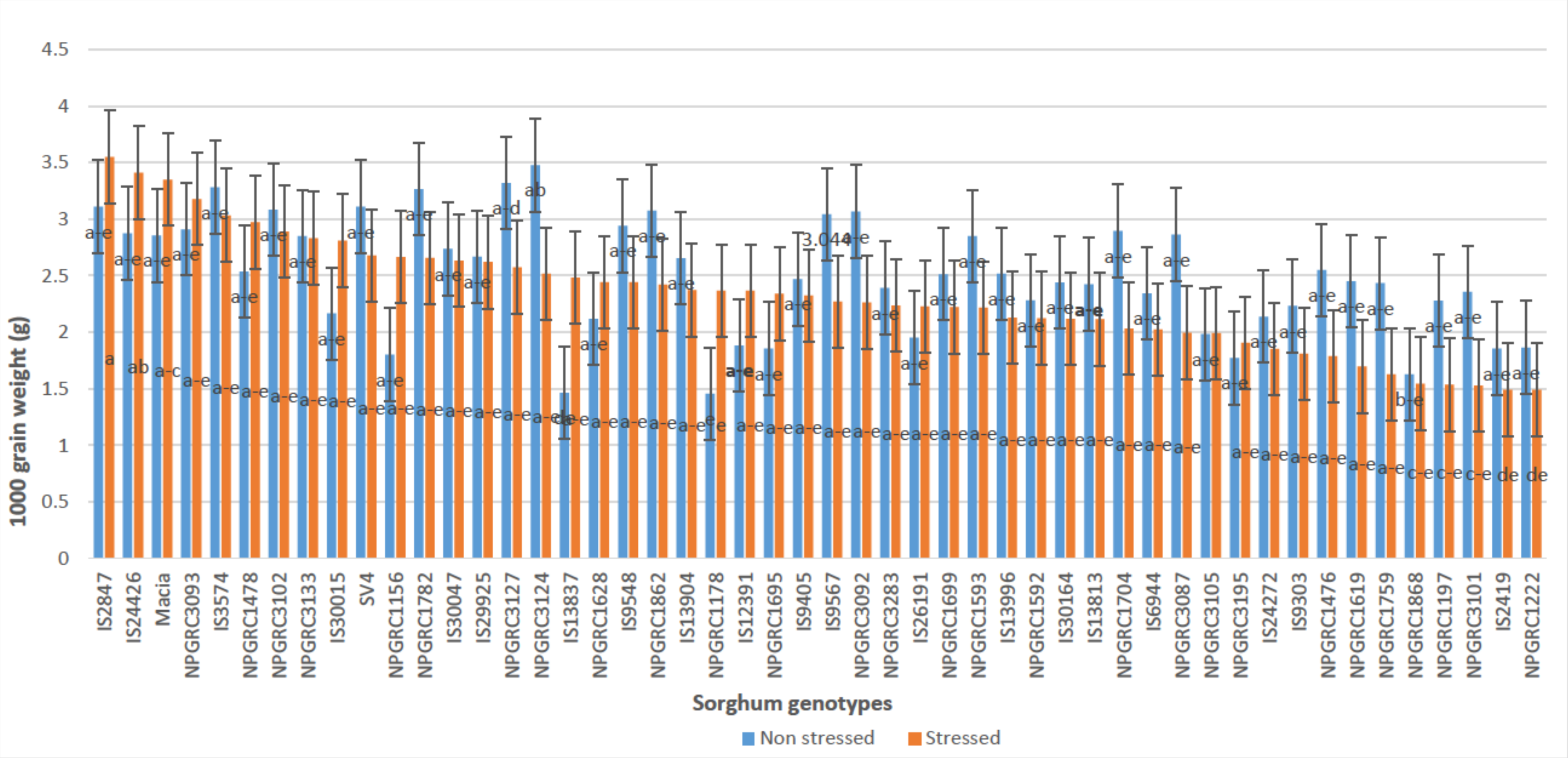

Figure 13. 1000 grain weight of 50 tropical sorghum genotypes assessed in a pot experiment under combined heat and drought stress and no stress in 2021/22. Different letter(s) on bars denote significant differences in means and the vertical bars at the apex of each bar denote standard error of differences in means. Bars arranged in descending order of yield of genotypes under stressed treatments.

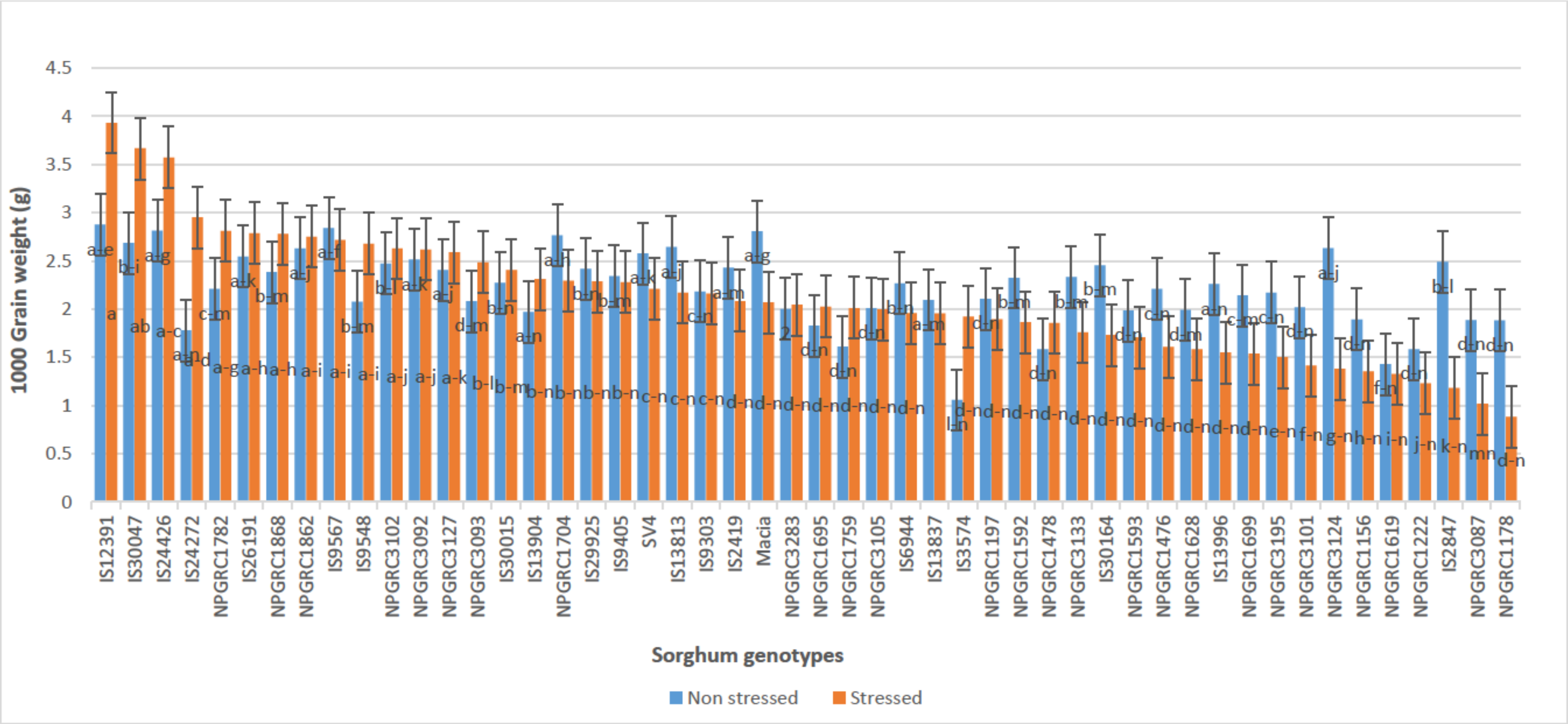

Figure 14. 1000 grain weight of 50 tropical sorghum genotypes assessed in a pot experiment under combined heat and drought stress and no stress in 2022/23. Different letter(s) on bars denote significant differences in means and the vertical bars at the apex of each bar denote standard error of differences in means. Bars arranged in descending order of yield of genotypes under stressed treatments.

6.3.5. Yield stress tolerance indices for sorghum genotypes based on yield performance under combined heat and drought stress and no stress induced environments in a pot experiment for 2021/2 and 2022/23

Highly significant ($p \leq 0.001$) differences in genotypes were observed for all four selected indices in two consecutive seasons **(Table 17)**. In 2021/22 genotypes IS12837, IS12391, NPGRC1178 and NPGRC1156 had the highest yield stability index (YSI) between 1.5 and 1.7 against an overall mean of 0.99. The four top genotypes outperformed 64% of the assessed genotypes. As expected the same genotypes had the lowest stress susceptibility indices (SSI) ranging between -.07 and -0.6. The above-mentioned superior genotypes also showed drought tolerance efficiency (DTE) of more than 100%. Overall, no genotype had DTE less than 50%. The least stable and most susceptible genotypes as indicated by low YSI and high SSI were NPGRC1197 and NPGRC1759 though not significantly different from 80% of the assessed genotypes. In the second season genotype IS12391 maintained its superiority and the other genotypes that dominated were IS3574, IS24272, IS30015, and IS9548 with YSI values between 1.2 and 1.8 **(Table 17)**. Genotypes IS3574 and IS24272 outperformed over 20% of the assessed genotypes in the second season. The observed less stable genotypes were IS2847 and NPGRC1178 with DTE less than 50%.

Table 17. Analysis of Variance and mean comparison for yield stress tolerance indices for 50 tropical sorghum genotypes based on yield performance under combined heat and drought stress and no stress environments induced in 2021/2 and 2022/23.

	YSI		SSI		MPI		DTE	
Genotype	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
IS12391	1.67^{ab}	1.37a-c	-0.67hi	-0.37b-d	2.12a-e	3.40a	166.6ab	136.7a-c
IS13813	0.87^{e-i}	0.83b-d	0.13a-e	0.17a-e	2.27a-e	2.41a-i	86.8e-i	83.26b-d
IS13837	1.71^{a}	0.93b-d	-0.71i	0.07a-c	1.97b-e	2.03d-j	171.1a	92.65b-d
IS13904	0.94^{e-i}	1.18a-d	0.06a-e	-0.18a-d	2.51a-e	2.14d-j	93.6e-i	118.1a-d
IS13996	0.84^{e-i}	0.69cd	0.16a-e	0.31ab	2.32a-e	1.91d-j	84.2e-i	68.67cd
IS2419	0.91^{e-i}	0.86b-d	0.09a-e	0.15a-c	1.67de	2.26b-j	90.7e-i	85.5b-d
IS24272	0.93^{a-i}	1.64ab	0.07a-e	-0.64cd	1.20b-e	2.37b-j	92.6e-i	163.5ab
IS24426	1.21^{a-g}	1.27a-d	-0.21c-i	-0.27a-d	3.1a-e	3.19ab	121.1a-g	126.6a-d
IS26191	1.16^{c-i}	1.09a-d	-0.16a-g	-0.10a-d	2.09a-e	2.67a-e	116c-i	109.5a-d
IS2847	1.16^{c-i}	0.48d	-0.16a-g	0.52a	3.33a	1.84d-j	115.9c-i	47.89d
IS29925	0.98^{e-i}	0.94b-d	0.017a-e	0.063a-c	2.64a-e	2.35b-j	98.3e-i	93.75b-d
IS30015	1.31a-e	1.06a-d	-0.31e-i	-0.06a-d	2.49a-e	2.34b-j	131.1a-e	105.9a-d
IS30047	1.08d-i	1.36a-c	-0.08a-f	-0.36b-d	2.69a-e	3.18a-c	108.0d-i	136.5a-c
IS30164	0.87e-i	0.71cd	0.13a-e	0.29ab	2.28a-e	2.09d-j	87.18e-i	70.58cd
IS3574	0.93e-i	1.82a	0.067a-e	-0.82d	3.16ab	1.49g-j	93.26e-i	181.9a
IS6944	0.88e-i	0.86b-d	0.119a-e	0.14a-c	2.18a-e	2.11d-j	88.12e-i	86.41b-d
IS9303	0.88e-i	1.05a-d	0.124a-e	-0.05a-d	2.02b-e	2.17c-j	87.59e-i	104.6a-d
IS9405	1.04d-i	0.96b-d	-0.043a-f	0.037a-c	2.40a-e	2.31b-j	104.34i	96.3b-d
IS9548	0.85e-i	1.29a-d	0.15a-e	-0.29a-d	2.69a-e	2.38b-j	84.55e-i	129.3a-d
IS9567	0.75g-i	0.96b-d	0.25a-c	0.044a-c	2.66a-e	2.78a-d	74.93g-i	95.63b-d
“Macia”	“1.18b-h	0.74cd	-0.18b-h	0.262ab	3.1ab	2.44a-h	118.2bh	73.77cd
NPGRC1156	1.51a-d	0.71cd	-0.51f-i	0.28ab	2.23a-e	1.62f-j	150.8a-d	71.82d
NPGRC1178	1.66a-c	0.47d	-0.66g-i	0.53a	1.91b-e	1.38j	166.1a-c	46.88b-d
NPGRC1197	0.68i	0.90b-d	0.32a	0.10a-c	1.91b-e	2.002ij	67.63i	89.95cd
NPGRC1222	0.82e-i	0.78cd	0.18a-e	0.22ab	1.68de	1.41d-j	81.71e-i	77.68cd
NPGRC1476	0.73g-i	0.73cd	0.27a-c	0.27ab	2.17a-e	1.91e-j	73.47g-i	72.82a-d
NPGRC1478	1.17b-i	1.23a-d	-0.17a-h	-0.23a-d	2.76a-e	1.72d-j	117.3b-i	123.4b-d
NPGRC1592	0.94e-i	0.81b-d	0.05a-e	0.19a-c	2.20a-e	2.10d-j	94.49e-i	81.09b-d

NPGRC1593	0.80f-i	0.94b-d	0.20a-d	0.06a-c	2.54a-e	1.85d-j	80.34f-i	93.66b-d
NPGRC1619	0.77g-i	0.92b-d	0.23a-c	0.08a-c	2.07b-e	1.38j	77.08g-i	91.9b-d
NPGRC1628	1.15d-i	0.84b-d	-0.15a0f	0.16a-c	2.28a-e	1.79d-j	115.5d-i	84.25b-d
NPGRC1695	1.30a-f	1.14a-d	-0.30d-i	-0.14a-d	2.10a-e	1.93d-j	130.1a-f	113.8a-d
NPGRC1699	0.94e-i	0.72cd	0.056a-e	0.28ab	2.37a-e	1.84d-j	94.38e-i	72.02cd
NPGRC1704	0.70h-i	0.83b-d	0.30ab	0.17a-c	2.47a-e	2.53a-f	69.63hi	82.91b-d
NPGRC1759	0.68i	1.25a-d	0.32a	-0.25a-d	2.03b-e	1.81d-j	67.67i	125a-d
NPGRC1782	0.83e-i	1.28a-d	0.17a-e	-0.28a-d	2.96a-c	2.51a-f	82.87e-i	127.8a-d
NPGRC1862	0.78g-i	1.05a-d	0.22a-c	-0.05a-d	2.75a-e	2.69a-e	78.09g-i	105.2a-d
NPGRC1868	1.05d-i	1.17a-d	-0.05a-f	-0.17a-d	1.59e	2.58a-f	105.1d-i	116.6a-d
NPGRC3087	0.7g-i	0.54cd	0.27a-c	0.46ab	2.43a-e	1.45h-j	73.14g-i	54cd
NPGRC3092	0.73g-i	1.04a-d	0.27a-c	-0.04a-d	2.67a-e	2.57a-f	72.75g-i	104.a-d
NPGRC3093	1.15d-i	1.19a-d	-0.15a-f	-0.19a-d	3.05a-c	2.28b-j	115.2d-i	119.4a-d
NPGRC3101	0.68hi	0.72cd	0.32ab	0.28ab	1.94b-e	1.72e-j	67.91hi	72.17cd
NPGRC3102	0.96e-i	1.19a-d	0.036a-e	-0.19a-d	2.99a-c	2.55a-f	96.45e-i	118.5a-d
NPGRC3105	1.03d-i	0.10a-d	-0.025a-f	0.004a-d	1.99b-e	2.00d-j	102.5d-i	99.6a-d
NPGRC3124	0.74g-i	0.55cd	0.26a-c	0.45ab	2.30a-c	2.01d-j	73.83g-i	54.7cd
NPGRC3127	0.77g-i	1.08a-d	0.23a-c	-0.08a-d	2.95a-c	2.5a-g	77.43g-i	107.7a-d
NPGRC3133	0.99e-i	0.74cd	0.014a-e	0.26ab	2.84a-d	2.05d-j	98.59e-i	74.01cd
NPGRC3195	1.10d-i	0.69cd	-0.102a-f	0.31ab	1.84c-e	1.84d-j	110.3d-i	69.14cd
NPGRC3283	0.94e-i	1.03a-d	0.062a-e	-0.03a-d	2.32a-e	2.02d-j	93.78e-i	102.7a-d
“SV4”	0.86e-i	0.86b-d	0.142a-e	0.14a-c	2.90a-d	2.39b-i	85.78e-i	85.99b-d
Overall mean	0.99	0.97	0.01	0.03	2.41	2.71	98.7	96.9
F-pr	0.001	<0.001	0.001	<0.001	<0.001	<0.001	0.001	<0.001
$LSD_{0.05}$	0.51	0.39	0.51	0.39	0.58	0.47	50.4	39.3
%CV	31.5	25.0	54.2	82.2	14.8	13.3	31.5	25

Means with similar letter(s) in the same column were significantly different at $p<0.05$. YSI= Yield stability index; SSI= Stress susceptibility index; MPI= Mean productivity index; DTE= Drought tolerance index.

6.3.5. Principal component analysis of the morpho-physiological parameters of sorghum genotypes

Variation in tolerance to combined heat and drought stresses induced at pre-flowering stage in 50 diverse tropical sorghum genotypes was shown in eight selected morphological and physiological traits that were assessed. Results of the PCA revealed that most variation was expressed in the first three principal components (PCs 1, 2 and 3) with Eigen values greater than 1 **(Table 18)**. The three components explained 45% of the total variation in data amongst the 50 sorghum genotypes that were assessed for eight selected traits. The first component (PC1) contributed 18% of total variance and showed high positive correlation with plant stem height, 1000 grain weight, panicle length and CTD. The second principal component (PC2) accounted for fourteen % of the total variability in data and canopy temperature depression, cellular membrane stability and leaf area were the major negative contributors. Fourteen percent was explained by the third major component (PC3) with basal stem diameter, leaf area, 1000 grain weight and CTD as key positive contributors.

Table 18. Principal component analysis in 50 diverse sorghum genotypes for the assessed morphological and physiological traits indicating their standard deviation, proportion of variance and Eigen values.

Variable	PC 1	PC 2	PC3
PH	0.451	0.257	-0.231
BD	-0.205	0.004	0.737
LA	0.355	-0.483	0.421
RLWC	0.289	0.260	0.165
CMS	0.192	-0.498	0.069
CTD	0.371	-0.453	-0.267
PL	0.506	0.256	0.051
1000 GW	0.341	0.338	0.347
Eigen values	1.40	1.142	1.10
Proportion of variance	0.175	0.14	0.14
Cumulative proportion	0.175	0.317	0.454

PH= Plant height; BD= Basal diameter; LA= Leaf area; RLWC= Relative leaf water content; CMS= Cellular membrane stability; CTD= Canopy temperature depression; PL= Panicle length; GW= Grain weight.

6.3.6. Correlation analysis of the assessed morphological and physiological traits of sorghum grown under combined heat and drought stressed and non-stressed conditions

The coefficients of the Pearson's correlation analysis and the associated t-probability values among the eight morpho-physiological traits of sorghum that were assessed under combined heat and drought stresses and non-stressed treatments in a pot experiment in two seasons revealed that there were few significant and weak correlations amongst the traits. Canopy temperature had a weak positive (r^2 = 0.2) and highly significantly correlation with leaf area. A significant positive correlation (r^2 = 0.12) between panicle length and 1000 grain weight was also observed while canopy temperature was found to be negatively correlated (r^2 = -0.12) with basal stem diameter **(Table 19)**.

Table 19. Correlation analysis of the assessed morphological and physiological traits of sorghum.

Traits	PH	LA	BD	RLWC	CMS	PL	CTD
LA	0.026^{ns}						
BD	-0.062^{ns}	0.075^{ns}					
RLWC	0.053^{ns}	0.051^{ns}	-0.040^{ns}				
CMS	-0.012^{ns}	0.101^{ns}	-0.032^{ns}	-0.003^{ns}			
PL	0.092^{ns}	0.082^{ns}	-0.049^{ns}	0.052^{ns}	0.046^{ns}		
CTD	0.083^{ns}	-0.152^{**}	-0.117^{*}	0.009^{ns}	0.052^{ns}	0.020^{ns}	
GW	0.048^{ns}	0.037^{ns}	0.018^{ns}	0.105^{ns}	-0.008^{ns}	0.129^{*}	0.028^{ns}

PH= Plant height; LA= Leaf area; BD= Basal diameter; RLWC= Relative leaf water content; CMS= Cellular membrane stability; PL= Panicle length; CTD = Canopy temperature depression; GW= 1000 Grain weight; * significant at 0.05; ** significant at 0.01; ns= not significant.

6.3.7. Path coefficient analysis for 1000 grain yield of sorghum

Partitioning of the effects of explanatory variables of 1000 grain weight into direct and indirect effects through path coefficient analysis revealed that panicle length had the greatest positive direct effect (0.12) on 1000 grain weight **(Table 20)**. Furthermore, it had significant correlation though it was weak at 0.13. Relative leaf water content had an almost equal (0.1) direct effect on 1000 grain weight. The highest indirect positive effects of plant stem height via panicle length (0.02), panicle length via leaf area, basal diameter and relative leaf water content were noted while the greatest negative indirect effect (-0.004) of relative leaf water content through basal stem diameter was observed as well **(Table 20)**.

Table 20. Path coefficient analysis direct and indirect effects of various quantitative traits of 50 diverse tropical sorghum genotypes on 1000 grain yield.

	PH	LA	BD	RLWC	CMS	PL	CTD	GW correlations
PH	0.019	0.0004	-0.002	0.005	0.0002	0.02	0.002	0.05^{ns}
LA	0.0005	0.017	0.002	0.005	-0.001	0.01	0.002	0.04^{ns}
BD	-0.001	0.001	0.03	-0.004	0.0004	0.01	-0.003	0.02^{ns}
RLWC	0.001	0.0009	-0.001	0.1	0.00004	0.01	0.0002	0.11^{ns}
CMS	-0.0002	0.0002	-0.001	-0.0003	-0.01	0.001	0.001	-0.01^{ns}
PL	0.004	0.001	0.002	0.005	-0.001	0.12	0.0001	0.13*
CTD	0.002	0.003	0.004	0.0009	-0.001	0.002	0.03	0.03^{ns}

Where PH= Plant height; LA= Leaf area; BD= Basal diameter; RLWC= Relative leaf water content; CMS= Cellular membrane stability; PL= Panicle length; CTD= Canopy temperature depression; GW= Grain weight, * significant correlation at $p<0.05$.

6.3.8. Cluster analysis of the morphological and physiological traits of the 50 sorghum genotypes investigated

The hierarchical agglomerate cluster analysis grouped the 50 genotypes that were assessed into seven distinct major groups based on the eight traits that were measured under combined heat and drought stresses and non-stressed environments **(Figure 14)**. Clusters A and B had the highest number of 12 and 11 genotypes respectively while the least number of three genotypes was noted in Cluster G followed by Clusters D and F which included four genotypes **(Figure 14)**. Each of seven Clusters had two sub groups.

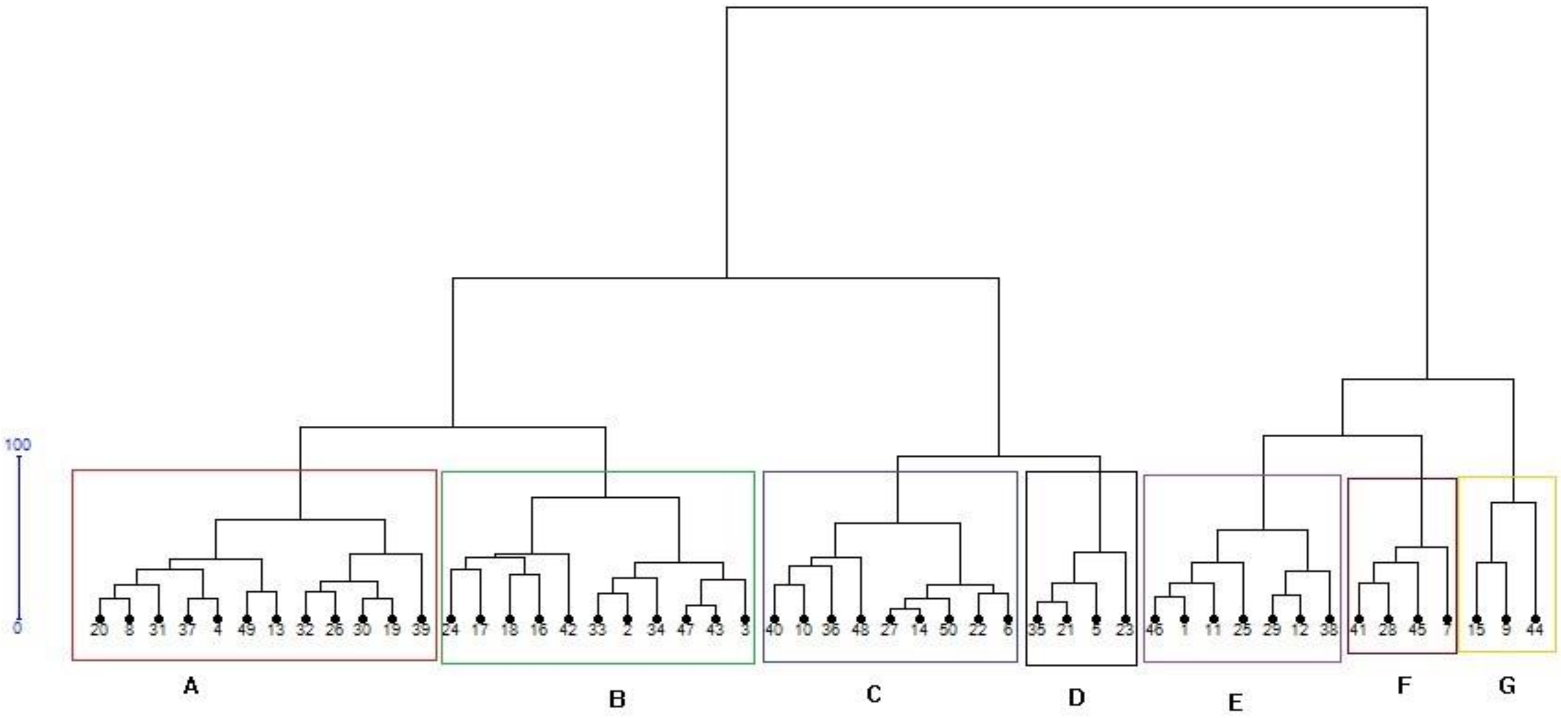

Figure 15. Dendrogram by hierarchical agglomerate cluster analysis of combined stress response of the 50 assessed sorghum genotypes (names of genotypes supplied in Supplementary **Table S1** in Appendices).

6.3.9. Comparison of the performance of clusters in assessed morphological and physiological traits

A detailed analysis of the performance of each identified cluster in the seven traits that were measured revealed that Cluster F with four genotypes namely IS24272, NPGRC1592, NPGRC3093 and NPGRC3124 had an outstanding mean 1000 grain weight of 2.56 g. The same cluster yielded the highest average mean plant stem height of 139.9 cm **(Table 21)**. Other clusters that had a noteworthy performance in 1000 grain weight were Clusters A and C both with a mean of 2.51 g. The least performing in terms of grain weight was Cluster B with 11 genotypes that yielded a mean 1000 grain weight of 2.31 g. Cluster G had the shortest plants with a mean stem height of 112.3 cm. The greatest mean leaf area was observed in Cluster 3 with a mean of 4064 mm^2 while Cluster D had the lowest mean of 2678.8 mm^2. Cluster D had the coolest canopies with the highest mean CTD of 0.13 while the highest relative leaf water content of 96% was noted in Cluster E **(Table 21)**.

Table 21. Mean performance of seven clusters for different morphological and physiological traits in 50 sorghum genotypes.

Clusters	BD (mm)	PH (cm)	CMS (%)	CTD (°C)	RLWC (%)	LA (mm^2)	PL (mm)	GW (g)	No. of genotypes	Names of Genotypes
A	20.32	130.5	57.48	-0.20	73	3119.8	170.0	2.51	12	IS13904;IS9567;IS24426;NPGRC3283;IS30047;IS9548,NPGRC1476; NPGRC1619;NPGRC1628;NPGRC1695;NPGRC1862; NPGRC3283
B	22.01	132.3	62.34	-0.27	73	3349.8	185.2	2.31	11	IS13813;IS13837;IS6944;IS9303;IS9405;NPGRC1197;NPGRC1699; NPGRC1704;NPGRC3101;NPGRC3102;NPGRC3133
C	20.56	120.8	63.01	0.08	78	2874.2	183.3	2.51	9	IS2419;IS2847;IS30164;NPGRC30164;NPGRC1156;NPGRC147; NPGRC1782;NPGRC3092;"SV4";NPGRC3195
D	21.09	123.6	58.89	0.13	55	2678.8	155.4	1.34	4	IS13996;"Macia"; NPGRC1178;NPGRC759
E	23.89	129.0	64.18	-0.25	96	3816.3	177.9	2.29	7	IS12391;IS29925;IS30015;NPGRC1222;NPGRC1593; NPGRC1868;NPGRC3127
F	21.66	139.6	58.54	-0.29	69	3575	164.1	2.56	4	IS24272;NPGRC1592;NPGRC3093;NPGRC3124
G	23.40	112.3	58.32	-0.58	77	4064	174.5	2.41	3	IS26191;1S3574;NPGRC3105

BD= Basal diameter; PH= Plant height; CMS= Cellular membrane stability; CTD= Canopy temperature depression; RLWC= Relative leaf water content; LA= Leaf area; PL= Panicle length; GW= Grain weight.

6.4. Discussion

Yield is widely accepted as the most desirable trait used for selecting genotypes for crops that are productive under the increasingly existing combinatory heat and drought stresses. Stress tolerance without any yield is indeed not beneficiary, thus in our current study yield was the main focus but it cannot be dissected in isolation, disregarding the morpho-physiological proxy traits. In the current study the performance of 48 diverse sorghum genotypes and two check varieties were assessed on morphological, physiological traits and final grain weight under combined HS and DS, and non-stress environment in a pot trial for two consecutive cropping seasons. The observed significant inhibitory main effects of combined heat and drought stresses on almost all the traits except stem basal diameter shows the detrimental effects of combined stresses on various physiological processes that contribute to growth and reproduction of sorghum. Similar results were shown for other crops as reported in several earlier research findings **(KHATON et al., 2016; MACHADO and PAULSEN, 2016; BIJU et al., 2018; ZANDALINAS et al., 2020; YOUSAF et al., 2022)**. The observed significant interaction between genotype and stress treatments suggest the well documented differential response of sorghum genotypes to HS and DS explained by the differences in expression of genes that regulate the mechanisms of tolerance and resistance to stress **(OGBAGA et al., 2016; LAWAS et al., 2018; AZZOUZ-OLDEN et al., 2020)**.

Cell division and expansion is reportedly disrupted and delayed by combined heat and drought stresses **(DE SOUZA et al., 2021; KUMAR et al., 2022)**, which have retarded the assessed vegetative growth parameters i.e. leaf area and stem height in the current study. Normally the stem diameter is expected to shrink due to reduced internal water status caused by a combination of excessive water loss due to HS and reduced water uptake in soil water deficient conditions **(HUSSAIN et al., 2019; RAD et al., 2023)**. However, the current study, stem diameter in stressed plants remained higher than in unstressed plants. In some instances, if stem height is reduced then basal diameter increases. **PERAZZO et al. (2014)**, also observed a negative correlation between stem height and stem diameter.

In most crop plants including sorghum, stomata closes under combined excessive atmospheric temperatures and soil water deficit **(ZANDALINAS et al., 2020; KHANTHAVONG et al., 2022)**. This results in increased canopy temperature **(BIJU et al., 2018)**, hence the observed significantly lower and negative CTD in stressed plants as compared to the controls in both seasons. A large positive difference between the ambient temperature and canopy temperature results in a high positive CTD which symbolizes cooler canopies **(SRIVASTAVA et al., 2017; LAWAS et al., 2018)**. This was noted in some genotypes even under combined HS and DS marking their tolerance. **MUTAVA et al. (2012)** observed significantly higher CTD means for irrigated sorghum plants with some genotypes under stressed conditions showing significantly cooler canopies. The genotypes are able to keep their stomata open to allow transpiration which has a cooling effect **(SASTRY and BARUA, 2017; MESEKA et al., 2018)**. However, sudden changes of atmospheric conditions and even the micro climate differences within plants of the same treatments renders the high variability noted in CTD and RLWC **(LEPEKHOV, 2022)**. The aforementioned researcher found coefficient of variation values between 10 and 43%. Canopy temperature has been related to RLWC in many research findings **(AL-GHZAWI et al., 2018**; **BIJU et al., 2018)**, which is also a good indicator of stress tolerance to the dual stresses under investigation. Surprisingly no statistical differences were observed in our study though the unstressed treatments had quite higher RLWC values in both seasons. **MUTAVA et al. (2012)**, in a similar study, observed significant differences on RLWC between dual stressed and non-stressed sorghum but no significant genotype × stress interactions.

The significant detrimental effects of combinatory stress that was noted on cellular membrane stability of stressed plants in the current study is a clear manifestation of lipid peroxidation of cell membranes fueled by increased production of ROS and other harmful derivatives during stress **(GUO et al., 2018; PRASAD et al., 2021b; LIU et al., 2023)**. However, the extent still varied with genotypes thus some genotypes were able to maintain a relatively higher membrane stability even under stress which can be attributed to enhanced antioxidant defense mechanisms.

Significant yield reductions were recorded in both seasons though the magnitude in the range of 5-7% lower compared to other studies. Nonetheless the observed reductions are an indication of the negative effect of combined stresses on yield. Yield formation is a complex process that is affected by many anatomical, biochemical, physiological and environmental factors stretching from the vegetative stages of growth. **HATFIELD and PRUEGER (2015)** reported that disruption and retardation of the vegetative stage which affects leaf expansion, plant height and many others as a drought escaping strategy resulting in reduced florets and consequently a lower kernel number. **MOSHELION (2020)** reiterated that good vegetative establishment is the foundation for yield production thus a detrimental episode just before flowering is likely to upset formation of yield apparatus and accumulation of reserves. The leaves are the main photosynthetic apparatus hence if reduced in size, assimilation of photosynthates is compromised. Stems and panicles are the temporary reservoirs of photo-assimilates while the primary yield sinks are developing **(WANG et al., 2012)**. Thus plant stem height which is determined by internode length plays a critical role in preparation of yield formation, though, **BARNABÁS et al. (2008)** argued that stem basal diameter determines the capacity of pre-anthesis reserve accumulation ahead of the stem height but certainly there is a critical length: diameter ratio.

The key processes in accumulation of pre-anthesis reserves are nutrient uptake and photosynthesis both affected by availability of water **(AHMADI et al., 2018; QUEIROZ et al., 2019; BEGNA, 2022)**. Drought stress is said to dominate the antagonistic effect of combined heat and drought stresses on stomatal behavior resulting in closure of stomata to conserve water under DS over opening of the stomata to reduce canopy temperature under HS **(MOSHELION, 2020)**. As such carbon assimilation is negatively affected resulting in reduced accumulated reserves which later compromises yield **(ABREHA et al., 2022)**. Genotypic differences led to the observed differential performance of the assessed genotypes in yield under the two tested environments. The sensitivity of threshold of genotypes to combined HS and DS differs, with more tolerant lines having improved stomatal aperture and prolonged periods of opening even under stressed conditions, hence higher yield **(EGGEN et al., 2019; QUEIROZ et al., 2019**; **KHANTHAVONG et al., 2022)**.

Generally most sorghum genotypes are said to be anisohydric with lower stomata closure (stomatal resistance) for increased uptake of carbon dioxide exposing them to desiccation under severe drought and excessive atmospheric temperatures **(RIVERO et al., 2022; LIU et al., 2023)**. Results on the performance of the genotypes based on the yield are supported by the yield-based indices that were calculated and analyzed. The indices are more effective and reliable indicators as they take into account the performance of the genotypes under stressed and non-stressed environments. High values in YSI, DTE and MPI resemble tolerance while high values in SSI resemble less tolerance hence these genotypes can be differentiated and selected using these indices **(BAZZAZ et al., 2015; MICKKY et al., 2019; ZUFFO et al., 2022; YAHAYA et al., 2023)**.

A lot of variation remains unexplained in the PCA, with the first three principal components explaining less than 50% of variability in data. This could be indicative of the complex nature of Hs and DS control mechanisms. Several reports show that these traits are polygenic and greatly influenced by the environment **(VELAZCO et al., 2019)**.

A negative correlation of CTD and LA confirmed the relationship between the two traits as confirmed in the PCA. This implies that genotypes with a smaller leaf area can afford to keep their stomata open for an extended duration even under heat and drought stressed environments which keeps their canopy cool, thus a high CTD **(MUTAVA et al., 2012)**. Panicle length certainly contributes in a positive manner to grain weight, thus the positive correlation that was observed. **GALICIA-JU et al. (2021)** observed up to 40% reduction in plant height, significant reductions in panicle length and similarly significant 1000 grain weight losses in sorghum exposed to stress conditions at the pre-anthesis stage. The relationship was confirmed in the current study by the observed direct effects of panicle length to grain weight in the path coefficient analysis and its indirect effect via several attributes like leaf area, basal diameter and relative leaf water content. Therefore, selecting for panicle length can result in increased grain yield under DS conditions. It was found to be influenced by plant height as well as to contribute positively to grain weight. All this certainly points to the enhanced accumulation and supply of photosynthates during grain filling, indicating that selection for these traits in sorghum will lead to either directly or indirectly enhanced yield under combined HS and DS. These factors will be beneficial in plant breeding for sorghum.

Cluster analysis also confirmed the existence of the relationship between panicle length, RLWC, CTD and CMS. Analysis of the Clusters showed that Cluster C had the second highest average grain weight after Cluster E, and at par with Cluster A, had higher means for PL, RLWC, CTD and CMS. However, the Cluster had a lower plant stem height average which indicates that genotypes in the cluster may be more suitable for grain purposes, while genotypes in Cluster F may be suitable for dual purposes i.e. grain and fodder due to their superior plant stem height, leaf area and grain weight. The noted classification of check varieties into different clusters implicates that they are different with “SV4” showing more tolerance than “Macia”.

6.5. Conclusion and future perspectives

The study interrogated the effects of combined heat and drought stresses induced at pre-flowering stage on morphological, physiological and yield traits of 50 sorghum genotypes in two consecutive seasons. The combined stresses negatively affected early growth of sorghum in terms of stem height, leaf expansion, physiologically affecting canopy temperature depression, cellular membrane stability as well and consequently panicle length and final grain weight. Genotypic effects were noted in both seasons except on stem basal diameter and relative leaf water content. The genotypes differentially responded to the two treatments i.e. combined HS and DS and non-stressed in both seasons for CTD, BD, panicle length and yield. Under the stressed conditions which was the major focus, genotypes NPGRC3124 was identified as the most superior in stem plant height, IS24426 in basal diameter, NPGRC3122, NPGRC1222 and IS9405 in leaf area. Genotypes IS3574 and NPGRC3092 showed superior performance in CTD, while NPGRC1704, IS30015 and IS24426 had the longest panicle length under the stressed conditions. The yield-based indices confirmed genotypes IS24426, IS12391 and NPGRC3093 as the most stable and tolerant to pre-flowering combined HS and DS. Further analysis of the performance of genotypes in both seasons showed that genotypes NPGRC3102, IS30015 and IS3574 maintained consistent superiority in almost all the morpho-physiological traits that were assessed.

Furthermore, PCA revealed that stem plant height, panicle length, canopy temperature depression and grain weight were the most affected parameters by the assessed test environment in the first component. Canopy temperature depression was found to be positively correlated to leaf area, panicle length, and to grain weight. Canopy temperature was negatively correlated to basal diameter in the correlation analyses. Panicle length and relative leaf water content had the highest direct effects on grain weight while plant height had a notable indirect effect through panicle length. Genotypes were grouped into seven distinct clusters with one of the clusters with four genotypes earmarked for grain and forage purposes due to their high biomass and grain yield potential. Further studies on multi-environment trials and stability analysis are recommended for a more informed, precise evaluation and deployment of the genotypes for adaptability and productivity in sets of semi-arid environments characterized by combined HS and DS.

CHAPTER 7: GENERAL CONCLUSIONS

Diversity in sorghum is the key driver of variations in response of genotypes in morphological and physiological traits to abiotic stresses like drought and excessive atmospheric temperatures throughout the ontogeny of the crop. Indeed, when combined, HS and DS, have been proven to be detrimental at any stage of growth and development of even the known resilient crops like sorghum. The affected traits and extent of injury vary depending on the stage of growth and genotype. This is coupled with co-evolutionary adaptation mechanisms of the genotypes to their places of origin. In the current study combined HS and DS suppressed germination in all the assessed genotypes. More genotypes resisted HS than DS when they occurred separately. The energy and time taken for germinating seeds to osmotically adjust to instant DS renders a more pronounced effect of DS than HS in germinating seeds which is of course genotype dependent.

In the current study it was noted that acclimatization of sorghum seedlings improves tolerance to heat shock. More so, genetic diversity for basal and acquired thermotolerance exists suggesting the potential use of some assessed genotypes in tropical areas. The existence of superior performance and specific adaptation in shoot elongation under heat and drought stresses applied separately and in combination is an indication of tolerance to stresses at the seedling stage and higher chances of increased survival to maturity on deployment in marginal areas of the world.

Selection of certain traits like PL can result in increased grain yield under drought and heat stressed conditions. Grain yield was found to be positively influenced by PL as well. Saliently opines to enhanced accumulation and supply of photosynthates during grain filling, implies that selection for these traits in sorghum will lead to either directly or indirectly enhanced yield under combined HS and DS. Potential donor genotypes are likely endowed with genetic factors that contribute towards the expression of biomolecules that directly contribute or control mechanisms that enhance resilience to the concerned stress factors.

Genotypes NPGRC1593, NPGRC1782, NPGRC1476, and IS224426 performed remarkably well in terms of extent and speed of germination under HS and DS induced separately and even when the two stresses were combined. Genotypes NPGRC1704, IS956, NPGRC1197, NPGRC1868 and NPGRC1782 exhibited potential in both basal and acquired thermotolerance. Genotypes NPGRC1478 and IS30164 exhibited dynamic stability and specific adaptability to HS and combined HS and DS at both seedling stages. Genotypes IS24426, IS12391 and NPGRC3093 emerged as the most stable and resilient to pre-flowering HS and DS as shown by their performance in terms of grain weight. Based on overall performance in the assessed morpho-physiological traits the 50 genotypes were grouped into seven distinct clusters using the hierarchical clustering technique with one cluster with four genotypes identified for dual purposes (grain and forage) characterized by PH, LA and GW. Genotypes IS24426, NPGRC1782, IS24272 and IS12391 showed some notable resilience to HS and DS in all the growth stages and traits that were assessed in this study. Multi-environment trials are recommended for a more informed, precise evaluation and deployment of the identified high potential genotypes.

APPENDICES

Table S1. Profiles of 50 selected African sorghum genotypes, their biological status and origins used in the study.

Entry No	Genotype code	Genotype identity	Biological status	Locality/ Province	Country of origin
1	“Macia”	“Macia”	Commercial variety	_ _ _ _ _	_ _ _ _ _
2	ICR61	IS13813.	Landrace	KwaZulu-Natal	South Africa
3	“SV4”	“SV4”	Commercial variety	_ _ _ _ _	_ _ _ _ _
4	GB 56	NPGRC3092	Landrace	Tsholotsho	Zimbabwe
5	ICR 34	IS3574	Landrace	Kassala	Sudan
6	ICR5	IS12391			
7	ICR 20	IS3904	Landrace	KwaZulu-Natal	South Africa
8	ICR 21	IS24272	Research Material	_ _ _ _ _	Tanzania
9	GB 61	NPGRC3124	Landrace	Murehwa	Zimbabwe
10	ICR 19	IS29925	Landrace	Hwange	Zimbabwe
11	ICR 17	IS9405	Landrace	Maseru	Lesotho
12	GB 13	NPGRC3127	Landrace	Chipinge	Zimbabwe
13	ICR 8	IS30047	Landrace	Hwange	Zimbabwe
14	IGR 52	IS26191	Landrace	Savanes	Togo
15	GB 92	NPGRC3093	Research Material	Chiredzi	Zimbabwe
16	GB 98	NPGRC1178	Research Material	Chiredzi	Zimbabwe
17	GB 185	NPGRC1699	Research Material	Chiredzi	Zimbabwe
18	GB 82	NPGRC3102	Research Material	Chiredzi	Zimbabwe
19	GB 172	NPGRC1695	Landrace	Murehwa	Zimbabwe
20	GB 175	NPGRC1593	Research Material	Chiredzi	Zimbabwe
21	GB 30	NPGRC1862	Landrace	Nyanga	Zimbabwe
22	GB 43	NPGRC3283	Landrace	Nyanga	Zimbabwe
23	GB 15	NPGRC3195	Landrace	Chiredzi	Zimbabwe
24	GB 144	NPGRC3087	Landrace	Murehwa	Zimbabwe
25	GB 102	NPGRC3133	Landrace	Chiredzi	Zimbabwe
26	GB 136	NPGRC1156	Landrace	Nyanga	Zimbabwe

27	GB 124	NPGRC3105	Landrace	Murehwa	Zimbabwe
28	GB 94	NPGRC1759	Research Material	Nyanga	Zimbabwe
29	GB 175	NPGRC1593	Research Material	Chiredzi	Zimbabwe
30	ICR46	IS9303	Research material	Pretoria	South Africa
31	GB59	NPGRC1628	-		
32	GB81	NPGRC3101	-		
33	GB82	NPGRC1197	-		
34	GB84	NPGRC1868	-		
35	GB93	NPGRC1222	-		
36	GB94	NPGRC1704	-		
37	ICR 116	IS13837	Landrace	KwaZulu-Natal	South Africa
38	GB111	NPGRC1619	-		
39	ICR 142	IS2419	Landrace	_ _ _ _ _	Tanzania
40	ICR 7	IS6944	Landrace	_ _ _ _ _	Sudan
41	ICR134	IS9567	Research Material	Pretoria	South Africa
42	ICR 65	IS9548	Research Material	_ _ _ _ _	South Africa
43	ICR145	IS13996	-		
44	GB 88	NPGRC1478	Landrace	Murehwa	Zimbabwe
45	GB92	NPGRC1782	-		
46	GB62	NPGRC1476	-		
47	ICR138	IS30164	Landrace	Sanyatwe	Zimbabwe
48	ICR 16	IS30015	Landrace	Hwange	Zimbabwe
49	ICR104	IS2847	-		
50	ICR76	IS24426	-		

REFERENCES

Abreha K.B., Enyew M., Carlsson A.S., Vetukuri R.R., Feyissa T., Motlhaodi T., Ng'uni, D. and Geleta M. (2022). Sorghum in dryland: morphological, physiological, and molecular responses of sorghum under drought stress. Planta, 255, 1-23. Springer Berlin Heidelberg. https://doi.org/10.1007/s00425-021-03799-7.

Abro, A. A., Eisawi, K. A. E., Batyrbek, M., Sial, N. Y., Akhtar, M., and Memon, S.A (2022). Identification of drought-tolerant wheat (*Triticum aestivum* L.) cultivars based on the associations of *in-vitro* and *in-vivo* predictors through polyethylene glycol (PEG 6000) mediated osmotic stress. Pakistan Journal of Biotechnology, 18, 69-80. https://doi.org/10.34016/pjbt.2022.19.1-2.69.

Abro, A. A., Memon, S., Abro, S. A., Sam, E. K., He, R. Y., Rind, M. H., Memon, S., Solangi, Z., Muhammad, T., Ali, B., Ahmed, W., Dev, W., Abro, M. A., Rajput, N., Nizamani, S., Nargis, M., and Kumbhar, R. A. (2020). Evaluation of drought tolerance in wheat (*Triticum aestivum* L.) cultivars at early seedling stage using polyethylene glycol induced osmotic stress. Journal of Animal and Plant Sciences, 30, 950-957. https://doi.org/10.36899/JAPS.2020.4.0111.

Abdel-Ghany, S. E., Ullah, F., Ben-Hur, A., and Reddy, A. S. N. (2020). Transcriptome analysis of drought-resistant and drought-sensitive sorghum (*Sorghum bicolor*) genotypes in response to peg-induced drought stress. International Journal of Molecular Sciences, 21, 1-26. https://doi.org/10.3390/ijms2103077.2.

Al-Ghzawi, A. L. A., Khalaf, Y. B., Al-Ajlouni, Z. I., Al-Quraan, N. A., Musallam, I., and Hani, N. B. (2018). The effect of supplemental irrigation on canopy temperature depression, chlorophyll content, and water use efficiency in three wheat (*Triticum aestivum* L. and *T. durum* Desf.) varieties grown in dry regions of Jordan. Agriculture, 8. https://doi.org/10.3390/agriculture8050067.

Adamcik, J., Tomaseck, J., Pulkrabek, J., Pazderu, K., and Dvorak, P. (2016). Stimulation sorghum seed leading to enlargement of optimum conditions during germination and emergence. Plant Soil Environment, 62, 547-551. Doi: 10.17221/556/2016-PSE.

Ahmad, M., Waraich, E. A., Skalicky, M., Hussain, S., Zulfiqar, U., Anjum, M. Z., Habib Rahman, M., Brestic, M., Ratnasekera, D., Lamilla-Tamayo, L., Al-Ashkar, I., and EL Sabagh, A. (2021). Adaptation strategies to improve the resistance of oilseed crops to heat stress under a changing climate: An overview. Frontiers in Plant Science, 12, 1-36. https://doi.org/10.3389/fpls.2021.767150.

Ahmad, M., Waraich, E. A., Zulfiqar, U., Ullah, A., and Farooq, M. (2021). Thiourea application improves heat tolerance in camelina (*Camelina sativa* L. Crantz) by modulating gas exchange, antioxidant defense and osmoprotection. Industrial Crops and Products, 170, 113826. https://doi.org/10.1016/j.indcrop.2021.113826.

Ahmad, S., Ahmad, R., Ashraf, M. Y., Ashraf, M., and Waraich, E. A. (2009). Sunflower (*Helianthus annuus* L.) response to drought stress at germination and seedling growth stages. Pakistan Journal of Botany, 41, 647-654.

Ahmadi, J., Pour-aboughadareh, A., Fabriki-ourang, S., Mehrabi, A., and Siddique, K. H. M. (2018). Screening wheat germplasm for seedling root architectural traits under contrasting water regimes : potential sources of variability for drought adaptation. Archives of Agronomy and Soil Science, 64, 1351–1365. https://doi.org/10.1080/03650340.2018.1432855.

Akman, Z. (2009). Comparison of high temperature tolerance in maize, rice and sorghum seeds by plant growth regulators. Journal of Animal and Veterinary Advances, 8, 358–361.

Al-Ghzawi, A. L. A., Khalaf, Y. B., Al-Ajlouni, Z. I., Al-Quraan, N. A., Musallam, I., and Hani, N. B. (2018). The effect of supplemental irrigation on canopy temperature depression, chlorophyll content, and water use efficiency in three wheat (*Triticum aestivum* L. and *T. durum* Desf.) varieties grown in dry regions of Jordan. Agriculture, 8. https://doi.org/10.3390/agriculture8050067.

Ali, A., Khan, M., Sharif, R., Mujtaba, M., and Gao, S. J. (2019). Sugarcane omics: an update on the current status of research and crop improvement. Plants, 8, 344.

Ali, M. A., Abbas, A., Awan, S. I., Jabran, K., and Gardezi, S. D. A. (2011). Correlated response of various morpho-physiological characters with grain yield in sorghum landraces at different growth phases. Journal of Animal and Plant Sciences, 21, 671–679.

Ali, M. A., Abbas, A., Niaz, S., Zulkiffal, M., and Ali, S. (2009). Morpho-physiological criteria for drought tolerance in Sorghum (*Sorghum bicolor*) at seedling and post-anthesis stages. International Journal of Agriculture and Biology, 11, 674–680.

Álvarez-Iglesias, L., Roza-Delgado, B. D. La, Reigosa, M. J., Revilla, P., and Pedrol, N. (2017). A simple, fast and accurate screening method to estimate maize (*Zea mays* L) tolerance to drought at early stages. Maydica, 62.

Ananda, N., Vadlani, P. V., and Prasad, P. V. V. (2011). Evaluation of drought and heat stressed grain sorghum (*Sorghum bicolor*) for ethanol production. Industrial Crops and Products, 33, 779-782. https://doi.org/10.1016/j.indcrop.2011.01.007.

Andrási, N., Pettkó-Szandtner, A., and Szabados, L. (2020). Variability of plant heat shock factors: regulation, interactions and functions. Authorea Preprints.

Arya, R., Singh, M., Yadav, A., Kumar, A., and Kumar, S. (2014). Advances in pearl millet to mitigate adverse environment conditions emerged due to global warming. Forage Research, 40, 57–70.

Assefa, Y., Staggenborg, S. A., and Prasad, V. P. V. (2010). Grain sorghum water requirement and responses to drought stress: A Review. Crop Management, 9, 1–11. https://doi.org/10.1094/cm-2010-1109-01-rv.

Azari Nasrabad, A., Mousavi Nik, S. M., Galavi, M., Siroosmehr, A., and Beheshti, S. (2016). Evaluation of water stress at different growth stages on yield, its components, cell membrane stability and leaf relative water content of grain sorghum (*Sorghum bicolor* (L. Moench) genotypes. Environmental Stresses in Crop Sciences, 9, 217-228.

Azzouz-Olden, F., Hunt, A. G., and Dinkins, R. (2020). Transcriptome analysis of drought-tolerant sorghum genotype SC56 in response to water stress reveals an oxidative stress defense strategy. Molecular Biology Reports, 47, 3291–3303. https://doi.org/10.1007/s11033-020-05396-5.

Babele, P. K., Kudapa, H., Singh, Y., Varshney, R. K., and Kumar, A. (2022). Mainstreaming orphan millets for advancing climate smart agriculture to secure nutrition and health. Frontiers in Plant Science, 13. https://doi.org/10.3389/fpls.2022.902536.

Barnabás, B., Jäger, A.K., and Fehér, A. (2008). The effect of drought and heat stress on reproductive processes in cereals. Plant, Cell and Environment, 31, 11–38. https://doi.org/10.1111/j.1365-3040.2007.01727.x.

Basha, M. H., and Mehta, A. K. (2016). Screening of oat (*Avena sativa* L.) mutant lines for drought tolerance using polyethylene glycol-6000 at seed ling stage. Progressive Research-An International Journal, 11, 5561-5569.

Bayu, W., Rethman, N. F., Hammes, P. S., Pieterse, P. A., Grimbeek, J., and Van der Linde, M. (2005). Water stress affects the germination, emergence and growth of different sorghum cultivars. Ethiopian Journal of Science, 28, 119-128.

Bazzaz, M. M., Khaliq, Q. A., Karim, M. A., Al-Mahmud, A., and Khan, M. S. A. (2015). Canopy temperature and yield based selection of wheat genotypes for water deficit environment. OALib, 2, 1–11. https://doi.org/10.4236/oalib.1101917.

Begna, T., Yali, W., and Gichile, H. (2022). Impact of drought stress in sorghum (*Sorghum bicolor* (L.) Moench) production and drought genetic resistance mechanisms. European Journal of Agriculture and Forestry Research, 10, 28-49. https://www.eajournals.org/.

Bernfur, K., Rutsdottir, G., and Emanuelsson, C. (2017). The chloroplast-localized small heat shock protein Hsp21 associates with the thylakoid membranes in heat-stressed plants. Protein Science, 26, 1773–1784. https://doi.org/10.1002/pro.3213.

Beyene, A., Hussien, S., Pangirayi, T., and Mark, L. (2015). Physiological mechanisms of drought tolerance in sorghum, genetic basis and breeding methods: A review. African Journal of Agricultural Research, 10, 3029-3040. https://doi.org/10.5897/ajar2015.9595.

Bhardwaj, A., Devi, P., Chaudhary, S., Rani, A., Jha, U. C., Kumar, S., Bindumadhava, H., Prasad, P. V. V., Sharma, K. D., Siddique, K. H. M., and Nayyar, H. (2023). 'Omics' approaches in developing combined drought and heat tolerance in food crops. Plant Cell Reports, 41, 3. https://doi.org/10.1007/s00299-021-02742-0.

Bheemanahalli, R., Ramamoorthy, P., Poudel, S., Samiappan, S., Wijewardane, N., and Reddy, K. R. (2022). Effects of drought and heat stresses during reproductive stage on pollen germination, yield, and leaf reflectance properties in maize. Plant Direct, 6, 434.

Bibi, A., Sadaqat, H. A., Tahir, M. H. N., and Akram, H. M. (2012). Screening of sorghum (*Sorghum bicolor* Var Moench) for drought tolerance at seedling stage in polyethylene glycol. The Journal of Animal and Plant Sciences, 22, 671-678.

Biju, S., Fuentes, S., and Gupta, D. (2018). The use of infrared thermal imaging as a non-destructive screening tool for identifying drought-tolerant lentil genotypes. Plant Physiology and Biochemistry, 127, 11–24. https://doi.org/10.1016/j.plaphy.2018.03.005.

Bobade, P. N., Amarshettivar, S. B., Rathod, T. H., Ghorade, R. B., and Kayande, N. V. (2019). Effect of polyethylene glycol induced water stress on germination and seedling development of rabi sorghum genotypes. Journal of Pharmacognosy and Phytochemistry, 8, 852-856.

Bokszczanin, K. L., and Fragkostefanakis, S. (2013). Perspectives on deciphering mechanisms underlying plant heat stress response and thermotolerance. Frontiers in Plant Science, 4, 1-20. https://doi.org/10.3389/fpls.2013.00315.

Bouslama, M., and Schapaugh, W. T. (1984). Stress tolerance in soybeans. I. evaluation of three screening techniques for heat and drought tolerance. Crop Science, 24, 933-937. https://doi.org/10.2135/cropsci1984.0011183x002400050026x.

Cairns, J. E., Crossa, J., Zaidi, P. H., Grudloyma, P., Sanchez, C., Luis Araus, J., Thaitad, S., Makumbi, D., Magorokosho, C., Bänziger, M., Menkir, A., Hearne, S., and Atlin, G. N. (2013). Identification of drought, heat, and combined drought and heat tolerant donors in maize. Crop Science, 53, 1335-1346. https://doi.org/10.2135/cropsci2012.09.0545.

Carvalho, H. D., Heilman, J. L., McInnes, K. J., Rooney, W. L., and Lewis, K. L. (2020). Epicuticular wax and its effect on canopy temperature and water use of Sorghum. Agricultural and Forest Meteorology, 284, 107893.

Chadalavada, K., Kumari, B. R., and Kumar, T. S. (2021). Sorghum mitigates climate variability and change on crop yield and quality. Planta, 253, 113. https://doi.org/10.1007/s00425-021-03631-2.

Chakrabarty, S., Mufumbo, R., Windpassinger, S., Jordan, D., Mace, E., Snowdon, R. J., and Hathorn, A. (2022). Genetic and genomic diversity in the sorghum gene bank collection of Uganda. BMC Plant Biology, 22, 1–11. https://doi.org/10.1186/s12870-022-03770-y.

Chaniago, I., Syarif, A., and Riviona, P. (2017). Sorghum seedling drought response: In search of tolerant genotypes. International Journal on Advanced Science, Engineering and Information Technology, 7, 892–897. https://doi.org/10.18517/ijaseit.7.3.1303.

Chanza, N., and Musakwa, W. (2022). Ecological and Hydrological Indicators of Climate Change observed by Dryland Communities of Malipati in Chiredzi, Zimbabwe. Diversity, 14, 541. https://doi.org/10.3390/d14070541.

Choinski, J. S. (1999). Laboratory studies of thermotolerance acquisition during seed imbibition and germination. American Biology Teacher, 61, 534–537. https://doi.org/10.2307/4450757.

Conde, A., Chaves, M. M., and Gerós, H. (2011). Membrane transport, sensing and signaling in plant adaptation to environmental stress. Plant and Cell Physiology, 52, 1583–1602. https://doi.org/10.1093/pcp/pcr107.

Costa, M. V. J. Da, Ramegowda, Y., Ramegowda, V., Karaba, N. N., Sreeman, S. M., and Udayakumar, M. (2021). Combined Drought and Heat Stress in Rice: Responses, Phenotyping and Strategies to Improve Tolerance. Rice Science, 28, 233–242. https://doi.org/10.1016/j.rsci.2021.04.003.

Cotrina Cabello, G. G., Ruiz Rodriguez, A., Husnain Gondal, A., Areche, F. O., Flores, D. D. C., Astete, J. A. Q., Camayo-Lapa, B. F., Yapias, R. J. M., Jabbar, A., Yovera Saldarriaga, J., Salas-Contreras, W. H., and Cruz Nieto, D. D. (2023). DOI Withdrawn: Plant adaptability to climate change and drought stress for crop growth and production. CABI Reviews. https://doi.org/10.1079/cabireviews.2023.004.

Craufurd, P. Q., and Peacock, J. M. (1993). Effect of heat and drought stress on sorghum (*Sorghum bicolor*). II. Grain yield. Experimental Agriculture, 29, 77–86. https://doi.org/10.1017/S0014479700020421.

Craufurd, P. Q., Ellis, R. H., Summerfield, R. J., and Menin, H. (1996). Development in cowpea (*Vigna unguiculata*). The influence of temperature on seed germination and seedling emergence. Experimental Agriculture, 32, 1–12.

Cvikrová, M., Gemperlová, L., Martincová, O., and Vanková, R. (2013). Effect of drought and combined drought and heat stress on polyamine metabolism in proline-over-producing tobacco plants. Plant Physiology and Biochemistry, 73, 7–15. https://doi.org/10.1016/j.plaphy.2013.08.005.

Das, D. R., Sarkar, M., and Paul, A. (2023). Tools and approaches for assessing stress-responsive secondary metabolites to design climate-smart crops. In *Biology and Biotechnology of Environmental Stress Tolerance in Plants* (pp. 305-370). Apple Academic Press.

De Souza, A. A., De Carvalho, A. J., Bastos, E. A., Cardoso, M. J., Júlio, M. P. M., Batista, P. S. C., Julio, B. H. M., Campolina, C. V., Portugal, A. F., De Menezes, C. B., and De Oliveira, S. M. (2021). Grain sorghum under pre- and post-flowering drought stress in a semi-arid environment. Australian Journal of Crop Science, 15, 1139–1145. https://doi.org/10.21475/ajcs.21.15.08.

De Vita, P., Mastrangelo, A. M., Matteu, L., Mazzucotelli, E., Virzì, N., Palumbo, M., Storto, M. Lo, Rizza, F., and Cattivelli, L. (2010). Genetic improvement effects on yield stability in durum wheat genotypes grown in Italy. Field Crops Research, 119, 68–77. https://doi.org/10.1016/j.fcr.2010.06.016.

Dehnavi, A. R., Zahedi, M., Agnieszka, L., Cardenas, P.S., and Piernik, A. (2020). Effect of salinity on seed germination and seedling development of sorghum (*Sorghum bicolor* (L.) Moench) genotypes. Agronomy, 10,859. https://doi.org/10.3390/agronomy10060859.

Devnarain, N., Crampton, B. G., Chikwamba, R., Becker, J. V. W., and Kennedy, M. M. (2016). Physiological responses of selected African sorghum landraces to progressive water stress and re-watering. South African Journal of Botany, 103, 61–69. https://doi.org/10.1016/j.sajb.2015.09.008.

Dewey, D. R., and Lu, K. H. (1959). A correlation and path-coefficient analysis of components of crested wheatgrass seed production. Agronomy Journal, 51, 515–518. https://doi.org/10.2134/agronj1959.00021962005100090002x.

dos Santos, T. B., Ribas, A. F., de Souza, S. G. H., Budzinski, I. G. F., and Domingues, D. S. (2022). Physiological responses to drought, salinity, and heat stress in plants: A Review. Stresses, 2, 113–135. https://doi.org/10.3390/stresses2010009.

Dugas, D. V., Monaco, M. K., Olsen, A., Klein, R. R., Kumari, S., Ware, D., and Klein, P. E. (2011). Functional annotation of the transcriptome of *Sorghum bicolor* in response to osmotic stress and abscisic acid. BMC Genomics, 12. https://doi.org/10.1186/1471-2164-12-514.

Eggen, M., Ozdogan, M., Zaitchik, B., Ademe, D., Foltz, J., and Simane, B. (2019). Vulnerability of sorghum production to extreme, sub-seasonal weather under climate change. Environmental Research Letters, 14. https://doi.org/10.1088/1748-9326/aafe19.

Ehdaie, B., Layne, A. P., and Waines, J. G. (2012). Root system plasticity to drought influences grain yield in bread wheat. Euphytica, 186, 219–232. https://doi.org/10.1007/s10681-011-0585-9.

Engelbrecht, B. M. J., Tyree, M. T., and Kursar, T. A. (2007). Visual assessment of wilting as a measure of leaf water potential and seedling drought survival. Journal of Tropical Ecology, 23, 497–500. https://doi.org/10.1017/S026646740700421X.

Enyew, M., Carlsson, A. S., Geleta, M., Tesfaye, K., Hammenhag, C., Seyoum, A., and Feyissa, T. (2022). Novel sources of drought tolerance in sorghum landraces revealed via the analyses of genotype-by-environment interactions. Frontiers in Plant Science, 13, 1-14. https://doi.org/10.3389/fpls.2022.1062984.

Fahad, S., Bajwa, A. A., Nazir, U., Anjum, S. A., and Farooq, A. (2017). Crop production under drought and heat stress: Plant responses and management options. Frontiers in Plant Science, 8. https://doi.org/10.3389/fpls.2017.01147.

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

Ferradini, N., Iannacone, R., Capomaccio, S., Metelli, A., Armentano, N., Semeraro, L., Cellini, F., Veronesi, F., and Rosellini, D. (2015). Assessment of heat shock protein 70 induction by heat in alfalfa varieties and constitutive overexpression in transgenic plants. PLoS ONE, 10, 1–15. https://doi.org/10.1371/journal.pone.0126051.

Finotello, F., Calura, E., Risso, D., Hautaniemi, S., and Romualdi, C. (2020). Multi-omic data integration in oncology. Frontiers in Oncology, 10, 1768.

Fischer, R. A., and Maurer, R. (1978). Drought resistance in spring wheat cultivars. I. Grain yield responses. Australian Journal of Agricultural Research, 29, 897–912. https://doi.org/10.1071/AR9780897.

Fischer, R. A., Rees, D., Sayre, K. D., Lu, Z. M., Condon, A. G., and Larque Saavedra, A. (1998). Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. Crop Science, 38, 1467–1475. https://doi.org/10.2135/cropsci1998.0011183X003800060011x.

Foti, S., Cosentino, S. L., Patane, C. D., and Agosta, G. M. (2002). Effects of osmo-conditioning upon seed germination of sorghum (*Sorghum bicolor* (L) Moench) under low temperatures. Seed Science Technology, 30, 521-533.

Galicia-Juárez, M., Sinagawa-García, S., Gutiérrez-Diez, A., Héctor, W.-A., Zavala-García, F., Villa, F., Ex, C., Canada, H. El, and León, N. (2020). Thermotolerance in sorghum lines [*Sorghum bicolor* (L.) Moench] for grain. Revista Mexicana Ciencias Agrícolas, 11, 221–227.

Galicia-Juárez, M., Zavala-García, F., Sinagawa-García, S.R., Gutiérrez-Diez, A., Williams-Alanís, H., Cisneros-López, M.E., Valle-Gough, R.E., Flores-Garivay, R., and Santillano-Cázares, J. (2021). Identification of sorghum (*Sorghum bicolor* (L.). Plants, 10, 2265. https://doi.org/10.3390/plants10112265.

Gano, B., Dembele, J. S. B., Tovignan, T. K., Sine, B., Vadez, V., Diouf, D., and Audebert, A. (2021). Adaptation responses to early drought stress of West Africa sorghum varieties. Agronomy, 11, 1–21. https://doi.org/10.3390/agronomy11030443.

Gavuzzi, P., Rizza, F., Palumbo, M., Campanile, R. G., Ricciardi, G. L., and Borghi, B. (1997). Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. Canadian Journal of Plant Science, 77, 523–531. https://doi.org/10.4141/P96-130.

Geilbyo, G., Toth, E., Farkas, C., Horel, A., Kasi, I., and Bakacsi, Z. (2018). Potential impacts of climate change on soil properties. Agrochemistry and Soil Science, 67, 21-141. DOI:10.1556/0088.2018.67.1.9.

George-Jaeggli, B., Mortlock, M. Y., and Borrell, A. K. (2017). Bigger is not always better: Reducing leaf area helps stay-green sorghum use soil water more slowly. Environmental and Experimental Botany, 138, 119-129. https://doi.org/10.1016/j.envexpbot.2017.03.002.

Georgii, E., Jin, M., Zhao, J., Kanawati, B., Schmitt-Kopplin, P., Albert, A., Winkler, J. B., and Schäffner, A. R. (2017). Relationships between drought, heat and air humidity responses revealed by transcriptome-metabolome co-analysis. BMC Plant Biology, 17, 1-23. https://doi.org/10.1186/s12870-017-1062-y.

Gong, M., Chen, B. O., Li, Z. G., and Guo, L. H. (2001). Heat-shock-induced cross adaptation to heat, chilling, drought and salt stress in maize seedlings and involvement of H_2O_2. Journal of Plant Physiology, 158, 1125–1130. https://doi.org/10.1078/0176-1617-00327.

Gosavi, G. U., Jadhav, A. S., Kale, A. A., Gadakh, S. R., Pawar, B. D., and Chimote, V. P. (2014). Effect of heat stress on proline, chlorophyll content, heat shock proteins and antioxidant enzyme activity in sorghum (*Sorghum bicolor*) at seedlings stage. Indian Journal of Biotechnology, 13, 356-363.

Govindaraj, M., Djanaguiraman, M., Djalovic, I., Shailani, A., Rawat, N., Singla-Pareek, S., and Prasad, P. (2021). Drought and high temperature stress in sorghum: Physiological, genetic and molecular insights, and breeding approaches. International Journal of Molecular Sciences, 22, 9826. https://doi.org/10.3390/ijms22189826.

Guihur, A., Rebeaud, M. E., and Goloubinoff, P. (2022). How do plants feel the heat and survive? Trends in Biochemical Sciences, 47, 824–838. https://doi.org/10.1016/j.tibs.2022.05.004.

Guo, Y. Y., Tian, S. S., Liu, S. S., Wang, W. Q., and Sui, N. (2018). Energy dissipation and antioxidant enzyme system protect photosystem II of sweet sorghum under drought stress. Photosynthetica, 56, 861–872. https://doi.org/10.1007/s11099-017-0741-0.

Hadebe, S. T., Modi, A. T., and Mabhaudhi, T. (2017). Drought tolerance and water use of cereal crops: A focus on sorghum as a food security crop in sub-Saharan Africa. Journal of Agronomy and Crop Science, 203, 177–191. https://doi.org/10.1111/jac.12191.

Handayani, T., and Watanabe, K. (2020). The combination of drought and heat stress has a greater effect on potato plants than single stresses. Plant, Soil and Environment, 66, 175–182. https://doi.org/10.17221/126/2020-PSE.

Hao, Z. F., Li, X. H., Su, Z. J., Xie, C. X., Li, M. S., Liang, X. L., Weng, J. F., Zhang, D. G., Li, L., and Zhang, S. H. (2011). A proposed selection criterion for drought resistance across multiple environments in maize. Breeding Science, 61, 101-108. https://doi.org/10.1270/jsbbs.61.101.

Hao, H., Li, Z., Leng, C., Lu, C., Luo, H., Liu, Y., and Wu, X. (2021). Sorghum breeding in the genomic era : opportunities and challenges. Theoretical and Applied Genetics, 134, 1899-1924. https://doi.org/10.1007/s00122-021-03789-z.

Hassan, A. M., Ahmed, M. F., and Rashed, M. A. (2022). Performance and transcriptomic analysis of *Sorghum bicolor* responding to drought stress. Sabrao Journal of Breeding and Genetics, 54, 814-825. https://doi.org/10.54910/sabrao2022.54.4.12.

Hatfield, J. L., and Prueger, J. H. (2015). Temperature extremes: Effect on plant growth and development. Weather and Climate Extremes, 10, 4–10. https://doi.org/10.1016/j.wace.2015.08.001.

Hein, N. T., Ciampitti, I. A., and Jagadish, S. V. K. (2021). Bottlenecks and opportunities in field-based high-throughput phenotyping for heat and drought stress. Journal of Experimental Botany, 72, 5102–5116. https://doi.org/10.1093/jxb/erab021.

Hossain, A. B. S., Sears, R. G., Cox, T. S., and Paulsen, G. M. (1990). Desiccation tolerance and its relationship to assimilate partitioning in winter wheat. Crop Science, 30, 622–627. https://doi.org/10.2135/cropsci1990.0011183x003000030030x.

Howarth, C. J., Pollock, C. J., and Peacock, J. M. (1997). Development of laboratory-based methods for assessing seedling thermotolerance in pearl millet. New Phytologist, 137, 129-139. https://doi.org/10.1046/j.1469-8137.1997.00827.x.

Hu, X., Li, Y., Li, C., Yang, H., Wang, W., and Lu, M. (2010). Characterization of small heat shock proteins associated with maize tolerance to combined drought and heat stress. Journal of Plant Growth Regulation, 29, 455–464. https://doi.org/10.1007/s00344-010-9157-9159.

Hussain, H. A., Men, S., Hussain, S., Chen, Y., Ali, S., Zhang, S., Zhang, K., Li, Y., Xu, Q., Liao, C., and Wang, L. (2019). Interactive effects of drought and heat stresses on morpho-physiological attributes, yield, nutrient uptake and oxidative status in maize hybrids. Scientific Reports, 9, 1–12. https://doi.org/10.1038/s41598-019-40362-7.

Ibraheem, O., Adigun, R. O., and Olatunji, I. T. (2018). Omics technologies in unraveling plant stress responses; using sorghum as a model crop, how far have we gone. Vegetos, 31, 2.

https://doi.org/10.4172/2229-4473.1000405.

Ivanova, N., Gugleva, V., Dobreva, M., Pehlivanov, I., Stefanov, S., and Andonova, V. (2016). We are Intech Open, the world's leading publisher of Open Access books Built by scientists for scientists Intech, i(tourism), 13.

Jagtap, V., Bhargava, S., Streb, P., and Feierabend, J. (1998). Comparative effect of water, heat and light stresses on photosynthetic reactions in *Sorghum bicolor* (L.) Moench. Journal of Experimental Botany, 49, 1715-1721. https://doi.org/10.1093/jxb/49.327.1715.

Jain, M., Chourey, P. S., Boote, K. J., and Allen, L. H. (2010). Short-term high temperature growth conditions during vegetative-to-reproductive phase transition irreversibly compromise cell wall invertase-mediated sucrose catalysis and microspore meiosis in grain sorghum (*Sorghum bicolor*). Journal of Plant Physiology, 167, 578–582.

Jeyasri, R., Muthuramalingam, P., Satish, L., and Pandian, S. K. (2021). An overview of abiotic stress in cereal crops: Negative Impacts, Regulation, Biotechnology and Integrated Omics. Plants, 10, 1472.

Jha, U. C., Bohra, A., Singh, N. P. (2014). Heat stress in crop plants: Its nature, impacts and integrated breeding strategies to improve heat tolerance. Plant Breeding, 133, 679-701. https://doi.org/10.1111/pbr.12217.

Joshi, C. P., Klueva, N. Y., Morrow, K. J., and Nguyen, H. T. (1997). Expression of a unique plastid-localized heat-shock protein is genetically linked to acquired thermotolerance in wheat. Theoretical and Applied Genetics, 95, 834-841. https://doi.org/10.1007/s001220050633.

Kader, M. (2005). A comparison of seed germination calculation formulae and the associated interpretation of resulting data. Journal and Proceedings of the Royal Society of New South Wales, 138, 65-75.

Kamal, N. M., Gorafi, Y. S. A., Abdelrahman, M., Abdellatef, E., and Tsujimoto, H. (2019). Stay-green trait: a prospective approach for yield potential, and drought and heat stress adaptation in globally important cereals. International Journal of Molecular Sciences, 20, 5837.

Kapoor, D., Savita, B., Marco, L., Sharma, A., Mathusany, R., and Sharma, A. (2020). The impact of drought in plant metabolism: How to exploit tolerance mechanisms to increase crop production. Applied Sciences, 10, 5692. https://doi.org/10.3390/app10165692.

Kaur, B., Sandhu, K. S., Kamal, R., Kaur, K., Singh, J., Röder, M. S., and Muqaddasi, Q. H. (2021). Omics for the improvement of abiotic, biotic, and agronomic traits in major cereal crops: Applications, challenges, and prospects. Plants, 10, 1989.

Kayalvizhi, V., and Antony, U. (2011). Microbial and physico-chemical changes in tomato juice subjected to pulsed electric field treatment. African Journal of Agricultural Research, 6, 6348–6353. https://doi.org/10.5897/A.

Keles, Y., and Oncel, I. (2002). Response of antioxidative defence system to temperature and water stress combinations in wheat seedlings. Plant Science, 163, 783–790.

Keskin, A., Tumer, E. I., Birinci, A. (2010). Analysis of the factors affecting the instrument and machinery assets in enterprises that deal with agricultural production: The case of Erzurum province. African Journal of Agricultural Research, 5, 600–605. https://doi.org/10.5897/AJAR10.027.

Khaeim, H., Kende, Z., Jolánkai, M., Kovács, G. P., Gyuricza, C., and Tarnawa, Á. (2022). Impact of temperature and water on seed germination and seedling growth of maize (*Zea mays* L.). Agronomy, 12, 397. https://doi.org/10.3390/agronomy12020397.

Khalifa, M., and Eltahir, E. A. B. (2023). Assessment of global sorghum production, tolerance, and climate risk. Frontiers in Sustainable Food Systems, 7, 1–20. https://doi.org/10.3389/fsufs.2023.1184373.

Khan, A., Ahmad, M., Ahmed, M., and Hussain, M. I. (2020). Rising atmospheric temperature impact on wheat and thermotolerance strategies. Plants, 10, 43. https://doi.org/10.3390/plants10010043.

Khanthavong, P., Yabuta, S., Malik, A. I., Hossain, M. A., Akagi, I., and Sakagami, J. I. (2022). Combinational variation temperature and soil water response of stomata and biomass production in maize, millet, sorghum and rice. Plants, 11. https://doi.org/10.3390/plants11081039.

Khaton, M., Sagar, A., Tajkia, J., Islam, M., Mahmud, M., and Hossain, A. (2016). Effect of moisture stress on morphological and yield attributes of four sorghum varieties. Progressive Agriculture, 27, 265–271. https://doi.org/10.3329/pa.v27i3.30806.

Kihara, J., Bationo, A., Mugendi, D. N., Martius, C., and Vlek, P. L. G. (2011). Conservation tillage, local organic resources and nitrogen fertilizer combinations affect maize productivity, soil structure and nutrient balances in semi-arid Kenya. Nutrient Cycling in Agroecosystems, 90, 213–225. https://doi.org/10.1007/s10705-011-9423-7.

Kong, W., Guo, H., Goff, V. H., Lee, T. H., Kim, C., and Paterson, A. H. (2014). Genetic analysis of vegetative branching in sorghum. Theoretical and Applied Genetics, 127, 2387-2403.

Krasenky, J., and Jonak, C. (2012). Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. Journal of Experimental Botany, 63, 1593-1608. https://doi.org/10.1093/jxb/err460.

Krupa, K. N., Dalawai, N., Shashidhar, H. E., Harinikumar, K. M., Manojkumar, H. B., Bharani, S., and Turaidar, V. (2017). Mechanisms of drought tolerance in Sorghum: a review. International Journal of Pure and Applied Bioscience, 5, 221-237. http://dx.doi.org/10.18782/2320-7051.2845

Kumar, D., As, N., Suresh, A., and Dogra, K. (2022). Morphological and physiological response of sorghum under drought stress condition : Review. The Pharma Innovation journal, 11, 3581–3586.

Kumar, M., Govindasamy, V., Rane, J., Singh, A. K., Choudhary, R. L., Raina, S. K., George, P., Aher, L. K., and Singh, N. P. (2017). Canopy temperature depression (CTD) and canopy greenness associated with variation in seed yield of soybean genotypes grown in semi-arid environment. South African Journal of Botany, 113, 230-238. https://doi.org/10.1016/j.sajb.2017.08.016.

Kumar, R. R., and Rai, R. D. (2014). Can wheat beat the heat: Understanding the mechanism of thermotolerance in wheat (*Triticum aestivum* L.). Cereal Research Communications, 42, 1–18. https://doi.org/10.1556/CRC.42.2014.1.1.

Kumar, A. A., Venkata, B., Reddy, S., Sharma, H. C., Hash, C. T., Rao, P. S., Ramaiah, B., and Reddy, P. S. (2011). Recent Advances in Sorghum Genetic Enhancement Research at ICRISAT. American Journal of Plant Sciences, 2, 589-600. https://doi.org/10.4236/ajps.2011.24070.

Larkindale, J., Hall, J. D., Knight, M. R., and Vierling, E. (2005). Heat stress phenotypes of Arabidopsis mutants implicate multiple signaling pathways in the acquisition of thermotolerance. Plant Physiology, 138, 882–897. https://doi.org/10.1104/pp.105.062257.

Lawas, L. M. F., Shi, W., Yoshimoto, M., Hasegawa, T., Hincha, D. K., Zuther, E., and Jagadish, S. V. K. (2018). Combined drought and heat stress impact during flowering and grain filling in contrasting rice cultivars grown under field conditions. Field Crops Research, 229, 66–77. https://doi.org/10.1016/j.fcr.2018.09.009.

Lepekhov, S. B. (2022). Canopy temperature depression for drought-and heat stress tolerance in wheat breeding. Vavilovskii Zhurnal Genetiki i Selektsii, 26, 196–201. https://doi.org/10.18699/VJGB-22-24.

Lopez, J. R., Erickson, J. E., Munoz, P., Saballos, A., Felderhoff, T. J., and Vermerris, W. (2017). QTLs associated with crown root angle, stomatal conductance, and maturity in sorghum. The Plant Genome, 10, 1-12. https://doi.org/10.3835/plantgenome2016.04.0038.

Liu, S., Zenda, T., Tian, Z., and Huang, Z. (2023). Metabolic pathways engineering for drought or/and heat tolerance in cereals. Frontiers in Plant Science, 14, 1111875. https://doi.org/10.3389/fpls.2023.1111875.

Lobell, D. B., Hammer, G. L., Chenu, K., Zheng, B., Mclean, G., and Chapman, S. C. (2015). The shifting influence of drought and heat stress for crops in northeast Australia. Global Change Biology, 21, 4115–4127. https://doi.org/10.1111/gcb.13022.

Machado, S., and Paulsen, G. M. (2001). Combined effects of drought and high temperature on water relations of wheat and sorghum. Plant and Soil, 233, 179–187. https://doi.org/10.1023/A:1010346601643.

Mackay, A. (2008). Climate Change 2007: Impacts, adaptation and vulnerability. Contribution of working group II to the fourth assessment report of the intergovernmental panel on climate change. Journal of Environmental Quality, 37, 2407–2407. https://doi.org/10.2134/jeq2008.0015br.

Magalhães, P. C., de Souza T. C., Lavinsky, A.O., Pereira, P. E., de Albuquerque, P. E. P., de Oliveira, L. L., and de Castro, E. M. (2016). Phenotypic plasticity of root system and shoots of *Sorghum bicolor* under different soil water levels during pre-flowering stage. Australian Journal of Crop science, 10, 81–87.

Makuvaro, V., Walker, S., Munodawafa, A., Masere, T. P., Murewi, C., and Chagonda, I. (2014). An overview of current agronomic practices of smallholder farmers in semi-arid Central and Western Zimbabwe. African Journal of Agricultural Research, 9, 2710–2720. https://doi.org/10.5897/ajar11.606.

Masetto, T., Oba, G., and Jesus, M. (2017). Germination of sweet sorghum seeds in different water potentials. American Journal of Plant Sciences, 8, 3062-3072. http://doi.org/10.4236/ajps.2017.812206.

Mehta, S., James, D., and Reddy, M. K. (2019). Omics technologies for abiotic stress tolerance in plants: Current status and prospects. Recent Approaches in Omics for Plant Resilience to Climate Change, 1-34. https://doi.org/10.1007/978-3-030-21687-0.

Meseka, S., Menkir, A., Bossey, B., and Mengesha, W. (2018). Performance assessment of drought tolerant maize hybrids under combined drought and heat stress. Agronomy, 8, 274.

Mickky, B., Aldesuquy, H., and Elnajar, M. (2019). Uni- and multi-variate assessment of drought response yield Indices in 10 wheat cultivars. Journal of Crop Science and Biotechnology, 22, 21–29. https://doi.org/10.1007/s12892-018-0221-0.

Mingli, L., Meng, L., Kaichang, L., and Na, S. (2015). Effects of drought stress on seed germination and seedling growth of different maize varieties. Journal of Agricultural Science, 7, 231. http://dx.doi.org/10.5539/jas.v7n5p231.

Mittler, R. (2006). Abiotic stress, the field environment and stress combination. Trends in Plant Science, 11, 15–19. https://doi.org/10.1016/j.tplants.2005.11.002.

Mittler, R., Finka, A., and Goloubinoff, P. (2012). How do plants feel the heat? Trends in Biochemical Sciences, 37, 118–125. https://doi.org/10.1016/j.tibs.2011.11.007.

Mongi, H., Majuule, A.E., and Lyimo, J. G. (2010). Vulnerability and adaptation of rainfed agriculture to climate change and variability in semi-arid Tanzania. African Journal of Environmental Science and Technology, 4, 371–381. https://doi.org/10.5897/ajest09.207.

Morris, G. P., Ramu, P., Deshpande, S. P., Hash, C. T., Shah, T., Upadhyaya, H. D., Riera-Lizarazu, O., Brown, P. J., Acharya, C. B., Mitchell, S. E., Harriman, J., Glaubitz, J. C., Buckler, E. S., and Kresovich, S. (2013). Population genomic and genome-wide association studies of agroclimatic traits in sorghum. Proceedings of the National Academy of Sciences of the United States of America, 110, 453–458. https://doi.org/10.1073/pnas.1215985110.

Moshelion, M. (2020). The dichotomy of yield and drought resistance. EMBO Reports, 21, 1–7. https://doi.org/10.15252/embr.202051598.

Moyo, R., Ndlovu, E., Moyo, N., Kudita, S., and Maphosa, M. (2015). Physiological parameters of seed vigour in ex situ stored sorghum germplasm. Journal of Cereals and Oil Seeds, 6, 31-38. http://dx.doi.org/10.5897/JCO15.

Muchow, R. C., and Carberry, P. S. (1990). Phenology and leaf-area development in a tropical grain sorghum. Field Crops Research, 23, 221–237. https://doi.org/10.1016/0378-4290(90)90056-H.

Mukherjee, A., Maheshwari, U., Sharma, V., Sharma, A., and Kumar, S. (2024). Functional insight into multi-omics-based interventions for climatic resilience in sorghum (*Sorghum bicolor*): a nutritionally rich cereal crop. Planta, 259, 1-21. https://doi.org/10.1007/s00425-024-04365-7.

Mutava, R. N. (2012). Evaluation of sorghum genotypes for variation in canopy temperature and drought tolerance. MSc Thesis, Kansas State University.

Muthamilarasan, M., and Kumar, N. (2019). Multi-omics approaches for strategic improvement of stress tolerance in underutilized crop species : A climate change perspective. Advances in Genetics, 103, 1-38. https://doi.org/10.1016/bs.adgen.2019.01.001.

Nadeem, M., Li, J., Wang, M., Shah, L., Lu, S., Wang, X., and Ma, C. (2018). Unraveling field crops sensitivity to heat stress: Mechanisms, Approaches, and Future Prospects. Agronomy, 8. https://doi.org/10.3390/AGRONOMY8070128.

Nahar, K., Hasanuzzaman, M., and Fujita, M. (2016). Heat stress responses and thermotolerance in soybean. In Abiotic and Biotic Stresses in Soybean Production: Soybean Production: 1, 261-284. Elsevier Inc. https://doi.org/10.1016/B978-0-12-801536-0.000128.

Naim, A. M. El, Jabereldar, A. A., El Naim, A. M., Abdalla, A. A., and Dagash, Y. M. (2017). Effect of water stress on yield and water use efficiency of sorghum (*Sorghum bicolor* L. Moench) in semi-arid environment. International Journal of Agriculture and Forestry, 7, 1–6. https://doi.org/10.5923/j.ijaf.20170701.01.

Nciizah, T., Nciizah, E., Mubekaphi, C., and Nciizah, A. D. (2021). Role of small grains in adapting to Climate Change: Zvishabane District, Zimbabwe. In *African handbook of Climate Change Adaptation* (pp. 581-599). Cham: Springer International Publishing.

Ndlovu, E., van Staden, J., and Maphosa, M. (2021). Morpho-physiological effects of moisture, heat and combined stresses on *Sorghum bicolor* [Moench (L.)] and its acclimation mechanisms. Plant Stress, 2, 100018. https://doi.org/10.1016/j.stress.2021.100018.

Ndlovu, E, Maphosa, M, and van Staden, J. (2023). Seedlings growth *in-vitro* and pot under drought and heat stress. Mendeley. https://doi.org/10.17632/dkff5gcyns.1.

Nhiwatiwa, T., Dalu, T., and Brendonck, L. (2017). Impact of irrigation based sugarcane cultivation on the Chiredzi and Runde Rivers quality, Zimbabwe. Science of the Total Environment, 587, 316-325. https://doi.org/10.1016/j.scitotenv.2017.02.155.

Nyoni, N., Ndlovu, E., and Maphosa, M. (2020). Effect of priming regimes on seed germination of field crops. African Crop Science Journal, 28, 169-176. https://doi.org/10.4314/acsj.v28i2.3.

Nxele, X., Klein, A., and Ndimba, B. K. (2017). Drought and salinity stress alters ROS accumulation, water retention, and osmolyte content in sorghum plants. South African Journal of Botany, 108, 261-266. https://doi.org/10.1016/j.sajb.2016.11.003.

Ogbaga, C. C., Stepien, P., Dyson, B. C., Rattray, N. J. W., Ellis, D. I., Goodacre, R., and Johnson, G. N. (2016). Biochemical analyses of sorghum varieties reveal differential responses to drought. PLoS ONE, 11, 1-20. https://doi.org/10.1371/journal.pone.0154423.

Ogden, A.J., Abdali, S., Engbrecht, K. M., Zhou, M., and Handakumbura, P. P. (2020). Distinct pre-flowering drought tolerance strategies of *Sorghum bicolor* genotype RTx430 revealed by subcellular protein profiling. International Journal of Molecular Sciences, 21, 1-17. https://doi.org/10.3390/ijms21249706.

Oguz, M. C., Aycan, M., Oguz, E., and Poyraz, I. (2022). Drought Stress Tolerance in Plants : Interplay of Molecular, Biochemical and Physiological Responses in Important Development Stages. Physiologia, 2, 180-197.

Osmolovskaya, N., Shumilina, J., Kim, A., Didio, A., Grishina, T., Bilova, T., Keltsieva, O. A., Zhukov, V., Tikhonovich, I., Tarakhovskaya, E., and Frolov, A. (2018). Methodology of drought stress research : Experimental setup and physiological characterization. International Journal of Molecular Sciences, 19, 4089. https://doi.org/10.3390/ijms19124089.

Pachauri, R. K. (2014). Climate Change 2014 Synthesis Report Summary for Policymakers. Intergovernmental Panel on Climate Change (IPCC), 1-32. https://www.ipcc.ch/site/assets/uploads/2018/02/AR5_SYR_FINAL_SPM.pdf

Park, S. Y., Yu, J. W., Park, J. S., Li, J., Yoo, S. C., Lee, N. Y., Lee, S. K., Jeong, S. W., Hak, S. S., Koh, H. J., Jeon, J. S., Park, Y. Il, and Paek, N. C. (2007). The senescence-induced staygreen protein regulates chlorophyll degradation. Plant Cell, 19, 1649-1664. https://doi.org/10.1105/tpc.106.044891.

Patane, C., Saita, A., and Tubeileh, A. (2012). Seedling emergence response to early sowings in unprimed and osmoprimed seeds of fiber sorghums for energy biomass under semi-arid climate. Italian Journal of Agronomy, 7, 214-220. Doi:10.4081/ija.2012.e30.

Paupière, M. J., van Heusden, A. W., and Bovy, A. G. (2014). The metabolic basis of pollen thermo-tolerance: Perspectives for breeding. Metabolites, 4, 889-920. https://doi.org/10.3390/metabo4040889.

Paiva, C. L., Netto, D. A., Queiroz, V. A., and Gloria, M. B. A. (2022). Germinated sorghum (Sorghum bicolor L.) and seedlings show expressive contents of putrescine. *LWT*, *161*, 113367. https://doi.org/10.1016/j.lwt.2022.113367.

Pavli, O. I., Ghikas, D. V., Katsiotis, A., and Skaracis, G. N. (2011). Differential expression of heat shock protein genes in sorghum (*Sorghum bicolor* L.) genotypes under heat stress. Australian Journal of Crop Science, 5, 511–515.

Payne, R., Welham, S., and Harding, S. (2012). A Guide to REML in GenStat. VSN International, Hemel Hempstead, United Kingdom.

Peacock, J. M. (1982). Response and Tolerance of Sorghum to Temperature Stress. International Crops Research Institute for the Semi-Arid Tropics. Sorghum in the Eighties: Proceedings of the International Symposium on Sorghum, 143–158.

Pennisi, E. (2009). Plant genetics: How sorghum withstands heat and drought. Science, 323, 573. https://doi.org/10.1126/science.323.5914.573.

Perazzo, A. F., de Carvalho, G. G. P., Santos, E. M., Pinho, R. M. A., Campos, F. S., Macedo, C. H. O., Azevêdo, J. A. G., and Tabosa, J. N. (2014). Agronomic evaluation of 32 sorghum cultivars in the Brazilian semi-arid region. Revista Brasileira de Zootecnia, 43, 232-237. https://doi.org/10.1590/S1516-35982014000500002.

Phiri, K., Nhliziyo, M., Madzivire, S. I., Sithole, M., and Nyathi, D. (2020). Understanding climate smart agriculture and the resilience of smallholder farmers in Umguza district, Zimbabwe, (R. Robert, Ed.) Cogent Social Sciences, 70, 1970425. https://doi.org/10.1080/23311886.2021.1970425.

Phuong, N., Afolayan, G., Stützel, H., Uptmoor, R., and El-Soda, M. (2019). Unraveling the genetic complexity underlying sorghum response to water availability. PLoS ONE, 14, 1-15. https://doi.org/10.1371/journal.pone.0215515.

Pirasteh-Anosheh, H., Saed-Moucheshi, A., Pakniyat, H., and Pessarakli, M. (2016). Stomatal responses to drought stress. In Water Stress and Crop Plants: A Sustainable Approach, 1-2, 24-40. https://doi.org/10.1002/9781119054450.ch3.

Prasad, P. V. V, and Staggenborg, S. A. (2008). Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. Response of crops to limited water: Understanding and modeling water stress effects on plant growth processes. Advances in Agricultural Systems Modeling, 1, 301–355.

Prasad, P. V. V., Djanaguiraman, M., Perumal, R., and Ciampitti, I. A. (2015). Impact of high temperature stress on floret fertility and individual grain weight of grain sorghum: Sensitive stages and thresholds for temperature and duration. Frontiers in Plant Science, 6, 1–11. https://doi.org/10.3389/fpls.2015.00820.

Pyngrope, S., Bhoomika, K., and Dubey, R. S. (2013). Reactive oxygen species, ascorbate-glutathione pool, and enzymes of their metabolism in drought-sensitive and tolerant indica rice (*Oryza sativa* L.) seedlings subjected to progressing levels of water deficit. Protoplasma, 250, 585–600. https://doi.org/10.1007/s00709-012-0444-0.

Qaseem, M. F., Qureshi, R., and Shaheen, H. (2019). Effects of pre-anthesis drought, heat and their combination on the growth, yield and physiology of diverse wheat (*Triticum aestivum* L.) genotypes varying in sensitivity to heat and drought stress. Scientific Reports, 9, 1–12. https://doi.org/10.1038/s41598-019-43477-z.

Queiroz, M. S., Oliveira, C. E. S., Steiner, F., Zuffo, A. M., Zoz, T., Vendruscolo, E. P., Silva, M.V., Mello, B. F. F. R., Cabral, R. C., and Menis, F. T. (2019). Drought stresses on seed germination and early growth of maize and sorghum. Journal of Agricultural Science, 11, 310-318. https://doi.org/10.5539/jas.v11n2p310.

R Core Team (2021) ‘R: a language and environment for statistical computing. Version 4.1.2.’ (R Foundation for Statistical Computing: Vienna, Austria).

Rad, R. D., Sharifabad, H. H., Torabi, M., Azizinejad, R., Salemi, H., and Soltanabadi, M. H. (2023). Drought stress tolerance based on selection indices of resistant crops variety. Global Journal of Environmental Science and Management, 9, 287–298. https://doi.org/10.22034/gjesm.2023.02.08.

Rajendra P.V. B., Govindaraj, M., Djanaguiraman, M., Djalovic, I., Shailani, A., Rawat, N., Singla-Pareek, S. L., Pareek, A., and Vara Prasad, P. V. (2021). Drought and high temperature stress in sorghum: Physiological, genetic, and molecular insights and breeding approaches. International Journal of Molecular Sciences, 22, 1–25. https://doi.org/10.3390/ijms22189826.

Raza, A., Tabassum, J., Kudapa, H., and Varshney, R. K. (2021). Can omics deliver temperature resilient ready-to-grow crops? Critical Reviews in Biotechnology, 41, 1209-1232. https://doi.org/10.1080/07388551.2021.1898332.

Rivero, R. M., Mittler, R., Blumwald, E., and Zandalinas, S. I. (2022). Developing climate-resilient crops: Improving plant tolerance to stress combination. Plant Journal, 109, 373–389. https://doi.org/10.1111/tpj.15483.

Rizhsky, L., Liang, H., and Mittler, R. (2002). The combined effect of drought stress and heat shock on gene expression in tobacco. Plant Physiology, 130, 1143–1151. https://doi.org/10.1104/pp.006858.

Rizhsky, L., Liang, H., Shuman, J., Shulaev, V., Davletova, S., and Mittler, R. (2004). When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress. Plant Physiology, 134, 1683–1696. https://doi.org/10.1104/pp.103.033431.

Sanchez-Diaz, M. F., and Kramer, P. J. (1971). Behavior of corn and sorghum under water stress and during recovery. Plant physiology, 48, 613-616.

Sankar, S. M., and Satyavathi, C. T. (2014). Genetic diversity analysis for high temperature stress tolerance in pearl millet [*Pennisetum glaucum* (L.) R. Br]. Indian Journal of Plant Physiology, 19, 324-329. https://doi.org/10.1007/s40502-014-0099-2.

Sari, A. (2023). Germination characteristics of sorghum (*Sorghum bicolor* L.) affected by temperature variation. Agronomy Research, 21, 347-356. https://doi.org/10.15159/AR.23.021.

Sarshad, A., Talei, D., Torabi, M., Rafiei, F., and Nejatkhah, P. (2021). Morphological and biochemical responses of *Sorghum bicolor* (L.) Moench under drought stress. SN Applied Sciences, 3, 1–12. https://doi.org/10.1007/s42452-020-03977-4.

Sastry, A., and Barua, D. (2017). Leaf thermotolerance in tropical trees from a seasonally dry climate varies along the slow-fast resource acquisition spectrum. Scientific Reports, 7, 1-11. https://doi.org/10.1038/s41598-017-11343-5.

Satyavathi, C. T., Ambawat, S., Khandelwal, V., and Srivastava, R. K. (2021). Pearl millet: A climate-resilient nutricereal for mitigating hidden hunger and provide nutritional security. Frontiers in Plant Science, 12, 659938, 1-18. https://doi.org/10.3389/fpls.2021.659938.

Scarpeci, T. E., Zanor, M. I., and Valle, E. M. (2008). Investigating the role of plant heat shock proteins during oxidative stress. Plant Signaling and Behavior, 3, 856–857. https://doi.org/10.4161/psb.3.10.6021.

Selvaraj, M. G., Burow, G., Burke, J. J., Belamkar, V., Puppala, N., and Burow, M. D. (2011). Heat stress screening of peanut (*Arachis hypogaea* L.) seedlings for acquired thermotolerance. Plant Growth Regulation, 65, 83–91. https://doi.org/10.1007/s10725-011-9577-y.

Setimela, P. S., Lunduka, R., Zaman-Allah, M., Ndoro, O., and Cairns, J. E. (2017). Performance of elite drought tolerant maize varieties across eastern and southern Africa under smallholder farmers' conditions, season 2015-16. International Maize and Wheat Improvement Center (CIMMYT), 2015, 1-16.

Setimela, P. S., Andrews, D. J., Eskridge, K. M., and Partridge, J. (2007). Genetic evaluation of seedling heat tolerance in sorghum. African Crop Science Journal, 15, 33-42. https://doi.org/10.4314/acsj.v15i1.54411.

Shahriari, S., Ali Adam, B. P., Ghizan, S., Addul, R. A., and Ali Adam, B. P. (2014). Germination at low osmotic potential as a selection criteria for drought stress tolerance in sweet corn. African Journal of Biotechnology, 13, 294-300. https://doi.org/10.5897/AJB11.3435.

Shaar-Moshe, L., Hayouka, R., Roessner, U., and Peleg, Z. (2019). Phenotypic and metabolic plasticity shapes life-history strategies under combinations of abiotic stresses. Plant Direct, 3. https://doi.org/10.1002/pld3.113.

Shivhare, R., and Lata, C. (2019). Assessment of pearl millet genotypes for drought stress tolerance at early and late seedling stages. Acta Physiologiae Plantarum, 41, 39. https://doi.org/10.1007/s11738-019-2831-z.

Singh, R. K., Sood, P., Prasad, A., and Prasad, M. (2021). Advances in omics technology for improving crop yield and stress resilience. Plant Breeding, 140, 719-731. https://doi.org/10.1111/pbr.12963.

Song, L., Jiang, Y., Zhao, H., and Hou, M. (2012). Acquired thermotolerance in plants. Plant Cell, Tissue and Organ Culture, 111, 265-276. https://doi.org/10.1007/s11240-012-0198-6.

Srivastava, A., Srivastava, P., Sharma, A., and Sarlach, R. S. (2017). Canopy temperature an effective measure of drought stress tolerance in RIL population of wheat. Vegetos, 30, 59–63. https://doi.org/10.5958/2229-4473.2017.00011.8.

Stickler, F. C., Wearden, S., and Pauli, A. W. (1961). Leaf area determination in grain sorghum. Agronomy Journal, 53, 187–188. doi.org/10.2134/agronj1961.00021962005300030018x.

Sud, S., and Bhagwat, S. G. (2010). Assessment of acquired thermotolerance in Indian bread wheat and association with yield and component traits under heat stress environment. Journal of Food, Agriculture and Environment, 8, 622–627.

Surbhaiyya, S. D., Gahukar, S. J., Jadhav, P. V, Bhagat, S. Y., Moharil, M. P., Potdukhe, N. R., and Singh, P. K. (2018). *In vitro* based screening of promising wheat (*Triticum aestivum* L.) genotypes for osmotic stress imposed at seedling stage. International Journal of Current Microbiology and Applied Sciences, 6, 2500–2508.

Suzuki, N., Rivero, R. M., Shulaev, V., Blumwald, E., and Mittler, R. (2014). Abiotic and biotic stress combinations. New Phytologist, 203, 32–43. https://doi.org/10.1111/nph.12797.

Tack, J., Lingenfelser, J., and Jagadish, S. V. K. (2017). Disaggregating sorghum yield reductions under warming scenarios exposes narrow genetic diversity in US breeding programs. Proceedings of the National Academy of Sciences of the United States of America, 114, 9296–9301. https://doi.org/10.1073/pnas.1706383114.

Tandzi, L. N., Bradley, G., and Mutengwa, C. (2018). Morphological responses of maize to drought, heat and combined stresses at seedling stage. Journal of Biological Sciences, 19, 7–16. https://doi.org/10.3923/jbs.2019.7.16.

Tang, R. S., Zheng, J. C., Jin, Z. Q., Zhang, D. D., Huang, Y. H., and Chen, L. G. (2008). Possible correlation between high temperature-induced floret sterility and endogenous levels of IAA, GAs and ABA in rice (*Oryza sativa* L.). Plant Growth Regulation, 54, 37–43. https://doi.org/10.1007/s10725-007-9225-8.

Tari, I., Laskay, G., Takács, Z., and Poór, P. (2013). Response of sorghum to abiotic stresses: A review. Journal of Agronomy and Crop Science, 199, 264-274. https://doi.org/10.1111/jac.12017.

Thapa, S., Jessup, K. E., Pradhan, G. P., Rudd, J. C., Liu, S., Mahan, J. R., Devkota, R. N., Baker, J. A., and Xue, Q. (2018). Canopy temperature depression at grain filling correlates to winter wheat yield in the U.S. Southern high plains. Field Crops Research, 217, 11-19. https://doi.org/10.1016/j.fcr.2017.12.005.

Tovignan, T. K., Fonceka, D., Ndoye, I., Cisse, N., and Luquet, D. (2016). The sowing date and post-flowering water status affect the sugar and grain production of photoperiodic, sweet sorghum through the regulation of sink size and leaf area dynamics. Field Crops Research, 192, 67–77. https://doi.org/10.1016/j.fcr.2016.04.015.

Tovignan, T. K., Adoukonou-Sagbadja, H., Diatta, C., Clément-Vidal, A., Soutiras, A., Cisse, N., and Luquet, D. (2020). Terminal drought effect on sugar partitioning and metabolism is modulated by leaf stay-green and panicle size in the stem of sweet sorghum (*Sorghum bicolor* L. Moench). CABI Agriculture and Bioscience, 1, 1–11. https://doi.org/10.1186/s43170-020-00003-w.

Tripathi, D., Nam, A., Oldenburg, D. J., and Bendich, A. J. (2020). Reactive oxygen species, antioxidant agents, and DNA damage in developing maize mitochondria and plastids. Frontiers in Plant Science, 11. https://doi.org/10.3389/fpls.2020.00596.

Tsago, W., Andargie, M., and Takele, A. (2014). *In vitro* selection of sorghum (*Sorghum bicolor* (L) Moench) for polyethylene glycol (PEG) induced drought stress. Plant Science Today, 1, 62-68. http://dx.doi.org/10.14719/pst.2014.1.2.14.

Tsuji, W., Inanaga, S., Araki, H., Morita, S., An, P., and Sonobe, K. (2005). Development and distribution of root system in two grain sorghum cultivars originated from Sudan under drought stress. Plant Production Science, 8, 553–562. https://doi.org/10.1626/pps.8.553.

Tu, M., Du, C., Yu, B., Wang, G., Deng, Y., Wang, Y., Chen, M., Chang, J., Yang, G., He, G., and Xiong, Z. (2023). Current advances in the molecular regulation of abiotic stress tolerance in sorghum via transcriptomic, proteomic, and metabolomic approaches. Frontiers in Plant Science, 14, 1147328. https://doi.org/10.3389/fpls.2023.1147328.

Van Oosterom, E. J., Mahalakshmi, V., Bidinger, F. R., and Rao, K. P. (1996). Effect of water availability and temperature on the genotype-by-environment interaction of pearl millet in semi-arid tropical environments. Euphytica, 89, 175–183. https://doi.org/10.1007/BF00034603.

Velazco, J. G., Jordan, D. R., Mace, E. S., Hunt, C. H., Malosetti, M., and van Eeuwijk, F. A. (2019). Genomic prediction of grain yield and drought-adaptation capacity in sorghum is enhanced by multi-trait analysis. Frontiers in Plant Science, 10, 1–12. https://doi.org/10.3389/fpls.2019.00997.

Vile, D., Pervent, M., Belluau, M., Vasseur, F., Bresson, J., Muller, B., Granier, C., and Simonneau, T. (2012). Arabidopsis growth under prolonged high temperature and water deficit: independent or interactive effects? Plant, Cell and Environment, 35, 702–718. https://doi.org/10.1111/j.1365-3040.2011.02445.x.

Wang, X., Cai, J., Liu, F., Jin, M., Yu, H., Jiang, D., Wollenweber, B., Dai, T., and Cao, W. (2012). Pre-anthesis high temperature acclimation alleviates the negative effects of post-anthesis heat stress on stem stored carbohydrates remobilization and grain starch accumulation in wheat. Journal of Cereal Science, 55, 331–336. https://doi.org/10.1016/j.jcs.2012.01.004.

Wang, X., Cai, X., Xu, C., Wang, Q., and Dai, S. (2016). Drought-responsive mechanisms in plant leaves revealed by proteomics. International Journal of Molecular Sciences, 17, 1706. https://doi.org/10.3390/ijms17101706.

Wasaya, A., Zhang, X., Fang, Q., and Yan, Z. (2018). Root phenotyping for drought tolerance: a review. Agronomy, 8, 241. https://doi.org/10.3390/agronomy8110241.

Weatherley, P. E. (1950). Studies in the water relations of the cotton plant. I. The field measurement of water deficits in leaves. The New Phytologist, 49, 81-97.

Wen, B. (2015). Effects of high temperature and water stress on seed germination of theinvasive species Mexican sunflower. PLoS ONE, 10, e0141567. https://doi.org/10.1371/journal.pone.0141567.

Wipf, H. M. L., Bùi, T. N., and Coleman-Derr, D. (2021). Distinguishing between the impacts of heat and drought stress on the root microbiome of *Sorghum bicolor*. Phytobiomes Journal, 5, 166–176. https://doi.org/10.1094/PBIOMES-07-20-0052-R.

Woldesemayat, A. A., Modise, D. M., Gemeildien, J., Ndimba, B. K., and Christoffels, A. (2018). Cross-species multiple environmental stress responses: An integrated approach to identify candidate genes for multiple stress tolerance in sorghum (*Sorghum bicolor* (L.) Moench) and related model species. PLoS ONE, 13, 1-30. https://doi.org/10.1371/journal.pone.0192678.

Worku, M., De Groote, H., Munyua, B., Makumbi, D., Owino, F., Crossa, J., Beyene, Y., Mugo, S., Jumbo, M., Asea, G., Mutinda, C., Kwemoi, D. B., Woyengo, V., Olsen, M., and Prasanna, B. M. (2020). On-farm performance and farmers' participatory assessment of new stress-tolerant maize hybrids in Eastern Africa. Field Crops Research, 246, 107693. https://doi.org/10.1016/j.fcr.2019.107693.

Yadav, A. K., and Arya, R. (2013). Evaluation of pearl millet (*Pennisetum glaucum*) genotypes and validation of screening methods for supra-optimal temperature tolerance at seedling stage. Indian Journal of Agricultural Sciences, 83, 260-271.

Yadav, A. K., Arya, R. K., and Narwal, M. S. (2014). Screening of Pearl Millet F_1 Hybrids for Heat Tolerance at Early Seedling Stage. Advances in Agriculture, 2014. https://doi.org/10.1155/2014/231301.

Yadav, A. K., Arya, R. K., Singh, M. K., Kumar, D., and Panchta, R. (2016). Heat Tolerance in Pearl Millet : A Review. Forage Research, 42, 65–81.

Yahaya, M. A., Shimelis, H., Nebié, B., Mashilo, J., and Pop, G. (2023). Response of african sorghum genotypes for drought tolerance under variable environments. Agronomy, 13. https://doi.org/10.3390/agronomy13020557.

Yan, W., and Tinker, N. A. (2006). Biplot analysis of multi-environment trial data: Principles and applications. Canadian Journal of Plant Science, 86, 623–645. https://doi.org/10.4141/P05-169.

Yang, Y., Saand, M. A., Huang, L., and Abdelaal, W. B. (2021). Applications of multi-omics technologies for crop improvement. Frontiers in Plant Science, 12, 563953. https://doi.org/10.3389/fpls.2021.563953.

Ye, H., Roorkiwal, M., Valliyodan, B., Zhou, L., Chen, P., and Varshney, R. K. (2018). Genetic diversity of root system architecture in response to drought stress in grain legumes. Journal of Experimental Biology, 69, 3267-3277. https://doi.org/10.1093/jxb/ery082.

Yeh, C., Kaplinsky, N. J., Hu, C., and Charng, Y. (2012). Some like it hot, some like it warm : Phenotyping to explore thermotolerance diversity. Plant Science, 195, 10-23. https://doi.org/10.1016/j.plantsci.2012.06.004.

Yousaf, M. I., Riaz, M. W., and Jiang, Y. (2022). Concurrent effects of drought and heat stresses on physio-chemical attributes, antioxidant status and kernel quality traits in maize (*Zea mays* L.) Hybrids. Frontiers in Plant Science, 13, 898823. https://doi.org/10.3389/fpls.2022.898823.

Yuan, Y., Qian, H., and Yu, Y. (2011). Thermotolerance and antioxidant response induced by heat acclimation in *Freesia* seedlings. Acta Physiologiae Plantarum, 33, 1001-1009. https://doi.org/10.1007/s11738-010-0633-4.

Zargar, S. M., Mir, R. A., Ebinezer, L. B., Masi, A., Hami, A., Manzoor, M., Salgotra, R. K.,

Sofi, N. R., Mushtaq, R., Rohila, J. S., and Rakwal, R. (2022). Physiological and multi-omics approaches for explaining drought stress tolerance and supporting sustainable production of rice. Frontiers in Plant Science, 12, 803603. https://doi.org/10.3389/fpls.2021.803603.

Zahra, K. (2012). Evaluation of maize (*Zea mays* L.) hybrids, seed germination and seedling characters in water stress conditions. African Journal of Agricultural Research, 7, 6049-6053. https://doi.org/10.5897/ajar12.2113.

Zandalinas, S. I., Mittler, R., Balfagón, D., Arbona, V., and Gómez-Cadenas, A. (2018). Plant adaptations to the combination of drought and high temperatures. Physiologiae Plantarum, 162, 2-12. https://doi.org/10.1111/ppl.12540.

Zandalinas, S. I., Fichman, Y., Devireddy, A. R., Sengupta, S., Azad, R. K., and Mittler, R. (2020). Systemic signaling during abiotic stress combination in plants. Proceedings of the National Academy of Sciences of the United States of America, 117, 13810–13820. https://doi.org/10.1073/pnas.2005077117.

Zandalinas, S. I., Fritschi, F. B., and Mittler, R. (2020). Signal transduction networks during stress combination. Journal of Experimental Botany, 71, 1734-1741. https://doi.org/10.1093/jxb/erz486.

Zegada-Lizarazu, W., and Monti, A. (2013). Photosynthetic response of sweet sorghum to drought and re-watering at different growth stages. Physiologiae Plantarum, 149, 56-66. https://doi.org/10.1111/ppl.12016.

Zhao, F., Zhang, D., Zhao, Y., Wang, W., Yang, H., Ta, F., Li, C., and Hu, X. (2016). The difference of physiological and proteomic changes in maize leaves adaptation to drought, heat, and combined both stresses. Frontiers in Plant Science, 7, 1471. https://doi.org/10.3389/fpls.2016.01471.

Zulfiqar, F., Akram, N. A., and Ashraf, M. (2020). Osmoprotection in plants under abiotic stresses: new insights into a classical phenomenon. Planta, 251, 1-17. https://doi.org/10.1007/s00425-019-03293-1.